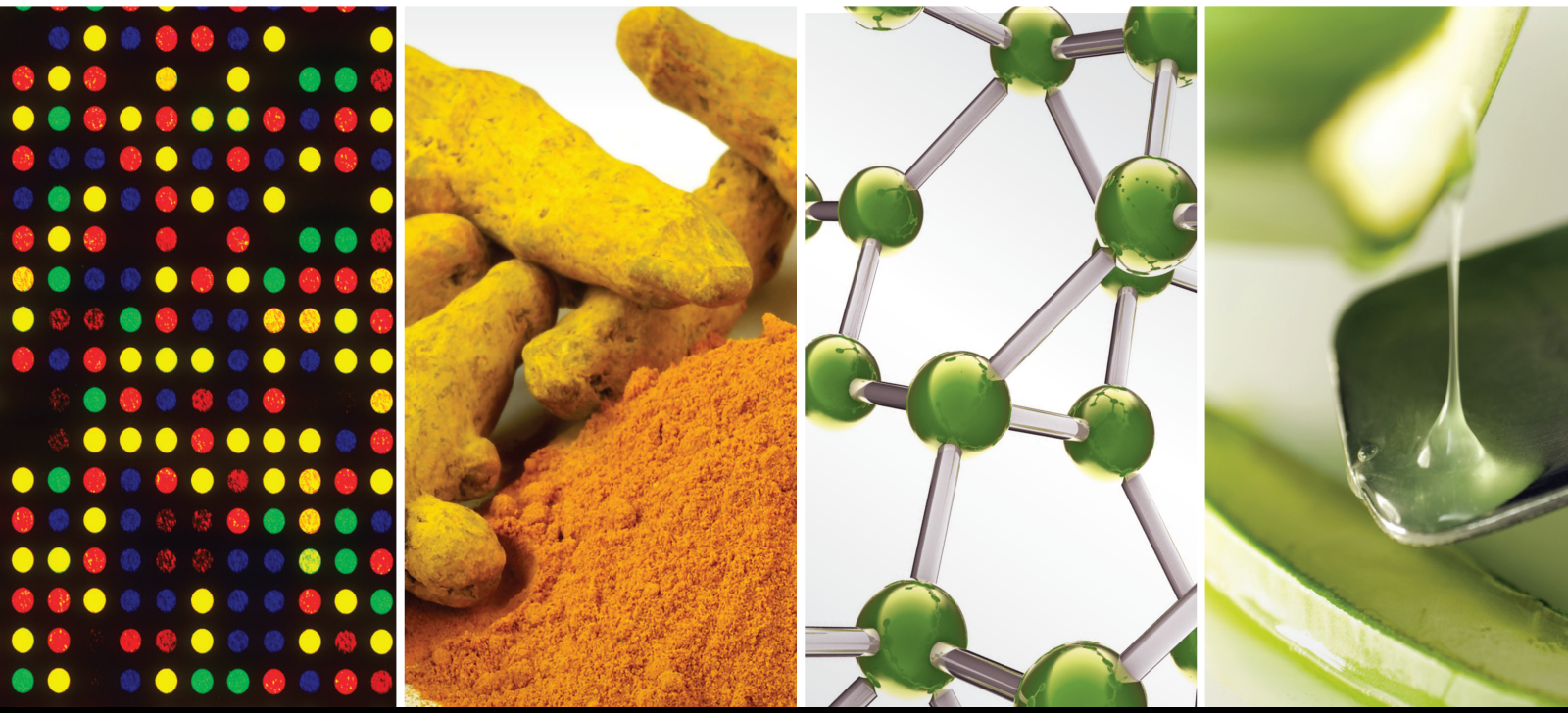
Evidence-Based Complementary and Alternative Medicine

Complementary and Alternative Medicine in Reproductive Endocrine Diseases 2021

Lead Guest Editor: Yuehui Zhang
Guest Editors: Xiaoke Wu, Linus R. Shao, Yong Wang, Tian-Bo Liu, and Min Hu



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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1989401, 10 pages
https://doi.org/10.1155/2022/1989401

Review Article

Research Trends of Acupuncture Therapy on Polycystic Ovary Syndrome: A Bibliometric Analysis

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Received 20 April 2022; Revised 25 July 2022; Accepted 13 September 2022; Published 12 October 2022

Academic Editor: Yue Hui Zhang

Background. Acupuncture has been confirmed as a suitable therapy for treating polycystic ovary syndrome (PCOS). However, there is no bibliometric analysis of the global use of acupuncture for PCOS. Our study used CiteSpace (5.8.R3) to provide a profile of the current state and trends in this field. *Methods*. Articles regarding acupuncture therapy for treating PCOS were retrieved from the Web of Science Core Collection. CiteSpace was used to analyze the number of publications, countries, institutions, journals, authors, cited references, and keywords by using standard bibliometric indicators. *Results*. A total of 159 publications were considered for the final analysis. The number of publications has slowly increased with fluctuations between years, and the most active countries, institutions, journals, and authors concerning acupuncture therapy for PCOS were identified. *Evidence-Based Complementary and Alternative Medicine* was the most productive journal, and Fertil Steril was the most cited. China and Heilongjiang University of Chinese Medicine were considered the most prolific countries and institutions in this field, respectively. Elisabet Stener Victorin became the most influential author and most cited author. Jedel E. published the most cited article. "Polycystic ovary syndrome" was the most frequent keyword, and the top three frontiers mentioned were research method, intervention, and outcome. *Conclusion*. The current status and trends in clinical research of acupuncture therapy on PCOS patients are revealed according to the results of this bibliometric study, which may facilitate researchers to identify hot topics and new directions for future study in this field.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of childbearing age. It is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphologic features, usually with manifestations of irregular menses, infertility, hirsutism, and acne [1, 2]. Besides, patients with PCOS are generally burdened with insulin resistance, leading to a higher incidence of type 2 diabetes and cardiovascular and cerebrovascular diseases [3, 4]. The pathogenesis of PCOS has not been well defined concerning the multiple complicated factors, including genetic, environmental, and transgenerational components [5, 6]. Depending on different diagnostic criteria, the worldwide prevalence of PCOS ranges from 4% to 21%, 4%–6.6% according to NIH 1990 criteria, and 4%–21% by Rotterdam 2003 criteria [7]. This disease has severely affected the quality of patients' daily lives and significantly increased the odds of moderate to severe depression and anxiety [8, 9].

To date, there is no good treatment option to improve the symptoms of PCOS. Education, self-empowerment, multidisciplinary care, lifestyle intervention, and weight management are prioritized treatment options [10]. However, few studies have shown that they positively impact ovulation and live births despite their actual improvement in patients' weight, menstrual cycles, and emotions to a certain extent [11–13]. Besides, long-term adherence to treatments is challenging. As second-line therapy, pharmacological treatment always chooses combined oral contraceptives (COCs) to alleviate menstrual irregularity and hyperandrogenism, which have been proven to increase the risk of

arterial thrombosis and venous thromboembolism [10, 14]. Meanwhile, letrozole, as the first-line pharmacological therapy for infertility, has been associated with musculoskeletal adverse events, vasomotor symptoms, vaginal dryness, hot flashes, and osteoporosis [15–17]. Furthermore, surgical procedures should only be used after all other treatment options have failed, as they will expose patients to surgery-related risks such as anesthetic difficulties, infection, and adhesions [6]. Apart from the interventions above, acupuncture, as a complementary and alternative therapy, has been confirmed as a suitable treatment for PCOS [18]. Clinical evidence shows that acupuncture has a specific effect on improving the symptoms of PCOS [19–22], but the treatment method and efficiency still need further research.

Bibliometrics is an interdisciplinary science that uses mathematical and statistical methods to analyze all knowledge carriers quantitatively. It is used to evaluate a specific discipline's social and scientific importance during a given period [23]. CiteSpace is an information visualization software jointly developed by Professor Chaomei Chen of Drexel University and the WISE Laboratory of the Dalian University of Technology. It can reveal hot spots and frontier directions in a particular field by visualizing the literature [24–26] and is widely applied in bibliometric analysis for its powerful functions and convenient operation.

As of yet, there has been no specific bibliometric analysis of the global use of acupuncture for PCOS, which presents an obstacle for researchers in obtaining a comprehensive understanding of the current status in this area. Therefore, it is necessary to conduct a bibliometric analysis, through which we can confirm the most influential countries, institutions, journals, and authors on acupuncture therapy for PCOS. This study aims to perform a macrolevel overview of the relevant academic literature through bibliometric analysis, thus evaluating the research trends of acupuncture treatment of PCOS, identifying the hot topics and frontiers, and paving the way for researchers to acquire relevant knowledge overall.

2. Materials and Methods

CiteSpace was utilized to do the bibliometric analysis of the current status and research trends of the global application of acupuncture for PCOS treatment. The literature eligible for retrieval was found in the Web of Science Core Collection.

All data of this study were retrieved from the Web of Science Core Collection on August 11, 2022, including Science Citation Index Expanded (2008-present), Current Chemical Reactions (1985-present), and Index Chemicus (1993-present). The search strategy included the topics "polycystic ovary syndrome" and "acupuncture therapy" without restrictions on the countries, categories, or languages. The literature published in 2022 was excluded from the analysis for incomplete results. The specific search strategies and results are shown in Table 1.

A bibliometric analysis was conducted on the identified publications through CiteSpace (5.8.R3) to reveal the annual output counts, prolific journals, authors, institutions, and countries, and explore the trends and patterns [27]. Meanwhile, we also investigated the collaborative relationships, such as co-occurrence analysis of institutions, authors, references, and keywords. The current research foundation, cutting-edge knowledge, and research trends of acupuncture treatment for PCOS were uncovered through bibliometric visualization.

CiteSpace was configured as follows: time slicing was done from 2008 to 2021, according to the search results, one year per slice (1); the term source was selected in its entirety; node types were selected one at a time; and the top 50 objects were applied in the selection criteria, and pathfinder was chosen in pruning. The visual knowledge figure was mainly composed of nodes and lines. Every node in the figure represented one element, such as country, institution, or author, and the bigger the node size, the higher the frequency of occurrence. Different colors suggest different years. From the interior to the outside nodes, the circles of various colors indicated the years 2008 to 2021. The importance of each node was roughly evaluated by the indicator of betweenness centrality (BC), which Freeman defined in 1977. A node with a high BC (≥ 0.1) was usually considered a pivotal point and marked with a purple circle. Moreover, lines between the nodes manifested cooperation, co-occurrence, or co-citation relationships [28–30].

3. Results

3.1. Annual Publications. A total of 175 publications were retrieved from the Web of Science Core Collection, including 111 records in articles, 48 records in review articles, 8 records in meeting abstracts, 6 records in letters, 1 record in correction, 1 record in book chapters, and 1 record in editorial material, according to the document types of the Web of Science Core Collection. Only records in articles and review articles, a total of 159 publications, were included in the analysis after removing duplicates [31]. Figure 1 depicts the number of specific articles published each year and reveals that the first paper on the application of acupuncture in treating PCOS was published in 2008. The figure indicated that the number of relevant publications had slowly increased, with some fluctuations. From 2008 to 2013, the number of publications increased from 5 to 14, despite reductions in 2010 and 2012. A plummet appeared in 2014 when the number was 2, the lowest in years, and then it was restored to 10 in 2015. Later, the number of published articles kept increasing, except for a shrink in 2018, and reached a peak of 25 in 2021.

3.2. Analysis of Journals and Cited Journals. In total, 159 papers were published in 76 different journals. The top five journals on acupuncture treatment for PCOS are listed in Table 2. The average impact factor (IF) of the top 5 journals was 2.516. Evidence-Based Complementary and Alternative Medicine had the highest IF of 3.014, and was the most productive journal with 15 publications. CiteSpace generated a visualized map (Figure 2 and Table 3) of the cited journals based on 1699

Table 1: The topic search query.

Set	Results	Search query
#1	15143	(TS = (acupuncture OR acupuncture therapy OR acupuncture treatment OR acupuncture treatments OR body acupuncture OR needle acupuncture OR manual acupuncture OR warm acupuncture OR electroacupuncture OR electroacupuncture)) Indexes = Web of Science Core Collection, Timespan = 1985–2021
#2	16127	(TS = (PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome)) Indexes = Web of Science Core Collection, Timespan = 1985–2021
#3	175	#1 AND #2

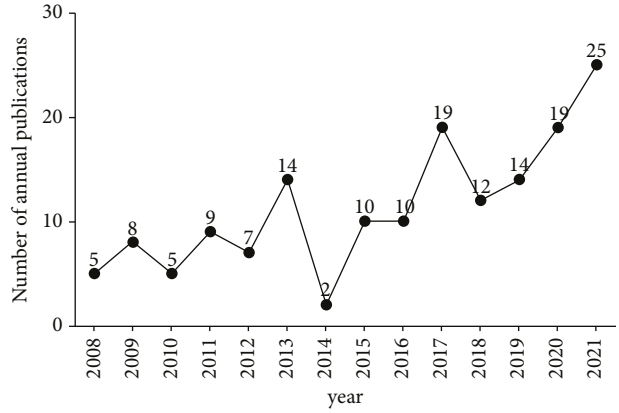

Figure 1: The annual number of publications on acupuncture therapy for PCOS.

Table 2: Top 5 scholarly journals related to acupuncture therapy on PCOS.

Rank	Publications	Journal	IF (Five years)
1	15	Evidence-Based Complementary and Alternative Medicine	3.014
2	10	Medicine	2.227
3	10	Acupuncture in medicine	2.192
4	8	Chinese Journal of Integrative Medicine	2.395
5	6	Trials	2.754

references. The nodes in the map represented journals, and links between the nodes reflected co-citation relationships. Additionally, the purple-ringed nodes that emphasized the centrality of literature were usually regarded as the key locations. Hence, Fertil Steril ranked first in frequency, and Ann Intern Med had the highest centrality.

3.3. Distribution of Countries and Institutions. A map concerning countries' distribution was generated, composed of 26 nodes and 64 links, as shown in Figure 3. Researchers from 26 countries and regions wrote 159 articles on acupuncture therapy for PCOS. From Table 4, Sweden (0.20), the United States (0.19), China (0.17), Australia (0.11), and England (0.09) were identified as the top five nations in terms of centrality as well as the number of publications, regardless of the difference in rankings.

Figure 4 depicts a distribution map of institutions with 230 nodes and 685 lines. There were 230 institutions dedicated to the field of acupuncture therapy for PCOS, with Heilongjiang University of Chinese Medicine (35), University of Gothenburg (29), Karolinska Institute (20), University of Hong Kong (10), and Guangzhou Medical University (10) being the top five in terms of publication numbers (Table 5). Interestingly, Heilongjiang University of Chinese Medicine not only owned the highest publication numbers but also the greatest centrality of 0.25, equal to Karolinska Institute (0.25) and followed by Beijing University of Chinese Medicine (0.17), University of Gothenburg (0.16), and Peking University (0.15).

3.4. Analysis of Authors and Cited Authors. The visualization map of authors aims to uncover the most productive author or co-author and reveal the cooperative relationship among them, which can provide valuable information regarding core research teams and potential collaborators and assist researchers in building new collaborative relationships [32]. The authors' map was generated after analyzing 159 publications, and 701 nodes and 2509 lines (Figure 5) were included. The 5 most published authors were Elisabet

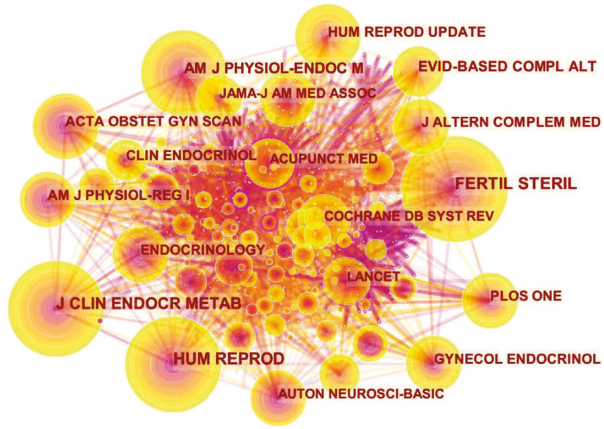

Figure 2: The cited journal map related to research of acupuncture treatment for PCOS. The nodes in the map represent journals, and links between the nodes signify cooperation relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of co-citations. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

Table 3: Top 5 cited journals and centrality related to acupuncture therapy on PCOS.

Rank	Frequency	Cited journal	Rank	Centrality	Cited journal
1	126	Fertil Steril	1	0.32	Ann Intern Med
2	112	Hum Reprod	2	0.13	Acupunct Med
3	100	J Clin Endocr Metab	3	0.12	Am J Public Health
4	90	Am J Physiol-Endoc M	4	0.11	Brit Med J
5	72	Evid-Based Compl Alt	5	0.10	Chin J Integr Med

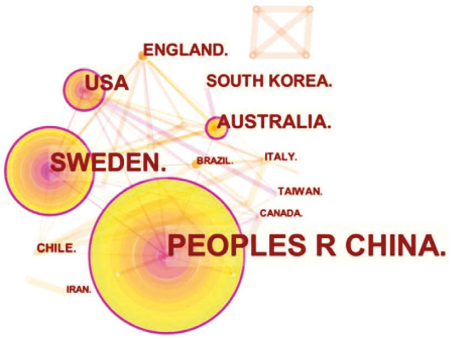

Figure 3: map of countries concerning research of acupuncture treatment for PCOS. The nodes in the map represent countries or territories, and links between the nodes signify cooperation relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of publications. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

Table 4: Top 5 publications and centrality of countries related to acupuncture therapy on PCOS.

Rank	Publications	Country	Rank	Centrality	Country
1	96	Peoples R China	1	0.20	Sweden
2	42	Sweden	2	0.19	USA
3	24	USA	3	0.17	Peoples R China
4	16	Australia	4	0.11	Australia
5	10	England	5	0.09	England

Figure 4: A map of institutions related to acupuncture treatment for PCOS. The nodes in the map represent institutions, and the links between the nodes signify collaborative relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of publications. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

Stener Victorin, Xiaoke Wu, Julia Johansson, Hongxia Ma, and Anna Benrick, and Elisabet Stener Victorin, Rong Li, Fan Qu, Richard S. Legro, and Yi Feng had the top 5 centralities (Table 6). Elisabet Stener Victorin from the Karolinska Institute in Sweden was the most prolific author in terms of publications and centrality. A

Table 5: Top 5 publications and centrality of institutions related to acupuncture therapy on PCOS.

Rank	Publications	Institution	Rank	Centrality	Institution
1	35	Heilongjiang Univ. Chinese Med	1	0.25	Heilongjiang Univ. Chinese Med
2	29	Univ. Gothenburg	2	0.25	Karolinska Inst.
3	20	Karolinska Inst.	3	0.17	Beijing Univ. Chinese Med
4	10	Univ. Hong Kong	4	0.16	Univ. Gothenburg
5	10	Guangzhou Med Univ.	5	0.15	Peking Univ.

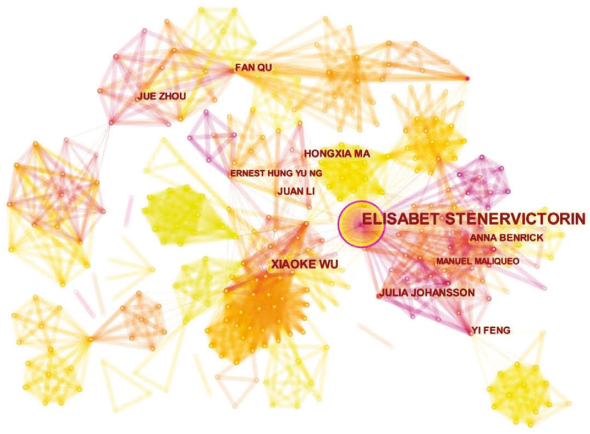

Figure 5: A map of authors dedicated to acupuncture treatment for PCOS. The nodes in the map represent authors, and the links between the nodes signify collaborative relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of publications. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

randomized controlled trial, performed by Elisabet Stener Victorin, on the impact of electroacupuncture (EA) and physical exercise on hyperandrogenism and oligoamenorrhea in PCOS women found that low-frequency EA and physical exercise both significantly improved hyperandrogenism and menstrual frequency, and the effect of low-frequency EA was superior to physical exercise [33]. However, another randomized controlled trial of hers demonstrated that acupuncture, with or without clomiphene (the first-line treatment for infertility), did not increase live births of PCOS women in China compared with acupuncture and placebo in the control group [34]. Despite this conclusion, one of her reviews still believed that acupuncture was a recommendable treatment for women with PCOS, which could improve PCOS-related symptoms, including irregular menstruation, health-related quality of life, emotions, and insulin sensitivity with minimal side effects [35].

A map of the cited authors was produced, which consisted of 1705 nodes and 7553 lines (Figure 6). Interestingly, Elisabet Stener Victorin was the most cited author about acupuncture therapy for PCOS with 101 counts in total, followed by Johansson J (65), Jedel E (53), Manneras L (43), and Legro RS (38) (Table 7). As for centrality, the top 5 cited authors were Barber TM (0.19), Andersson S (0.12), Avis NE (0.12), Andersen CL (0.11), and Blank SK (0.10) (Table 7). Then, Barber TM, who had the highest centrality, served in the Oxford Centre for Diabetes, Endocrinology, and Metabolism (OCDEM), a world-class center for clinical research on diabetes, endocrine, and metabolic disorders, along with clinical treatment and education. Some of his reviews and studies explored the genetics, pathogenesis, and metabolic characteristics of PCOS, which were often referred to by related researchers as basic information [36–39].

3.5. Analysis of Cited References. The map of cited references with 1699 nodes and 6229 links was acquired by analyzing 159 publications (Figure 7). The top 5 cited references in frequency and centrality are shown in Tables 8 and 9. The four of the top five most frequently cited references were all randomized controlled trials of acupuncture therapy for PCOS, three of which had been mentioned previously. The remaining article demonstrated that low-frequency EA and physical exercise could lower high sympathetic nerve activity, which was considered one of the causes of PCOS [40, 41]. Moreover, the review published by Lim CED in 2019, which ranked first in centrality, concluded that compared to sham acupuncture, receiving true acupuncture may improve the number of menstrual days of PCOS women [42]. The following publication, which was still a review of Lim's CED, suggested that acupuncture may have affected PCOS by increasing blood flow to the ovaries, reducing ovarian volume and number of ovarian cysts, improving insulin sensitivity, lowering cortisol levels, assisting in weight loss, etc [43].

3.6. Analysis of Keywords. It was believed that keywords could reveal the research frontiers and emerging trends in a specific field with an increasing frequency of citations in a given period [44]. The keyword co-occurrence map was generated in Figure 8 with 598 nodes and 3159 links. Table 10 shows the top 5 keywords with the highest frequency and centrality related to acupuncture therapy for PCOS. The most popular keywords included "polycystic ovary syndrome," "women," "electroacupuncture," "acupuncture," "insulin resistance," and "adipose tissue." Besides, the top 10 keywords with the strongest citation burst were identified by using burst detection (Figure 9). The most recent keyword bursts were "acupuncture," "polycystic ovary syndrome," "quality of life," "ovulation," and "randomized controlled trial," which indicated that the three

Table 6: Top 5 publications and centrality of authors related to acupuncture therapy on PCOS.

Rank	Publications	Author	Rank	Centrality	Author
1	38	Elisabet Stener Victorin	1	0.18	Elisabet Stener Victorin
2	13	Xiaoke Wu	2	0.10	Rong Li
3	9	Julia Johansson	3	0.07	Fan Qu
4	9	Hongxia Ma	4	0.06	Richard S Legro
5	8	Anna Benrick	5	0.03	Yi Feng

Figure 6: A map of cited authors dedicating to acupuncture treatment for PCOS. The nodes in the map represent co-cited authors, and links between the nodes signify co-citation relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of co-citations. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

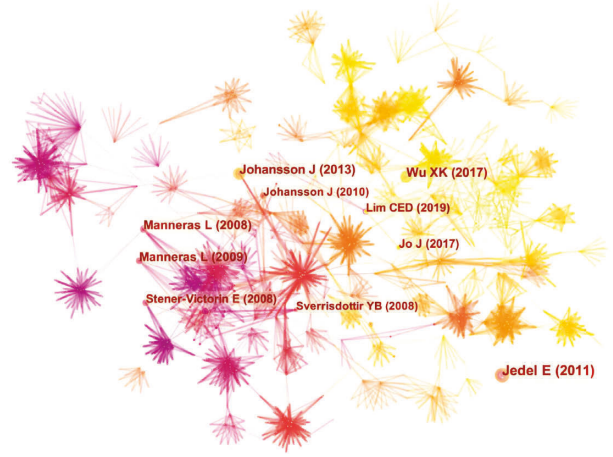

Figure 7: A map of cited references related to acupuncture treatment for PCOS. The nodes in the map represent cited references, and the links between the nodes signify co-citation relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of co-citations. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

Table 7: Top 5 frequency and centrality of cited authors related to acupuncture therapy on PCOS.

Rank	Frequency	Cited author	Rank	Centrality	Cited author
1	101	Stener Victorin E	1	0.19	Barber TM
2	65	Johansson J	2	0.12	Andersson S
3	53	Jedel E	3	0.12	Avis NE
4	43	Manneras L	4	0.11	Andersen CL
5	38	Legro RS	5	0.10	Blank SK

frontiers in burst strength were research method, intervention, and outcome.

4. Discussion

According to the above results, the status of acupuncture therapy on PCOS can be exposed as follows.

The fluctuant growth in the number of articles suggests that acupuncture, as a supplementary and alternative medicine, has received increasing attention in recent years. More studies have been done to determine its effectiveness in PCOS treatment. However, the amount of literature is limited overall, and no sign of rapid growth.

Evidence-Based Complementary and Alternative Medicine is the most productive journal, with the highest IF of 3.014, indicating its maximum impact in the area. Furthermore, the average IF of 2.516 in the top five journals indicates limited attention to the field. Fertil Steril broadly influences infertility and human reproductive disorders, while Ann Intern Med possesses authority in the field.

China ranks first in the aspect of publications with a total of 96 references, probably because China is the origin of acupuncture. As the second-ranked country, Sweden has 42 references, indicating the country's broad application of acupuncture treatment for PCOS. While the Heilongjiang University of Chinese Medicine, the most prolific and critical institution, may own the most influential position in this field. Besides, Karolinska Institute and the University of Gothenburg are in the top five in terms of publications and centrality and are located in Sweden. Thus, it can be assumed that Chinese and Swedish institutions give more priority to the research on acupuncture therapy for PCOS.

Elisabet Stener Victorin, the most productive and cited author, undoubtedly holds substantial influence in the field of acupuncture treatment on PCOS, and the effectiveness of electroacupuncture in improving PCOS-related symptoms is one of her research directions. Besides, Julia Johansson and Richard S Legro are among the top five productive and cited authors, indicating they are active researchers and excellent

Table 8: Top 5 frequency of cited references related to acupuncture therapy on PCOS.

Rank	Frequency	Cited reference	Author and publication year
1	32	Impact of electroacupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial	Jedel (2011)
2	25	Effect of acupuncture and clomiphene in Chinese women with polycystic ovary syndrome: a randomized clinical trial	Wu (2017)
3	23	Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial	Johansson (2013)
4	21	Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome	Stener Victorin (2009)
5	20	Acupuncture and exercise restore adipose tissue expression of sympathetic markers and improve ovarian morphology in rats with dihydrotestosterone-induced PCOS	Manneras (2009)

Table 9: Top 5 centrality of cited references related to acupuncture therapy on PCOS.

Rank	Centrality	Cited reference	Author and publication year
1	0.13	Acupuncture for polycystic ovarian syndrome	Lim (2019)
2	0.12	Current evidence of acupuncture on polycystic ovarian syndrome	Lim (2010)
3	0.11	Longitudinal antimüllerian hormone in women with polycystic ovary syndrome: an acupuncture randomized clinical trial	Franasiak (2012)
4	0.11	Anxiety and depression in polycystic ovary syndrome: a comprehensive investigation	Deeks (2010)
5	0.10	Reproductive and metabolic phenotype of a mouse model of PCOS	Leonie (2012)

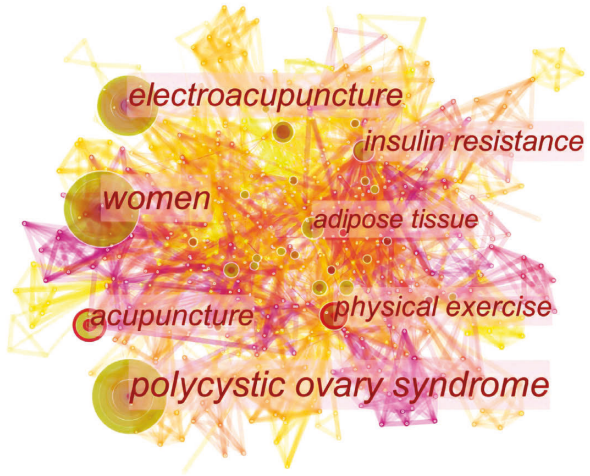

Figure 8: A map of keywords related to acupuncture treatment for PCOS. The nodes in the map represent keywords, and the links between the nodes signify co-occurrence relationships. The diverse colors of the nodes represent different years. The larger the node area, the greater the number of co-occurrences. The purple ring indicates the centrality of literature, and nodes with high centrality are considered pivotal points.

potential collaborators in the field. Regrettably, there is little clinical research regarding acupuncture treatment on PCOS among the publications of the influential cited authors, which indicates a dearth of high-quality clinical evidence to confirm the effectiveness of acupuncture in the treatment of PCOS.

According to the top five cited references, it can be considered that the comprehensive analysis of acupuncture treatment for PCOS is mainly concerned with randomized controlled trial, review, and endocrine metabolic-based regulatory mechanism. Nevertheless, the limited number of randomized controlled trial and the lack of high-quality clinical evidence have made it challenging to accurately evaluate acupuncture therapy's effectiveness for PCOS.

As the most important and popular research method of evaluating the efficacy of acupuncture therapy on PCOS, randomized controlled trial can provide high-quality evidence. The keyword "acupuncture," emerging from 2019 and possessing the strength of citation burst of 4.04, suggests that acupuncture therapy has gained increasing attention in recent years. And the keyword "low-frequency electroacupuncture," which existed from 2013 to 2014 with the strength of citation burst of 3.88, indicates that low-frequency electroacupuncture is the most commonly applied acupuncture therapy in the treatment of PCOS. Furthermore, more relevant randomized controlled trials have been conducted to evaluate the effects of acupuncture on improving the quality of life, ovulation rates, and insulin resistance of PCOS patients [22, 45, 46]. Regrettably, there is a lack of convincing evidence for the efficacy of acupuncture in treating women with PCOS [47]. Therefore, more well-designed randomized controlled trials should be carried out for further investigation.

In addition, our research had several drawbacks. First and foremost, because Web of Science was the only database we used to search for relevant publications, the results of our study were inherently incomplete. As a result, additional

Table 10: Top 5 frequency and centrality of cited authors related to acupuncture therapy on PCOS.

Rank	Frequency	Keyword	Rank	Centrality	Keyword
1	82	Polycystic ovary syndrome	1	0.25	Insulin resistance
2	56	Women	2	0.21	Electroacupuncture
3	45	Electroacupuncture	3	0.19	Acupuncture
4	35	Acupuncture	4	0.17	Adipose tissue
5	32	Insulin resistance	5	0.15	Women

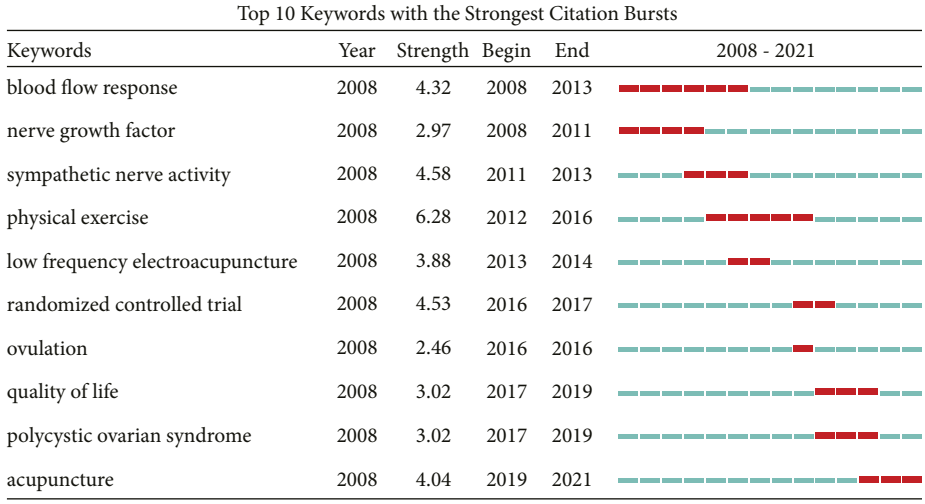

Figure 9: The top 10 keywords with the strongest citation bursts. The red bars indicate that the keyword was cited frequently; the green bars mean that the keyword was cited infrequently.

analysis of articles in other databases, particularly some Chinese databases, is critical. Besides, although the research topic has been defined during searching, there is no guarantee that all retrieved literature is totally relevant to the issue. Despite this, we believe this study retains some credibility in describing the overall state and potential prospects in this field.

5. Conclusions

Acupuncture has been proven to be a good treatment for PCOS as a supplementary and alternative therapy and has the advantages of clinical efficacy and few side effects. Much of the relevant literature in the study was published in Evidence-Based Complementary and Alternative Medicine journals. China was the most productive country in this area, and the Heilongjiang University of Chinese Medicine was the most prolific institution. While Elisabet Stener Victorin of the Karolinska Institute in Sweden was named the most influential author and the most cited author simultaneously, which implied that acupuncture therapy was increasingly accepted worldwide. Additionally, randomized controlled trials were commonly carried out to explore the efficacy of acupuncture treatment on PCOS, and low-frequency electroacupuncture was the most popular acupuncture therapy. The primary outcome measures were quality of life, ovulation rates, and insulin sensitivity. Nevertheless, the limited number of randomized controlled trials and a lack of high-quality clinical evidence constrained researchers from evaluating the effectiveness of acupuncture therapy for PCOS. Thus, more well-designed randomized controlled trials should be conducted for further investigation.

Overall, this study first fills the gap of bibliometric analysis on acupuncture therapy for PCOS and then reveals the present state and trends in this field, which may help researchers identify hot topics and explore new research paths.

Data Availability

The raw data can be directly obtained from the Web of Science Core Collection (WoSCC).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors would like to express their appreciation to Professor Chaomei Chen for inventing CiteSpace and making it free to use. This work was supported by the scientific research project of the Zhejiang Provincial Department of Education (grant no. 2022ZZ029). The trial sponsor is Hangzhou Third Hospital Affiliated to the Zhejiang Chinese Medical University (No. 38, West Lake Road, Shangcheng District, Hangzhou City, Zhejiang Province 310000, China, 86-571-87823126).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 5076306, 21 pages
https://doi.org/10.1155/2022/5076306

Review Article

The Application of Complementary and Alternative Medicine in Polycystic Ovary Syndrome Infertility

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Received 20 April 2022; Revised 7 July 2022; Accepted 12 September 2022; Published 6 October 2022

Academic Editor: Vijaya Anand

Polycystic ovary syndrome (PCOS) is a lifelong reproductive endocrine disease, which is the most common cause of anovular infertility. Modern medicine mainly treats infertile patients with PCOS by improving living habits, ovulation induction therapy, and assisted reproductive technology (ART), but the effect is not satisfied. Complementary alternative medicine (CAM) has conspicuous advantages in the treatment of PCOS infertility due to its good clinical efficacy, wide mechanism of action, and no obvious adverse reactions, but its safety and effectiveness in the treatment of PCOS infertility have not been proved. Based on the existing clinical and experimental studies, this paper looks for the therapeutic effect and the mechanism behind it, and explores the safety and effectiveness of its treatment in PCOS infertility, in order to provide reference for future clinical treatment and experimental research.

1. Introduction

PCOS is a life-long reproductive endocrine disease characterized by anovulatory, hyperandrogenism, and polycystic ovary. It is one of the most common causes of infertility in women of childbearing age. It is usually associated with reproductive complications (irregular menstruation, ovulation dysfunction, and pregnancy complications), metabolic disorders (type 2 diabetes and cardiovascular disease), and even psychological risk factors [1–4]. Depending on the population investigated and the diagnostic criteria used, the prevalence of PCOS ranges from 6% to 15% and approximately 75% of women with PCOS suffer from infertility due to ovulation disorders, which makes infertility an urgent problem for PCOS patients [5–7]. Overexpression of proinflammatory factors and intense oxidative stress (OS) in PCOS patients inhibits follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptor expression, leading to oocyte dysplasia and eventually infertility [8, 9]. For infertile patients with PCOS who have fertility needs, first-line and second-line treatment such as lifestyle adjustment and ovulation induction therapy are usually preferred. For patients with ineffective treatment, they will eventually choose clinical third-line treatment to assist reproductive technology in order to achieve pregnancy. Due to hormone and metabolic problems in PCOS patients receiving ovulation induction, ART process is not good and accompanied by serious adverse reactions, it is necessary to find an alternative therapy to supplement or replace conventional western medicine treatment, in order to solve the poor efficacy of conventional western medicine treatment, adverse reactions, and other drawbacks. CAM refers to a group of medical systems used in combination with or in place of traditional medicine. According to the National Center for Complementary and Integrative Health, CAM models are divided into three categories: natural products, mind-body practices, and other complementary health approaches, and the specific classification varies from socio-

culturalbackground [10, 11]. According to international studies, 29–91% of infertile women are using CAM techniques [12–15]. Studies have shown that the use of CAM methods can significantly improve sex hormone levels, relieve IR, reduce weight, regulate mood, and improve ovulation in infertile patients with PCOS [16]. CAM is usually safe for physicians to prescribe on a patient-by-patient basis, but many treatment have contraindications. Since there is insufficient evidence on the safety and efficacy of CAM techniques in the treatment of PCOS, it is necessary to investigate the efficacy and safety of CAM techniques in the treatment of infertile patients with PCOS. All of the studies are listed in Table 1.

2. Chinese Medicine Treatment of PCOS Infertility

Traditional Chinese medicine (TCM) is a CAM model, which is guided by the theory of yin-yang and five elements, five viscera, and six fu organs, qi, blood, and fluid, and takes syndrome differentiation and treatment as the treatment principle, and provides individualized diagnosis and treatment for infertility patients with PCOS. TCM diagnosis and treatment is rooted in China's profound traditional cultural background, summarizes and sublimation of more than 5000 years of people's practical experience, combined with modern medical theory of medical diagnosis and treatment. TCM is not limited to improving "symptoms," but emphasizes the human body, society, and nature as a whole "holistic view," through a variety of ways to treat patients with physiological and psychological "disease." TCM has been spread to 196 countries and has achieved satisfactory results in more than one-third of the world's population. Chinese herbal medicine (CHM) is the most distinctive treatment of TCM, which is divided into monomer and compound. Due to its obvious therapeutic effect and high safety, it is widely used in the treatment of infertile patients with PCOS [17]. TCM theory believes that the pathogenesis of PCOS infertility is due to deficiency to excess kidney deficiency, resulting in abnormal liver and spleen function and pathological products such as qi stagnation, phlegm dampness, and blood stasis [18]. In the theory of TCM, kidney contains kidney essence, kidney essence is the key material to nourish the human body to promote the development of reproductive function; it is the most fundamental nutrients in the human body. Therefore, most of the TCMs for PCOS infertility are kidney-tonifying, supplemented by qi-expelling, phlegm-resolving, blood-activating, and stasis-resolving therapies, with good clinical results. Therefore, the majority of scholars have carried out a large number of in vitro and in vivo experiments on TCM, studies have shown that TCM by regulating sex hormone levels, improves IR and promotes follicle development [19–22]. We have listed some RCTs in Table 2.

2.1. Clinical Observation of Chinese Medicine in the Treatment of PCOS Infertility

2.1.1. Clinical Observation of Chinese Medicine Monomer in the Treatment of PCOS with Infertility

(1) Clinical Application of Berberine. Berberine (BBR), an iso quinoline alkaloid, is widely found in many plants of the Berberaceae family, such as Rhizoma Coptidis and Cortex Phellodendri. BBR was initially used in gastrointestinal infections such as diarrhea because of its excellent antibacterial effect. But in recent years, with the deepening of research, it has been found to be effective in improving IR, lowering androgen levels, and improving glucolipid metabolism, especially in PCOS patients with IR (IR) [23]. BBR can improve insulin sensitivity by regulating the signaling pathway of mTOR-IRS1 in patients to achieve the therapeutic effect of PCOS treatment [24]. For infertile patients with PCOS receiving assisted reproduction, BBR combined with ovulation induction drugs such as cyproterone acetate (CPA), clomiphene citrate (CC), and letrozole (LET) can improve the ovulation induction effect and reduce the incidence of adverse reactions, thus BBR has a high potential research value [25, 26]. However, this conclusion is controversial. Wu et al. found that the pregnancy and ovulation rates of BBR combined with LET in the observation group were similar to those of the control group using BBR alone, which could not indicate that BBR improves pregnancy and ovulation rates in PCOS patients [27]. Further studies are needed to comprehensively evaluate the effect and mechanism of BBR in improving reproductive function in the future.

(2) Clinical Application of Cryptotanshinone. Cryptotanshinone (CRY), as the main lipid-soluble component of Salvia miltiorrhiza, has various pharmacological effects such as antibacterial, anti-inflammatory, antioxidant and so on [28]. Modern pharmacological studies have shown that CRY has a regulatory effect on the reproductive endocrine of ovarian organs and can significantly reduce serum androgen levels in PCOS patients [29, 30]. In an experiment using dehydroepiandrosterone to induce PCOS in a rat model, CRY was shown to improve PCO status, regulate the estrous cycle, and reduce testosterone (T), LH, and androstenedione [31]. Yang et al. found that CRY can regulate sex hormone disorder and promote follicle development in PCOS by inhibiting the expression of HMGB1, TLR4, and NF-κB/p65 in ovarian tissue and reducing the level of inflammatory factors such as TNF-α [32].

(3) Clinical Applications of Quercetin. Quercetin (QUE) is a phytoestrogen commonly found in herbal medicine and has antioxidant, anti-inflammatory, immunoprotective, and even anticancer effects. The most important features of QUE are its estradiol-like structure and phytoestrogen activity, which can improve the clinical symptoms of obesity, infertility, and sex hormone disorders in PCOS [33]. Rezvan et al. have confirmed that QUE can increase adiponectin levels and reduce homeostasis model assessment of insulin resistance (HOMA-IR), T, LH, and insulin levels in PCOS patients [34], which is consistent with the results of Khorshidi [35] et al. The rat model suggests that QUE not only regulates the level of glucolipid metabolism and improves the level of sex hormones, but also has powerful antioxidant ability to improve ovarian PCO status [36].

Table 1: List of reference numbers corresponding to traditional Chinese medicine, acupuncture and other therapies.

CAM treatment modalities	Mechanism of action	Reference range
CHM (monomers, compound, compound enema)	Monomers (BBR, CRY, QUE), compound (BSD, GFP, STP, and ZYP) in the treatment of patients with PCOS infertility is to improve IR and glucose and lipid metabolism disorder, improve sex hormone levels, and promote follicular development	[17–60]
Acupuncture (general acupuncture, EA, moxibustion and warm acupuncture, other treatment modalities related to acupuncture)	Lose weight, improves depression, regulates the HPOA, promotes ovulation, and improves ER.	[61–112]
Other therapies (vitamin and trace element supplementation and other nutrients, weight loss, exercise, and other healthy lifestyles)	Nutrient supplementation improves FSH levels and chronic low-intensity inflammatory response, and micronutrient supplementation promotes paired follicle development in PCOS infertility patients. Weight loss can improve insulin sensitivity, HA, and follicular development in PCOS infertility patients. Exercise modulates the HPOA to improve sex hormone disorders and increase ovulation and pregnancy rates in patients with PCOS infertility.	[113–139]

Table 2: RCT and results of monomers of CHM.

ID	Design	Test subjects	Sample size	Grouping situation	Outcomes	Ethical clearance number
24	RCT	Human	78	Control group: Non-PCOS infertility Observation group A : PCOS infertility Observation group B: PCOS infertility + BBR	Observation group B: mTOR mRNA ↓ ($P = 0.001$), IRS-1 mRNA ↑ ($P = 0.009$)	[2017] No. 158
27	RCT	Human	644	Berberine group(A): BBR + LET placebo LET group(B): LET + BBR placebo Combination group(C): LET + BBR	The live birth rate, PR and OR of B and C were similar and significantly higher than those of berberine group.	ChiCTR-TRC-09000376
34	RCT	Human	84	Treatment group: QUE Control group: Placebo	Treatment group: adiponectin ↑ HOMA-IR ↓、 T ↓、 LH ↓、 FBS ↓ All <0.009	IRCT2013112515536N1
35	RCT	Human	78	Treatment group: QUE Control group: Placebo	Resistin concentration: 2.07 ± 0.23 vs. 2.88 ± 0.40 ng/ml mRNA levels: 0.64 ± 0.58 vs. 1 ± 0.56 T: 0.72 ± 0.15 vs. 0.76 ± 0.12 ng/ml LH: 8.05 ± 2.88 vs. 8.77 ± 1.99 mIU/ml All $p < 0.05$	IRCT2016082215536N4
31	RCT	Rats	60	Normal rats: PCOS rat A: Normal saline PCOS rat B: CRY	Observation group B: T↓、E2↓、 LH↓、 LH/FSH ↓ Inhibin B、 lutein mRNA and protein expression ↓ Activin A mRNA and protein expression ↑ All $P < 0.05$	All protocols were conducted in accordance with the guidance suggestions for the care and use of laboratory animals, formulated by the Ministry of Science and Technology of China.
32	RCT	Rats	60	Blank control group PCOS group PCOS + HMGB1 group PCOS + HMGB1 + CRY	Weight ↓, ovarian mass ↓, LH ↓, LH/FSH ↓, T ↓, TNF-α ↓	No. 20190103
36	RCT	Rats	24	Control group (C): carboxymethyl cellulose aqueous solution PCOS group (M): LET Metformin group ($M + L$): metformin + LET quercetin group ($Q + L$): QUE + LET	T↓ : $Q + L$:0.78 ng/ml ± 0.14 vs. M:1.69 ng/ml ± 0.17 E2↓ : $Q + L$:8.85 pg/ml ± 0.19 vs. M:1.61 pg/ml ± 0.29) P↑ : $Q + L$:34.47 ng/ml ± 1.65 vs. M:1.08 ng/ml ± 1.17 All $P < 0.05$	BAS#0256

2.1.2. Clinical Application of Commonly Used Compound Prescriptions

(1) Clinical Application of Liu Wei Di Huang Prescription. Liu Wei Di Huang Prescription (LDP, the main dosage form is decoction or pill) is a well-known formula for tonifying kidney yin, which was first recorded in Qian Yi's "Key to Therapeutics of Children's Diseases" in A.D.1114. LDP is composed of six Chinese medicines: *Rehmanniae Radix, Corni Fructus, Dioscoreae Rhizoma, Poria, Alismatis Rhizoma,* and *Moutan Cortex*, which have anti-inflammatory and antioxidant properties and can improve IR and HA [37]. LWP can regulate sex hormone levels and promote ovulation, so it is widely used in the treatment of infertility [38], PCOS [39], premature aging [40], diabetes [41] and other diseases.

(2) Clinical Application of Gui Zhi Fu Ling Pill. Gui Zhi Fu Ling Pill (GFP) is a classic formula for activating blood circulation and removing blood stasis, which was first published in Zhang Zhongjing's "Synopsis of Golden Chamber - Women's Chapter" in the Eastern Han Dynasty [42]. GFP is composed of *Cinnamomum cassia Presl, Poria*, *Paeonia lactiflora Pall.*, *Moutan Cortex* and *Persicae Semen*, etc. and is widely used in the treatment of PCOS, infertility, and endometriosis [45]. GFP can increase the pregnancy rate by improving the inflammatory response as well as regulating hormone levels and immune-related protein expression levels to alleviate PCOS-IR, improving ovarian function and promoting ovulation [42, 43]. Zhang found through experiments that the combined use of GFP and ovulation induction drugs for 3 months could significantly improve the ovulation situation and pregnancy rate of PCOS patients [44].

(3) Clinical Application of Shou Tai Pill and Zi Shen Yu Tai Pill. Shou Tai Pill (STP) is a classic formula for tonifying the kidneys and calming the fetus, which is taken from the book "Intergrating Chinese and Western Medicine" by the famous physician Zhang Xichun. STP is composed of four herbs: *Corii Asini Colla, Taxilli Herba, Dipsaci Radix, and Cuscutae Semen*, and is widely used in the treatment of abortion, assisted reproduction, and PCOS infertility [45]. Zi Shen Yu Tai Pill (ZYP) is a kind of Chinese medicine preparation based on Shoutai Pill, which is processed by the famous physician and professor Luo Yuankai, according to his personal experience, adding Chinese herbs such as *Polygoni Multiflori Radix, Atractylodis Macrocephalae Rhizoma, and Amomi Fructus*. ZYP is of great significance in assisted reproduction [46]. Due to its remarkable clinical efficacy and high safety without obvious side effects, which was included in the National Basic Medical List in 2018 [47]. ZYP has the function of tonifying the kidney and spleen, nourishing blood and calming fetus, strengthening body and health. Both STP and ZYP have been shown that they have good anti-inflammatory and antioxidant functions, and significantly increase pregnancy and live birth rates in PCOS patients by improving IR, promoting follicle development, and regulating hormone levels [48]. The above studies are listed in Table 3.

2.1.3. Clinical Application of Commonly Used Enema Formulae. Chinese medicine retention enema is a therapeutic means of introducing Chinese medicine with water decoction into the rectum through the anus with a catheter, so that the liquid is absorbed through the colorectum. It avoids the first-pass effect of the liver and increases the drug concentration in the uterus and adnexal areas. Therefore, enema is widely used in clinical infertility caused by PCOS, pelvic inflammatory disease, ovulation disorder, or tubal blockage [49, 50]. A RCT evaluated the efficacy and safety of prepregnancy enemas of Rehabin liquid (a Chinese patent medicine) in combination with mesalazine in women with active ulcerative colitis who had a need for fertility and found that it improved pregnancy outcomes and quality of life [51]. Duan and Lu found that Zichong granules (composed of Rehmanniae Radix Praeparata, Cuscutae Semen, and Chuanxiong Rhizoma, etc.) could increase serum estrogen (E) level and produce estrogen-like effect in mice by rectal administration, thus promoting follicle development [52]. Zhao, C. verified this conclusion that oral administration of Chinese medicine combined with medicine enemas could significantly enhance the ovulation rate in patients with luteinized unrupture follicle syndrome (LUFS) [53].

2.2. Mechanism of Chinese Medicine on PCOS Infertility

2.2.1. Improve Hormone Levels and Promote Follicle Development. PCOS patients usually show abnormal sex hormone levels, which can interfere with the normal development of follicles. Liu found that LDP combined with CC could significantly reduce the number of antral follicles, ovarian volume, T, LH, and improve endometrial thickness and E levels in infertile women with PCOS, and enhance pregnancy rate (37.50% vs. 15% $P < 0.05$) [54], which is consistent with Li[55] and Zhang [48]. STP can increase E and progesterone levels, reduce serum D-dimer levels, improve endometrial blood flow, and enhance blastocyst implantation rate to improve the success rate of pregnancy [45, 56]. QUE, the most active compound in ZYP, can improve the aromatase activity of ovarian granulosa cells in high insulin level environment, promote FSH receptor expression, and E synthesis to induce ovulation [57].

2.2.2. Improving IR and Promoting Follicle Development. IR refers to the decrease in the efficiency of insulin uptake and utilization of glucose for various reasons, and the body compensatory secretion of excessive insulin resulting in hyperinsulinemia, to maintain the stability of blood glucose. IR stimulates the production of T in the ovaries and decreases the production of sex hormone binding globulin in the liver, thereby increasing free testosterone (FT) levels in the body [58]. GFP reduces inflammation by altering the structure of the intestinal flora, thereby improving IR [43]. Qiu et al. found that LDP could inhibit PI3/AKT activation, down-regulate mRNA expression of FSHR and Cyp19a1 in ovaries, improve insulin resistance index (HOMA-IR) and sex hormone levels in vivo, alleviate polycystic changes in

Table 3: RCT and results of TCM compound.

ID	Design	Model	Sample size	Grouping situation	Outcome	Ethical clearance number
54	RCT	Human	80	Observation group: Daing 35 + CC Control group: Daying 35 + LDP	PR: 37.50% (15/40) vs. 15% (6/40) Number of follicles ↓、ovarian volume ↓、T ↓、 LH ↓ Endometrial thickness ↓、E2↑ $P < 0.05$	This trial was approved by the Ethics Committee of Zijin county maternal and Child Health hospital, Guangdong Province, and the ethical approval number is not explicitly mentioned in the text
44	RCT	Human	56	Observation group: metformin + ethinyl estradiol cyproterone tablets + GFP Control group: Metformin + ethinyl estradiol cyproterone tablets	Total efficiency: 96.4% (27/28) vs. 71.4% (20/28) pregnancy rate: 67.9% (19/28) vs. 35.6% (10/28) All $P < 0.05$	This trial was approved by the ethics committee of Anyang People's hospital in Henan Province; the ethics approval number is not explicitly mentioned in the text
55	RCT	Human	100	Observation group: ethinyl estradiol cyproterone tablets + ZYP Control group: ethinylestradiol cyproterone tablets	Total efficiency: 100% (50/50) vs. 80% (40/50) LH/FSH<3 : 100%- (50/50) vs. 80% (40/50) All $P < 0.05$	This trial was approved by the Ethics Committee of the Dazhou hospital of integrative medicine, the ethical approval number is not explicitly mentioned in the text
48	RCT	Human	64	Observation group: estradiol valerate tablets + progesterone capsules + ZYP Control group: estradiol valerate tablets + progesterone capsules	Pregnancy rate: 40.63% (13/32) vs. 15.63%(5/32) survival rate: 28.13% (9/32) vs. 3.13% (1/32) All $P < 0.05$	This trial was approved by the thics Committee of Nantong Chinese hospital, the ethical approval number is not explicitly mentioned in the text
49	RCT	Human	60	Observation group: Chinese herbal enema (*CyperusrotundusL, Lindera aggregata (Sims) Kosterm, Amomum villosum Lour, Radix Aucklandiae, Olibanum, Geosaurus, Bombyx Batryticatus, Curcuma phaeocaulis Valeton, Angelica sinensis (Oliv.) Diels, Salvia miltiorrhiza Bunge.)* Blank control group (K)	Uterine spiral artery RI: 0.63 ± 0.03 vs. 0.66 ± 0.03 S/D: 2.72 ± 0.17 vs. 3.06 ± 0.22) Ovarian spiral artery RI: 0.60 ± 0.04 vs. 0.56 ± 0.04 S/D: 2.47 ± 0.3 vs. 2.28 ± 0.08	This trial has been approved by the Ethics Committee of Suzhou hospital of traditional Chinese medicine, Jiangsu Province, and the ethics approval number is not explicitly mentioned in the text
39	RCT	Rats	50	Blank control group (K) High fat diet (HFD) + LET HFD + LET + metformin Low-dose group: HFD + LET + LDP High-dose group: HFD + LET + LDP	In PCOS-IR rats with upregulated ovarian FSHR and Cyp19a1 mRNA levels, LDP (3.6 g-kg-1-d-1) significantly reversed the upregulated phosphorylation of IRS-1 (S307) and the downregulated phosphorylation of PI3Kp85α, Akt and FoxO1a.	This trial has been approved by the ethics committee of the iangsu key laboratory for the evaluation and translation of traditional Chinese medicine, and the ethics approval number is not explicitly mentioned in the text
43	RCT	Rats	72	Blank control group (K) Model control group (M) GFP low dose group (D) GFP medium dose Group (Z) GFP high dose group (Group G) Positive drug (metformin) control group (group Y)	Experimental group HS-CPR ↓, IL-6 ↓, TNF-α ↓, ucg-008 ↑, nk4a136 ↑	SYXK2018-0126
42	RCT	Rats	84	Control group PCOS model group(M) low-dose GFP group.(D) The medium-dose GFP group.(Z) High-dose GFP group(G) Metformin group(group Y). Medium dose GFP plus LY294002 group	GFP treatment group: atresia follicles ↓, cystic follicles ↓, mature follicles ↑ and corpus luteum ↑ T ↓、 LH ↓、 FINS ↓、 LH/FSH↓、HOMA-IR ↓ Phosphorylation levels of PI3K, AKT and mTOR↑ All $P < 0.05$	SYXK2018-0126

Blank control group (K), model control group (M), low dose group (D), medium dose group (Z), high dose group (G), positive drug (Y).

ovaries, and inhibit premature follicular atresia [39]. Liu et al. came to a conclusion that GFP reduces T, LH and LH/FSH values in PCOS rats by activating the PI3K/AKT/mTOR signaling pathway and inhibiting autophagy of granulation cells, and promoting follicle development and alleviates ovulation disorders in PCOS-IR rats [42]. The network pharmacological analysis showed that ZYP could promote ovulation and improve IR in the treatment of PCOS by inhibiting OS and inflammation response [59].

2.2.3. Improved Pregnancy Outcomes in Ovulation Promotion and Assisted Reproduction. For infertile patients with PCOS who have fertility needs, first-line and second-line treatment such as lifestyle adjustment and ovulation induction therapy are usually preferred. For patients with ineffective treatment, they will eventually choose clinical third-line treatment to assist reproductive technology in order to achieve pregnancy. Chinese medicine significantly improves sex hormone levels, ovulation, and endometrial tolerance in PCOS patients during assisted reproduction, thereby increasing pregnancy and live birth rates. The number of high quality blastocysts significantly increased in 462 patients with expected poor ovarian response (POSEIDON Group 4) undergoing in vitro fertilization-embryo transfer (IVF-ET) after oral administration of Ding kun dan for 5–6 weeks [60]. In a double-blind, multicenter placebo, RCT with a sample size of 2265, administration of ZYP to infertile women undergoing IVF significantly increased the live birth rate compared to placebo before and after ovarian stimulation and ET (26.8% vs. 23.0% (rate ratio [RR], 1.16; 95% CI 1.01–1.34; $P = .038$) [46]. A metabolomic study suggested that ZYP may be involved in the regulation of endometrial proliferation, OS, and lipid metabolism, thus improving endometrial tolerance and oocyte quality and ultimately enhancing IVF live birth rates [140].

2.3. Safety of Chinese Medicine in Treating PCOS Infertility. The use of herbal medicines is common in infertile patients with PCOS, while some Chinese herbal medicine (CHM) may contain anthraquinones, flavonoids, and glycosides which are nephrotoxic as well as Polygonum multiflorum Thunb, Tripterygium wilfordii which are hepatotoxic and so on. In addition, they may affect maternal sex hormone levels and may have reproductive toxicity, teratogenic, and abortive adverse effects on the embryo. Most of the experiments did not consider the safety of CHMs. The Chinese medicines involved in this paper, such as BBR, ZYP, and Ding kun Dan, did not show any obvious adverse reactions and some mild adverse reactions such as gastrointestinal tract which occurred in a few patients but could be relieved spontaneously [27, 46, 60]. Therefore, in the treatment of PCOS infertility with TCM, studies on the adverse effects of Chinese medicine and the recommended doses are needed in the future.

3. Acupuncture for PCOS with Infertility

Acupuncture is a nondrug therapy in Chinese medicine, which refers to the use of acupuncture or moxibustion to stimulate special parts of the body to regulate the balance of yin and yang in the body and thus achieve the purpose of disease prevention and treatment. This medical method has a mature system and theory, and is based on the internal organs, meridians, qi and blood. As a symbol of TCM, it is being accepted by most countries in the world. Modern research shows that acupuncture cannot directly eliminate disease-causing factors or pathological tissues, but rather prevents or treats disease by activating complex regulatory systems and maintaining physiological homeostasis, thus improving the body's ability to heal itself [61]. The effect on the human body is more likely to promote the robustness of the human body.

In recent years, acupuncture has been increasingly used in the treatment of gynecological diseases due to the advantages of easy and quick operation, better efficacy, and less side effects [62]. Wang, included 27 studies containing 7676 subjects and found that acupuncture was effective in treating infertility, especially in ovulatory disorders and PCOS combined infertility [63]. Quan et al. [64] also concluded that acupuncture or combined with other therapies significantly increased pregnancy and live birth rates in women with PCOS [64]. When acupuncture is applied to gynecological disorders, it can regulate the function of hypothalamic-pituitary-ovarian axis (HPOA), promote ovulation, and improve endometrial tolerance. For summarizing the randomized clinical trials, please refer to Table 4.

3.1. Clinical Efficacy of Acupuncture in the Treatment of PCOS with Infertility. Acupuncture includes general acupuncture, electro-acupuncture (EA), warm acupuncture, moxibustion, acupoint injection, and auricular acupuncture. Among them, general acupuncture and EA are the most commonly used treatments in clinical practice, and they are the most effective and widely used; while moxibustion, warm acupuncture and other treatments related to acupuncture are seldom used alone, but mostly in conjunction with acupuncture or herbal supplemental treatments.

3.1.1. General Acupuncture. General acupuncture means inserting filiform needles into special parts of the body (acupoints) at an appropriate angle and using corresponding techniques, such as "lifting and inserting" and "twisting," to enhance stimulation and achieve better therapeutic effects. Pan et al. used CHM combined with acupuncture as the observation group in an RCT, while the control group was treated with CHM and sham acupuncture. The acupoints mainly included RN4, EX-CA1, and ST29. The results showed that the PR and OR of the observation group were higher than that of the control group (46.34% vs. 18.42%, 58.14% vs. 45.74% $P < 0.05$) [65]. Lai et al. found that CC combined with CHM and acupuncture points RN4, RN3, and EX-CA1 could increase E2 levels, decrease LH and T levels, which can significantly increase PR in PCOS infertile patients compared with CC alone (PR: 46.5% vs. 30.2% $P < 0.05$) [66]. Lai et al. also demonstrated that acupuncture could significantly improve ovulation and normal menstruation rates in PCOS patients compared to controls

Table 4: RCT and results of acupuncture.

ID	Design	Sample size	Interventions	Outcomes	Composition	Ethical clearance
67	RCT	86	Manual acupuncture: CHM + acupuncture sham acupuncture: CHM	PR:46.34% (19/41) vs. 18.42% (7/38) $P < 0.05$	Acupoints: RN4, EX-CA1, ST29, ST36, SP6 Prescription: not mentioned	2017-569-52–01
68	RCT	86	Observation group: CC + CHM + acupuncture Control group: CC treatment	PR: 46.5% (20/43) vs. 30.2% (13/43) Early miscarriage rate: 15.0% (3/20) vs. 38.5% (5/13) Observation group: E2↑, T↓, LH↓ All $P < 0.05$	Prescription: *Medicata Fermentata Massa, Citri Reticulatae Pericarpium, Aurantii Fructus, Cyperi Rhizoma, Atractylodis Rhizoma, Pinelliae Rhizoma, Zingiberis Recens Rhizoma, Glycyrrhizae Radix et Rhizoma, Poria, Arisaema cum Bile* Acupoints: RN4, RN3, EX-CA1, ST29, SP6, ST36, SP10	This trial was approved by the Ethics Committee of The First People's hospital of Foshan city, Guangdong Province, and the ethical approval number is not explicitly mentioned in the text
69	RCT	60	Observation group: LET + acupuncture Control group: LET	PR: 60.53% (23/38) vs. 27.03% (10/37) OR: 89.47% (34/38) vs. 67.57% (25/37) All $P < 0.05$	Acupoints: EX-CA1, SP6, RN3	This trial was approved by the Ethics Committee of Affiliated hospital of Shandong University of traditional Chinese medicine, and the ethical approval number is not explicitly mentioned in the text
72	RCT	60	Observation group: Daying 35 + LET + CHM Control group: Daing 35 + LET	Endometrial thickness: 9.58 ± 0.91 mm vs. 6.43 ± 0.87 mm PR: 36.67% (11/30) vs. 23.33% (7/30) observation group: RI↓, PI↓ All $P < 0.05$	Prescription: *Angelicae Sinensis Radix, Paeoniae Alba Radix, Rehmanniae Radix Praeparata, Dioscoreae Rhizoma, Cuscutae Semen, Dipsaci Radix, Epimedii Herba, Cremastrae seu, Pleiones, Pseudobulbus, Gleditsiae Spina, Salviae Miltiorrhizae, Radix et Rhizoma, Spatholobi Caulis* Acupoints: RN4, RN3, EX-CA1, ST28, KI3, ST40, SP6 Moxibustion points: RN4, RN3, EX-CA1, ST28.	Trial was approved by Ganzhou traditional Chinese medicine hospital and the ethical approval number is not explicitly mentioned in the text
73	RCT	120	A: Daphne + LET + left right return pill + EA group B: Daphne + left and right return pill + EA C: Daphne + LET + EA	Clinical efficacy: 85.0% (43/40) vs. 70.0% (28/40) vs. 60.0% (24/40) Type a endometrium: 65.0% (26/40) vs. 35.0% (14/40) vs. 35% (14/40) OR: 40.0% (16/40) vs. 30.0% (12/40) vs. 20.0% (8/40) PR: 22.5% (9/40) vs. 12.5% (5/40) vs. 10.0% (4/40) All $P < 0.05$	Acupoints: RN4, RN3, RN6, EX-CA1, SP10, ST36, SP6, KI3, KI6	Trial was approved by Southwest medical university affiliated traditional Chinese medicine hospital and the ethical approval number is not explicitly mentioned in the text

Table 4: Continued.

ID	Design	Sample size	Interventions	Outcomes	Composition	Ethical clearance
80	RCT	103	Observation group: CC + CHM + moxibustion Control group: CC	Observation group: The peak systolic flow rate↑, PI↑ RI ↓ OR: 84.62% (44/52) vs. 64.71% (33/51) PR: 48.8% (25/52) vs. 23.53% (12/51) All $P < 0.05$	Prescription: *Angelicae Sinensis Radix, Paeoniae Alba Radix, Rehmanniae Radix, Praeparata, Corni Fructus, Ligustri Lucidi Fructus, Testudinis, Carapax et Plastrum, Ecliptae Herba, Glycyrrhizae, Radix et Rhizoma* Moxibustion: DU2, BL23, DU4	Trial was approved by Department of Gynecology, directly under the authority No.2 outpatient department, Henan Province and the ethical approval number is not explicitly mentioned in the text
81	RCT	90	Observation group: Aspirin + CC + CHM + warm acupuncture Control group A: Aspirin + CC Control group B: Aspirin + CC + CHM	Endometrial thickness: 9.85 ± 1.27 mm vs. 7.29 ± 0.931 mm vs. 8.14 ± 1.12 mm Follicle diameter: 19.48 ± 2.40 mm vs. 16.36 ± 2.67 mm vs. 17.85 ± 2.28 mm OR: 90.0% (27/30) vs. 63.3% (19/30) vs. 70.0% (21/30) PR:46.7% vs. 16.7% (5/30)vs. 20.0% (6/30) All $P < 0.05$	Prescription: *Dioscoreae Rhizoma, Rehmanniae Radix, Praeparata, Lycii Fructus, Corni Fructus, Cuscutae Semen, Morindae Officinalis, Radix, Epimedii Herba, Hominis Placenta, Spatholobi Caulis, Salviae Miltiorrhizae Radix et Rhizoma, Dipsaci Radix,* Acupoints: ST25, RN12, RN4, RN3, RN6, SP10, EX-CA1, LR3, SP6, ST36	Trial was approved by Tangshan traditional Chinese medicine hospital and the ethical approval number is not explicitly mentioned in the text
82	RCT	82	Observation group: CC + Duoyuan acupuncture Control group: CC	PR: 51.2% (21/41) vs. 26.8% (11/41) Bilateral ovarian volume↓, follicle number↓, menstrual cycle↓, All $P < 0.05$	Acupoints: RN12, RN4, RN6, RN3, DU4, DU3, DU2	Trial was approved by Nanjing University of traditional Chinese medicine and the ethical approval number is not explicitly mentioned in the text
83	RCT	60	Observation group: Acupoint injection of urotropin injection Control group: Urinary gonadotropin injection into the gluteal muscle.	FSH: 11.36 ± 1.84 IU/L vs. 9.87 ± 1.75 IU/L T: 0.72 ± 0.1 μg/L vs. 1.18 ± 0.16 μg/L LH: 19.36 IU/L ± 4.25 vs. 24.18 ± 4.16 IU/L All $P < 0.05$	Acupoints: EX-CA1, RN3, RN4, SP6	Trial was approved by Chenghai district People's hospital, Shantou City, Guangdong Province and the ethical approval number is not explicitly mentioned in the text
84	RCT	80	Observation group: CC + Chinese medicine + acupuncture point injection of angelica injection Control group: CC	Total efficiency: 80.0% (32/40) vs. 52.5% (21/40) Endometrial thickness: 0.98 ± 0.06 cm vs. 0.74 ± 0.05 cm All $P < 0.05$ After treatment, there was a statistically significant difference in the TCM symptom scores between the treatment and control groups	Chinese herbs: *Cuscutae Semen, Morindae Officinalis Radix, Cistanches Herba, Cinnamomi Ramulus, Astragali Radix, Liquidambaris Fructus, Cudraniae Radix, Salviae Miltiorrhizae, Radix et Rhizoma, Cyperi Rhizoma, Curcumae Rhizoma, Arecae Pericarpium* Acupuncture point injection: BL23	Trial was approved by Panyu district central hospital, Guangzhou city, guangdong province and the ethical approval number is not explicitly mentioned in the text

Table 4: Continued.

ID	Design	Sample size	Interventions	Outcomes	Composition	Ethical clearance
85	RCT	125	Observation group: estradiol valerate tablets + acupuncture + ear acupuncture Control group: estradiol valerate tablets	Total efficiency: 93.65% (59/63) vs. 80.65% (50/62) Time to return to normal menstruation: 3.71 ± 0.84 months vs. 4.29 ± 1.06 months OR: 90.46% (57/63) vs. 77.42% (48/62) All $P < 0.05$	Acupoints: RN6, SP6, RN4, BL20, ST36, LR3, DU4, BL23 Ear points: liver, kidney, ovary, ovaries and endocrine, with matching points for spleen, lumbar, and pelvic	Trial was approved by Sichuan Nanchong traditional Chinese medicine hospital and the ethical approval number is not explicitly mentioned in the text
92	RCT	120	C: Daing 35 + acupuncture B: Daying 35 + CHM A : Daing 35	Total efficiency: 95.0% (39/40) vs. 85.0% (34/40) vs. 72.5% (29/40) PR: 80.0% (32/40) vs. 62.5% (25/40) vs. 52.5% (21/40) All $P < 0.05$	Prescription: *Epimedii Herba, Cuscutae Semen, Fluoritum, Atractylodis Rhizoma, Citri Reticulatae, Pericarpium, Pinelliae Praeparatum, Rhizoma, Coicis Semen, Poria, Angelicae Sinensis Radix, Paeoniae Alba Radix, Chuanxiong Rhizoma, Cyperi, Rhizoma, Aurantii, Fructus* Acupoints: RN6, RN4, EX-CA1, ST25, ST36, SP6, SP9, SP10, ST40	Trial was approved by Hubei maternal and child health hospital traditional Chinese medicine and the ethical approval number is not explicitly mentioned in the text
105	RCT	60	Observation group: Daying 35 + acupuncture Control group: Daing 35	OR: 93.3% (28/30) vs. 80.0% (24/30) Clinical PR 43.3% (13/30) vs. 33.3% (10/30) Observation group: E2↓、T↓、BMI↓ All $P < 0.05$	Acupoints: RN4, RN6, SP6, ST36, EX-CA1, BL23, BL20, BL21, BL18	Trial was approved by Lianyungang maternal and child health hospital reproductive medicine center and the ethical approval number is not explicitly mentioned in the text
106	RCT	96	Observation group: acupuncture Control group: LET	Observation group: PR↑, APN↑, BMI↓, WHR↓, HOMA-IR↓, LEP↓ All $P < 0.05$	Acupoints: RN9, RN7, ST25, ST26, ST24	Trial was approved by reproductive center of the first affiliated hospital of Tianjin University of traditional Chinese medicine and the ethical approval number is not explicitly mentioned in the text
93	RCT	76	Observation group: EA Control group: Sham acupuncture	Transferable embryo rate: 49.0% (284/580) vs. 41.9% (273/652) High quality embryo rate: 36.6% (104/284) vs. 27.8% (76/273) Live birth rate: 50% (19/38) vs. 26.3% (10/38) Phlegm-damp syndrome score↓, IR↓, IRS-1↑, PI3K↑, GLUT4 mRNA↑ All $P < 0.05$	Acupoints: RN12, ST25, SP15, GB26, RN6, RN4, SP10, ST40, ST36, SP9	SDSZYYSZ20170210
107.	RCT	60	Observation group: dietary control plus exercise + acupuncture Control group: dietary control plus exercise	Observation group: Fasting insulin↓, fasting glucose↓ and waist↓ $P < 0.05$	Acupoints: GB26, ST25, SP15, BL23, BL32, ST29, GB41, SJ5	no.2018019

Table 4: Continued.

ID	Design	Sample size	Interventions	Outcomes	Composition	Ethical clearance
76	RCT	80	Observation group: Daying 35 + CHM Control group: Daing 35	Fertility rate: 82.5% (33/40) vs. 60% (24/40) LUFS: 2.5% (1/40) vs. 20% (8/40) OHSS: 0% (0/40) vs. 15.0% (6/40) All $P < 0.05$	Prescription: *Atractylodis Rhizoma, Epimedii Herba, Cuscutae Semen, Paeoniae Alba Radix, Coicis Semen, Pinelliae Praeparatum, Rhizoma , Angelicae Sinensis, Radix, Fluoritum, Cyperi Rhizoma, Chuanxiong Rhizoma, Poria, Citri Reticulatae Pericarpium* Acupoints: RN17, SP6, LR3, ST25, ST36, EX-CA1, RN12, RN4, LR14	Trial was approved by Zhaoqing Gaoyao People's hospital and the ethical approval number is not explicitly mentioned in the text

[67–69]. In infertile patients with PCOS, abnormal hormone levels often result in reduced ER leading to low embryo implantation rate or biochemical pregnancy. Xie, H. also certified in an RCT that the combination of tonifying the kidney, resolving phlegm, and activating blood formula with acupuncture points RN6 and RN4 significantly thickened the endometrial thickness (9.58 ± 0.91 mm vs. 6.43 ± 0.87 mm $P < 0.05$), reduced PI and RI to improve ER and thus to increase PR [70]. He et al. [71] also concluded that acupuncture combined with LET could achieve a complementary and mutually reinforcing effect in significantly improving ER in infertile patients with PCOS [71].

3.1.2. Electroacupuncture. EA is the treatment of stimulating acupuncture points by connecting the needle handle to the electrode after the general acupuncture gets qi and using the EA instrument to output the microcurrent close to the human bio-electricity. The advantage is that the body is stimulated more strongly and consistently, and the acupuncturist is able to objectively control the amount of stimulation the acupuncture provides to the patient. Studies have shown that EA can promote oocyte growth in PCOS patients and increase oocyte maturation and fertilization rates [68, 69]. Li [72] found that EA combined with CHM not only improved the PR in PCOS patients (82.5% vs. 60% $P < 0.05$), but also reduced the incidence of adverse effects such as LUFS and OHSS during ovulatory treatment (2.5% vs. 20%,0% vs. 15.0% $P < 0.05$). Budihastuti et al. [73] draw a similar conclusion that LET combined with EA in ovulation induction therapy for PCOS patients could significantly improve uterine hemodynamics, promote follicle development (19.86 ± 0.7 mm vs. 13.92 ± 3.61 mm $P < 0.05$), and increased endometrial thickness (8.22 ± 1.76 mm vs. 6.95 ± 1.82 mm $P < 0.05$) [73]. In addition, Peng et al. [74] found that EA improved DAEA-induced IR, mitochondrial dysfunction, and endoplasmic reticulum stress in a rat model of PCOS by inhibiting the mTOR/4E-BP1 signaling pathway, and reversed the beneficial effects of EA on PCOS-like rats by inhibiting autophagy in a reversion experiment in which rats with improved symptoms were injected with 3-MA (autophagy inhibitor).

3.1.3. Moxibustion and Warm Acupuncture. According to TCM, deficiency of kidney yang, deficiency of cold in the cellular veins and stasis blocking the thoroughfare and conception vessels are important etiological mechanisms of infertility, and moxibustion is widely used in the improvement of assisted reproduction and metabolic abnormalities in infertile patients with PCOS, because of its effects on warming the menstrual channels and dispersing cold, reinforcing yang and prostration, eliminating stasis and resolving masses, as well as preventing disease and health care. [75] Yu et al. [76] found in an RCT that Chinese medicine combined with moxibustion at points BL23, DU2, DU4 and other acupoints could effectively regulate the level of sexual hormones, improve ovarian hemodynamics, increase OR, and thus improve PR (OR: 84.62% vs. 64.71%, PR: 48.8% vs. 23.53%, $P < 0.05$). Similar to moxibustion, warm acupuncture and moxibustion is a treatment in which moxa wool is twisted around the needle handle and ignited during needle retention, and the needle body transmits heat into the acupoint, which has the effect of warming the meridians and activating qi and blood. In an RCT, Liu et al. [77] found that on the basis of conventional western medicine treatment, the application of warm acupuncture points ST25, RN12 and other acupoints combined with tonifying of kidney and eliminating blood stasis decoction was significantly improved in endometrial thickness (9.85 ± 1.27 mm vs. 7.29 ± 0.93 mm vs. 8.14 ± 1.12 mm $P < 0.05$) and follicle diameter (19.48 ± 2.40 mm vs. 16.36 ± 2.67 mm vs. 17.85 ± 2.28 mm) compared with the two groups of western medicine and CHM, and it improved sex hormone levels, endometrial blood flow parameters, reduced plasma peripheral platelet aggregation rate and D-dimer levels, and increased OR and PRs, and this conclusion is also verified by Xu, et al. [78].

3.1.4. Other Acupuncture-related Therapies. Acupoint injection is a way to treat diseases by injecting drugs into acupoints and organically combining the dual stimulating effects of acupuncture and drugs, which has the characteristics of easy operation, small amount of drugs, and wide indications. Acupoint injection assisted treatment of female infertility can often achieve better therapeutic effects. Cai et al. [79]. found that the injection of urinary gonadotropins into EX-CA1, RN3, RN4 and other acupoint significantly improved the quality and quantity of oocytes compared with intramuscular injection in the buttocks. In addition, PR and OR (PR: 46.7% vs. 26.7, OR: 86.7% vs. 56.7%, $P < 0.05$) could also be significantly improved. Wen et al. verified this experimental conclusion [80]. Auricular acupoint pressing is used to continuously stimulate some specific areas of the auricle by using the seeds of Vaccariae Semen to prevent and treat diseases. Studies have shown that auricular acupoint pressing combined with acupuncture can improve the level of sex hormones in PCOS patients, promote the follicle development and the increase of endometrial thickness, and regulate menstrual cycle [81]. However, there are few trials on acupoint injection, acupoint application, and auricular point, mostly in combination with other therapies, and the level of evidence is not high enough to elucidate the mechanism of action, so future trials with larger sample sizes and more sophisticated designs are needed to prove its effectiveness.

3.2. Mechanism of Acupuncture on PCOS Infertility

3.2.1. Regulation of HPOA Function. Modern studies have shown that acupuncture stimulation acts on local skin, the excitation of peripheral nerves is transmitted to the central nervous system, releasing brain neurotransmitters or neuropeptides acting on the HPOA, promoting ovarian vascular dilatation and blood perfusion in ovarian arteries, elevating E and endorphin levels in peripheral blood, and regulating the serum levels of GnRH, LH, FSH, and PRL in PCOS patients [82]. EA improved PCOS-IR by down-regulating

hypothalamic NFκB protein expression and significantly reduced abdominal circumference, body weight, serum fasting glucose, fasting insulin, and IR in rats [83]. Zhu et al. [84] found that moxibustion could rescue the HPO axis of ovarian-injured rats, improve hypothalamic GnRH mRNA overexpression and abnormal secretion of reproductive hormones, and maintain normal ovarian function. Huang et al. [85] found that EA could activate the PI3K/AKT signaling pathway, inhibit autophagy-induced follicular atresia, and reduce serum T, LH, and anti-müllerian hormone (AMH) levels, thus improving ovulation. But on the other hand, some studies have also found that acupuncture can inhibit the PI3K/AKT/mTOR pathway by down-regulating LncMEG3 expression, reduce granulocyte autophagy to promote follicle proliferation, and to ensure the normal development of follicles [86]. In TCM theory, acupuncture therapy has a two-way regulation of "activation" and "inhibition" to maintain body homeostasis, so it is assumed that acupuncture treatment also has bidirectional regulation of HPOA function.

3.2.2. Promotion of Ovulation. EA not only promotes angiogenesis in the antral follicles of PCOS rats, but also promotes follicular maturation and ovulation [87]. Yin et al. [88] found that EA combined with ovulatory induction drugs significantly improved the menstrual cycle in infertile patients with PCOS, decreased serum LH, LH/FSH, T and AMH levels, and increased ovulation and PRs. Xiang et al. [89] further found that EA improved oocyte quality and embryonic development potential by activating the IRS-1/PI3K/GLUT4 signaling pathway. Li et al. [90] verified this conclusion that moxibustion improved ovarian function and inhibited ovarian granulosa cell apoptosis by activating the PI3K/AKT signaling pathway. However, this conclusion has also had opposite results. Lai et al. [91] found that EA was able to inhibit the expression of IRS1 and IRS2 mRNA through experiments. In the future, further studies are needed to explore the clinical efficacy and mechanism of acupuncture in PCOS patients.

3.2.3. Improvement of ER. PCOS infertile patients who do not conceive after lifestyle regulation and ovulation induction treatment will eventually opt for ART to achieve pregnancy. However, the cycle PR of assisted reproductive techniques is still hovering at 30%–40% [92]. Owing to sexual hormone disorder and ovulation disorder, PCOS patients often reduce the PR and live birth rate of assisted reproduction due to the low number and poor quality of oocytes obtained, poor ER, and adverse reactions during ovulation induction and embryo transfer. ER refers to the ability of endometrium to accept embryo implantation that changes with menstruation, the influence of sex hormone levels, endometrial thickness, endometrial PI/RI and other factors. EA improves ER by regulating hormone levels and promoting the expression of factors such as vascular endothelial growth factor (VEGF) in the endometrium and ovary, which makes ER present as "trilinear endometrium" and increases pregnancy and embryo transfer rates [93, 94]. Yuan et al. [95] further found that acupuncture combined with CHM can improve the ER of ovulation induction mice by activating the PI3K/Akt/mTOR signaling pathway, down-regulating the expression of miR-494-3p, increasing the expression of endometrial thickness, and ER-related factor HOXA10. Chen et al. [96] validated this conclusion that EA promoted endometrial angiogenesis and thus increased blastocyst implantation rate by activating VEGFR2/PI3K/AKT and VEGFR2/ERK signaling pathways. Shen et al. [97] used high-throughput RNA sequencing and bioinformatics to comparatively analyze patients treated with acupuncture or not and found that circ-SFMBT2, circ-BACH1, and circ-LPAR1 circRNAs were significantly upregulated in patients treated with acupuncture. Therefore, it was also speculated that acupuncture could affect ER by regulating the expression of circRNA, thereby improving the PR and the success rate of ART.

3.2.4. Lose Weight. Studies have shown that 30–70% of PCOS women present with overweight/obesity and visceral obesity, and higher BMI is associated with poorer fertility prognosis [98, 99]. Hypothalamus plays a key role in regulating food intake and energy homeostasis. Studies have shown that the key targets of acupuncture to improve obesity are mainly neurons or neuropeptides in the hypothalamic arcuate nucleus and peripheral hormones (leptin and insulin). Weight loss by reducing leptin and insulin expression to improve leptin and insulin sensitivity [100, 141]. Xu and Zuo [101] found that acupuncture could improve the body mass index (BMI) of infertile patients with PCOS, increase the response of ovulation induction in patients, and effectively shorten the cycle of pregnancy assistance (OR in ovulation promotion cycle: 93.3% vs. 80.0%, clinical PR 43.3% vs. 33.3% $P < 0.05$). Dou et al. [102]. found that acupuncture around the navel increased the PR in obese PCOS patients, which may be related to the increase of serum adiponectin and the decrease of BMI, waist-hip ratio (WHR), homeostasis model of IR, and serum leptin level. In addition, acupuncture can effectively reduce fasting insulin levels and waist-to-hip ratio (WHR) in abdominally obese patients with PCOS, and the therapeutic effect is better than that of diet control plus exercise alone [103]. The theory of TCM believes that acupuncture prescriptions should be "syndrome differentiation and treatment", and individualized acupuncture prescriptions should be issued according to the actual situation of patients. There may be individual differences in the clinical manifestations of PCOS infertility patients, so individualized acupuncture prescription has a more positive effect on improving pregnancy outcomes in infertile patients with PCOS [104].

3.2.5. Improving Mood. Infertile women with PCOS are often more prone to stress and anxiety due to obesity and infertility, which in turn affect pregnancy outcomes. Therefore, improving patients' mental health and adverse emotions has a significant role in the relief of PCOS symptoms and improvement of pregnancy outcomes. According to the theory of TCM, emotional disorder can

easily lead to liver qi stagnation, disorder of liver drainage can lead to deregulation of qi and blood, and disorder of thoroughfare and conception vessels lead to unacceptable pregnancy. Therefore, soothing liver qi and regulating emotions are of great significance for the treatment of infertility. Modern medicine has also clearly demonstrated the effectiveness of psychological interventions in improving anxiety, depression and other adverse emotions, and also can significantly increase the PR [105, 142]. For infertile women with PCOS who are under great social and family pressure, acupuncture has become their best choice due to its less side effects and wide range of action mechanisms, and the combined therapy of acupuncture and Western medicine has been widely used in clinical practice [106]. Wang et al. found that EA improved anxiety/depression symptoms and quality of life in patients with PCOS, and that acupuncture combined with selective 5-hydroxytryptamine (5-HT) inhibitors can significantly improve anxiety compared with selective 5-HT inhibitors alone [107, 108].

3.3. The Safety of Acupuncture in Treating PCOS Infertility. Acupuncture therapy is known as a "economic therapy" due to its low invasive, easy to operate, and no gastrointestinal irritation. With the widespread use of acupuncture around the world, there is an increasing trend of reported adverse reactions to acupuncture. Kim et al. [109] believed that adverse infectious events caused by acupuncture may lead to serious consequences, but can be largely avoided if formal acupuncture procedures are followed and aseptic operation is standardized. The safety of acupuncture in the hands of a qualified practitioner is also appreciable. Petra Bäumler's [110] meta-analysis showed that 9.31% (95% CI 5.10% to 14.62%, 11 studies) and 7.57% (95% CI 1.43% to 17.95%, 5 studies) of patients treated with acupuncture had at least one occurrence of a mild acupuncture adverse event, and half of the mild adverse events were pain, bleeding, and ecchymosis at the acupuncture site, while there was no adverse effect on the selection of IVF adjuvant therapy due to infertility. A retrospective cohort study in Korea reported no significant differences in delivery outcomes between the acupuncture and control groups of pregnant women, and the incidence of acupuncture-related adverse events during pregnancy was 1.3%, most of which were mild adverse events such as acupuncture pain [111]. Serious adverse events and fetal complications due to preterm delivery were rare, and the abortion rate was 5%, which was lower than the intervention of the control group [112]. This suggests that acupuncture will not have adverse effects and consequences for pregnant women, so acupuncture can be considered one of the safer medical treatment and can be widely used in patients with PCOS infertility through standardized practice by experienced practitioners.

4. Clinical Observation and Mechanism of Other Therapy for PCOS Infertility

In recent years, in addition to the above TCM and acupuncture, the supplement of nutrients and the cultivation of healthy lifestyle, which are also complementary and alternative therapies, have gradually attracted the attention of the researcher. They are impacting the traditional treatment of PCOS infertility and are increasingly accepted and used by infertility patients with PCOS [16].

4.1. Clinical Observation and Mechanism of Supplementing Nutrients in the Treatment of PCOS Infertility. Recently, a growing number of studies have found that the lack of vitamins and microelement is related to the occurrence and development of PCOS infertility. Vitamins, minerals, probiotic supplements and other dietary additives can significantly reduce PCOS-related symptoms [113]. The following mainly focuses on the effects of vitamin and microelement supplementation on infertile patients with PCOS.

4.1.1. Vitamin Supplementation. Vitamins are a kind of organic substances that are essential to maintain human life activities, which are mainly involved in the regulation of the body's metabolism. Once deficient, it will cause damage to human health. Currently, vitamin D (VD)and E are the main vitamins that have been studied in the treatment of PCOS infertility.

VD deficiency is very common in women with PCOS. A study by Samantha F Butts et al. found that ovulation and live birth rates were 15.2% and 40% lower, respectively, in VD deficient PCOS patients than in normal women [114]. Recent studies have shown that VD supplementation can increase FSH levels in PCOS patients, thereby decreasing LH/FSH values, and improving IR and hyperlipidemia, thus increasing ovulation and pregnancy rates in PCOS patients [115, 116]. Severe OS is one of the causes of PCOS. Pallavi Dubey et al. found that excessive reactive oxygen species (ROS) in PCOS patients lead to IR, hyperandrogenism (HA), chronic inflammation, and affect oocyte fertilization and blastocyst implantation [113, 117]. Therefore, antioxidative stress may be one of the methods for the treatment of PCOS with infertility. ROS is a natural byproduct of normal oxygen metabolism and plays an important role in cell signaling and homeostasis in the body, and is produced at abnormally high levels in patients with PCOS due to an imbalance between oxidation and antioxidation [118]. VD and vitamin E (VE) can regulate the abnormally high level of ROS in the body. Chen et al. found that short-term VE supplementation can improve OS and reduce ROS levels and reduce the amount of exogenous human menopausal gonadotropin [119]. In addition to the fat-soluble VD and VE, water-soluble vitamins also have antioxidant and anti-inflammatory effects. A study by Szczuko et al. found that water-soluble vitamins could improve the clinical symptoms of PCOS patients by reducing the low-intensity inflammatory response caused by multiple factors such as OS and chronic infection [120].

4.1.2. Microelement Supplementation. Microelements are mineral elements that are present in the body in amounts less than 0.01%. Although their content is minuscule, they play an indispensable role in the human body. Deficiency of a certain microelement inhibits sexual maturity and

ovulation function of women, resulting in infertility and threatened abortion. For example, low serum Cu is closely related to recurrent abortion, missed abortion, and spontaneous abortion; low serum Zn can cause a decline in the synthesis and secretion of FSH and LH by the pituitary gland, resulting in ovulation disorders [121]. Relevant studies have found that women can comprehensively play the regulatory function of multiple trace nutrients by supplementing multiple microelements, and jointly play the role of promoting follicle development, preventing multiple birth defects of offspring and improving pregnancy outcomes [122]. Yu et al. found that ovulation and pregnancy rates of infertile patients with PCOS were significantly higher after taking MaFuLong combined with multivitamin tablets compared to the control group taking MaFuLong alone (ovulation rate: 86.67% vs. 63.33%, pregnancy rate: 46.15% vs. 15.79%) [121]. Montanino Oliva et al. also confirmed this conclusion, where women with PCOS showed improvements in their menstrual cycle, ovulation, and body weight after 6 months of continuous administration of a compound with inositol 2 g, L-tyrosine 0.5 mg, folic acid 0.2 mg, selenium 55 mcg, and chromium 40 mcg [123].

4.2. The Clinical Efficacy and Mechanism of Healthy Lifestyle in the Treatment of PCOS Infertility. Patients with PCOS are often associated with poor lifestyle habits, and the 2018 edition of the Chinese guidelines for the management of PCOS recommends that the development of a healthy lifestyle (including diet, exercise, and behavioral interventions) should be the preferred treatment for PCOS patients [124].

4.2.1. Lose Weight. The prevalence of obesity in PCOS patients ranges from 30% to 70% and obese PCOS patients are often associated with low fertility and infertility [99, 125]. In addition to unreasonable diet and lack of exercise, PCOS patients are more likely to have abnormal glucose and lipid metabolism due to poor insulin sensitivity, so the probability of obesity is significantly higher than that of normal women [126]. Therefore, a reasonable and efficient weight loss is particularly important for infertile women with PCOS. Weight loss not only improves metabolic abnormalities and insulin sensitivity, but also improves hyperandrogenemia and promotes follicle development. Dietary adjustment is one aspect of the weight loss approach that should not be ignored. Studies have shown that high-carb diet can easily lead to low-level chronic inflammation and obesity in the body, which aggravates ovulation disorders in patients with PCOS infertility [127]. The ketogenic diet is a diet that reduces the proportion of carbohydrate in the diet, and appropriately increases the proportion of vegetables and proteins, which can significantly improve the weight loss effect of PCOS-IR patients in clinical practice. By reducing the absorption of monosaccharide, the ketogenic diet decreases the glucose level in PCOS patients, which in turn lowers insulin levels to regulate glucolipid metabolism and improves the endocrine status [128]. Paoli et al. recruited 24 overweight women with PCOS and after 12 weeks of ketogenic diet, the weight and BMI of the experimental group decreased significantly (pre- and post-treatment weight: 81.19 ± 8.44 kg vs. 71.76 ± 6.66 kg; $p < 0.0001$; pre- and post-treatment BMI: 28.84 ± 2.10 vs. 25.49 ± 1.69; $p < 0.0001$) [129]. A total of 254 PCOS patients with overweight or obesity (BMI ≥25) who were treated with IVF-ET assisted pregnancy by Tan et al. were randomly divided into the weight management strengthening group (80 cases with dietary intervention, the strengthening group), the weight management education group (80 cases, the education group), and the control group (94 cases). After 2 months of treatment, they entered the IVF cycle. BMI, waist circumference, waist-hip ratio, and HOMA-IR of visceral fat area in the strengthening group and the education group were significantly lower than those before weight loss ($P < 0.05$); the trend of the number of oocytes gained, total number of fertilization, total number of cleavage, number of high-quality embryos, and the clinical pregnancy rate among the three groups was strengthening group > education group > control group ($P < 0.05$) [130]. Therefore, weight loss is crucial for infertile patients with PCOS.

4.2.2. Exercise. For different clinical manifestations of PCOS, exercise and weight loss are still the first-line treatment in the world [131]. Li et al. found that aerobic exercise can improve T, E2, and FSH sex hormone disorders in PCOS rats by affecting the hypothalamus-pituitary-ovarian axis, so as to promote follicle development and increase ovulation rate in PCOS patients [132]. Moderate amounts of vigorous exercise are beneficial to most women and may also improve the fertility of infertile patients with PCOS [133]. In aspects of exercise intensity, Cory T. Richards et al. argue that moderate intensity steady state exercise is recommended for PCOS patients compared with high-intensity interval training [134].

In addition to moderate and high intensity exercise, Tai Chi, yoga, Qigong and other exercise methods have also attracted more and more attention from infertile patients with PCOS in recent years. They can not only reduce weight, but also relieve tension and improve immunity of patients. Tai Chi is a traditional Chinese boxing. According to TCM, kidney deficiency is the fundamental pathogenesis of ovulation disorder infertility [135]. Tai Chi focuses on the function of "activating the waist," "waist is the house of the kidney," and the exercise of the waist helps the accumulation of the essence of the kidney [136]. Therefore, the practice of Tai Chi helps to cultivate the essence and qi of the kidney, which has a positive effect on infertile patients with PCOS from the perspective of TCM. Yoga and Tai Chi are very similar limbs and trunk exercise therapies in the two ancient medical systems of China and India. Studies have found that infertile patients with PCOS are more prone to anxiety and depression than those with tubal factor infertility [137]. In a RCT conducted by Maryam Mohseni et al., they found that yoga practice could significantly reduce hypertrichosis and WHR in PCOS patients, and it was recommended to include it in the treatment strategy of PCOS women. In addition to Tai Chi and yoga, Qigong, as a unique Chinese gymnastics

with health care, wellness, and disease elimination effects, is now widely used in disease prevention, treatment, and rehabilitation. Qigong not only promotes the circulation of qi and blood, but also regulates the emotions and relieves negative emotions through exercise. Sun Xiaoling et al. found that exercise can reduce IR, promote the recovery of ovarian function, and increase ovulation rate in PCOS patients, thus improving pregnancy rate [139]. Therefore, regular and moderate exercise is very important for infertile patients with PCOS.

5. Conclusion

This review mainly discusses the therapeutic effects of CHMs, acupuncture, nutrient supplementation, healthy lifestyle, Tai Chi, yoga, and Qigong of CAM on infertile women with PCOS. The results showed that CAM can improve IR and sex hormone disorders, enhance endometrial thickness, increase ovulation rate, pregnancy rate, and improve anxiety status in infertile patients with PCOS. At present, few studies have reported the adverse effects of CAM on liver and kidney function and fertility outcomes of infertile women with PCOS, and few reports of abortion and malformation of embryos. CAM can not only regulate the current physical and mental health status of infertile patients with PCOS, but also regulate many long-term complications caused by PCOS. Overall, CAM is a safe treatment option for infertile patients with PCOS. However, due to the limited number of methods and trials included and the generally low quality, CAM needs to be studied in more depth and larger trials to demonstrate its efficacy and safety if it is to be used more widely in the clinic and mostly as an auxiliary to primary therapy for therapeutic purposes.

Abbreviations

PCOS: Polycystic ovary syndrome
IR: Insulin resistance
HOMA-IR: Insulin resistance index
VE: Vitamin E
OS: Oxidative stress
FSH: Follicle stimulating hormone
LH: Luteinizing hormone
ART: Assisted reproductive technology
VD: Vitamin D
5-HT: 5-hydroxytryptamine
CAM: Complementary and alternative medicine
HPOA: Hypothalamic-pituitary-ovarian axis
TCM: Traditional Chinese medicine
CHM: Chinese herbal medicine
BBR: Berberine
WHR: Waist-to-hip ratio
CC: Clomiphene
LET: Letrozole
CRY: Cryptotanshinone
PR: Pregnancy rate
ASD: Androstenedione
T: Testosterone
QUE: Quercetin
LDP: Liu Wei Di Huang Prescription
GFP: Gui Zhi Fu Ling Pill
PCOS-IR: Polycystic ovary syndrome with insulin resistance
STP: Shou Tai Pill
ZYP: Zi Shen Yu Tai Pill
LUFS: Luteinized unruptured follicle syndrome
E: Estrogen
CPA: Cyproterone acetate
OR: Ovulation rate
IVF-ET: In vitro fertilization-embryo transfer
RCT: Randomized controlled trial
PI: Perfusion index
RI: Resistive index
ER: Endometrial receptivity
EA: Electroacupuncture
OHSS: Ovarian hyperstimulation syndrome
GnRH: Gonadotropin-releasing hormone
AMH: Anti-müllerian hormone
VEGF: Vascular endothelial growth factor
BMI: Body mass index
ROS: Reactive oxygen species.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Disclosure

Yu-Qian Shi and Yi Wang are co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant no. 82074259), the Project of Cultivation Project of Outstanding Youth Fund of Heilongjiang University of Traditional Chinese Medicine (grant no. 2018jc02), Luo Yuankai Young and Middle-Aged Scientific Research Fund Project of Zishen Yutai Pill (No. 20190804), as well as "Outstanding Young Academic Leaders" Scientific Research Project of Heilongjiang University of Chinese Medicine to Y.Z.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3245663, 12 pages
https://doi.org/10.1155/2022/3245663

Review Article

Impact of Chinese Herbal Medicine on Glucolipid Metabolic Outcomes in Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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Received 19 April 2022; Revised 12 July 2022; Accepted 7 September 2022; Published 30 September 2022

Academic Editor: Tian-Bo Liu

Objective. This investigation was conducted to analyze and evaluate the impact of Chinese herbal medicine on glucolipid metabolism in women with polycystic ovary syndrome (PCOS). *Methods*. We used manual and computer-aided search methods, and the search scopes included Chinese databases (China National Knowledge Infrastructure, Wanfang, the China Science and Technology Journal Database, and the Chinese Biomedical Literature Database) and English databases (PubMed, Embase, Web of Science, and the Cochrane Library). We searched these eight databases for randomized controlled trials investigating the effects of Chinese herbal medicine on glucolipid metabolism in women with PCOS, with the retrieval deadline being June 2021. Two reviewers screened, selected, and extracted data and verified the results independently. The NoteExpress software was used to manage and screen the literature, the risk of bias assessment tool was used to evaluate the methodological quality of the included studies, and the RevMan 5.4 software was used for meta-analysis. *Results*. A total of 13 trials were included, including 825 patients with PCOS. Because the drugs used in the control group were different, we divided the results into two parts, with four trials using placebo and nine trials using metformin as the control. The results of the meta-analysis showed that fasting insulin (MD = −2.45, 95% CI = [−4.74, −0.17], $P = 0.04$), 2 h fasting plasma glucose (MD = −0.33, 95% CI = [−0.64, −0.02], $P = 0.04$), serum total cholesterol (MD = −0.38, 95% CI = [−0.58, −0.18], $P = 0.0002$), triglycerides (MD = −0.36, 95% CI = [−0.58, −0.14], $P = 0.001$), and low-density lipoprotein cholesterol (MD = −0.58, 95% CI = [−0.75, −0.41], $P < 0.00001$) were significantly improved in the Chinese herbal medicine group compared with the placebo group. In addition, compared with metformin, body mass index (MD = −1.04, 95% CI = [−1.55, −0.53], $P < 0.0001$), serum total cholesterol (MD = −0.27, 95% CI = [−0.46, −0.07] $P = 0.007$), and low-density lipoprotein cholesterol were significantly reduced (MD = −0.12, 95% CI = [−0.22, −0.02], $P = 0.02$) and high-density lipoprotein cholesterol (MD = 0.09, 95% CI = [0.02, 0.17], $P = 0.01$) was significantly improved after treatment with Chinese herbal medicine. *Conclusion*. Compared with the placebo group, Chinese herbal medicine had positive effects on glucolipid metabolism in women with PCOS. Chinese herbal medicine had a positive effect on lipid metabolism when the control group was metformin, but no effect on glucose metabolism. These findings need to be verified in high-quality, large-sample, randomized controlled trials in the future.

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine and metabolic disorder in reproductive-age women [1]. PCOS commonly manifests as ovulatory dysfunction, elevated androgen levels, polycystic ovaries, insulin resistance (IR), and obesity. Studies have shown that hyperandrogenism and IR are the core etiology

and main endocrine features of PCOS [2]. IR not only affects the reproductive function of PCOS patients, but also significantly increases the risk of chronic metabolic diseases—such as hyperlipidemia, hyperglycemia, cardiovascular disease, and type2 diabetes [3]—and metabolic disorders are considered to be the most important long-term concerns related to PCOS [4, 5]. In addition, the healthcare system bears a huge burden for treating the direct and indirect diseases related to IR [6]. Thus, IR has become the main focus of treatment for patients with PCOS.

There are no effective curative treatments for PCOS due to its requirement for long-term treatment. At present, syndrome differentiation dictates the main clinical treatments. Lifestyle interventions such as strength training, diet, and changing poor habits (for example, giving up smoking and drinking) have positive effects in patients with PCOS and are currently the first-line treatments [7]. However, lifestyle interventions face various challenges as the first-line management, for instance, suboptimal response and lack of adherence. Therefore, patients require additional pharmacological interventions. Metformin, the most extensively studied insulin-sensitizing agent for the treatment of women with PCOS, reduces serum insulin and androgen levels and improves ovulatory function [8]. In addition, metformin can reduce low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels to lower the risk of complications such as cardiovascular disease [9]. However, oral metformin has a high incidence of adverse reactions such as gastrointestinal distress, nausea, vomiting, anorexia, etc., and can even lead to death [10, 11]. It is difficult for some patients to accept these possible risks, and thus metformin is not an ideal choice for long-termfirst-line medication for patients. Therefore, the use of complementary treatments has increased in recent years [10, 11].

Traditional Chinese medicine (TCM), as a form of complementary medicine, has the clinical advantages of obvious curative effects, minimal side effects, low cost, and fewer complications in the treatment of IR in PCOS patients and has gradually become a new choice for people [12]. The treatment of PCOS should be sustainable and dynamic and should be adapted to the changing circumstances and expectations of the individual patient. In addition, patients with PCOS have different clinical symptoms, and TCM is amenable to individualized treatments. Doctors can analyze the physical condition of patients with PCOS and then formulate corresponding treatment plans. Owing to the lack of efficacy and the debilitating side effects of pharmaceuticals, complementary and alternative drugs are becoming more and more popular in the treatment of PCOS [13]. Clinical studies have further shown that Chinese herbal medicine has a definite effect in regulating glucolipid metabolism disorders [14, 15], and numerous experimental studies have found that Chinese herbal medicine also has the effect of improving glucolipid metabolism in animal models of PCOS and IR [16–18]. Overall, the available evidence suggests that Chinese herbal medicine has higher efficacy and safety for patients with PCOS compared to pharmaceutical treatments.

However, the effects of Chinese herbal medicine on glucolipid metabolism in PCOS patients have not been comprehensively analyzed and studied. Therefore, on the basis of the existing evidence, we conducted a comprehensive search of domestic and foreign literature to objectively evaluate the clinical efficacy and safety of Chinese herbal medicine on glucolipid metabolism in PCOS patients, aiming to provide the latest basis for clinical medication.

2. Methods and Materials

2.1. PRISMAP. Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMAP) was followed for the systematic review and meta-analysis.

2.2. Search Strategy. Two reviewers conducted a systematic literature search in English databases (PubMed, Embase, Web of Science, and the Cochrane Library) and Chinese databases (China National Knowledge Infrastructure (CNKI), Wanfang, the China Science and Technology Journal Database (VIP), and the Chinese Biomedical Literature Database (CBM)). Search terms were related to Chinese herbal medicine (e.g., "Oriental medicine," "East Asian medicine," and "Chinese herbal drugs"), PCOS (e.g., "polycystic ovary syndrome"), glucolipid metabolism (e.g., "insulin sensitivity," "glucose tolerance tests," "lipid profile," "HbA1c," "triglycerides," "total cholesterol," "high-density lipoprotein cholesterol," and "low-density lipoprotein cholesterol"), and randomized controlled trials (e.g., "clinical trial," "RCT," "random," "randomize," and "randomization"). Both text and MeSH terms were used. We searched all the above databases until June 22, 2021, and two reviewers screened, selected, and extracted data and cross-verified the results of the data extraction independently.

2.3. Eligibility Criteria. The PICOS (population, intervention, comparison, results, and study design) framework was used to establish the selection criteria.

2.3.1. Types of Studies. We only included randomized controlled trials (RCTs) in Chinese or English that used Chinese herbal medicine to treat PCOS patients. The status or date of the study had no effect on the systematic review.

2.3.2. Participants. Participants in all included RCTs were adult patients with PCOS, and all participants were diagnosed using the 2003 Rotterdam criteria [19]. This definition proposes that PCOS can be diagnosed in any woman presenting with at least two of the three following criteria: hyperandrogenism (either clinical or hyperandrogenemia), ovulation dysfunction, and polycystic ovaries on ultrasound plus the exclusion of other diagnoses that could result in hyperandrogenism or ovulatory dysfunction. The participants were not excluded by their race, background, or body size, but participants with other serious diseases (such as cancer, liver disease, or kidney disease) were excluded from the RCTs.

2.3.3. Intervention Groups. The included RCTs used various forms of Chinese herbal medicine treatment, including Chinese herbal decoctions or proprietary Chinese medicines derived from botanicals, minerals, animals, or chemicals. The dosage forms of Chinese medicine included decoctions, tablets, powders, pills, granules, capsules, ointments, oral liquids, plasters, and injections. Nonherbal interventions (such as massage, acupuncture, cupping, and other TCM treatments), herbal injections, or combined interventions using two or more different types of herbal medicines were excluded from the included RCTs. There were no restrictions on the herbal composition, dosage, frequency of intake, or duration of treatment in the included RCTs.

2.3.4. Comparison Groups. Patients in the control groups received Western medicine (including insulin sensitizers such as metformin), placebo, Western medicine combined with placebo, or lifestyle management, including weight loss through diet and exercise. There were no restrictions on the dosage form, quantity, or duration of the medicine or placebo.

2.3.5. Outcome Measures. The primary outcome was homeostatic model assessment of insulin resistance (HOMA-IR), and the secondary outcomes were fasting blood glucose (FPG), fasting plasma insulin (FINS), 2-hour fasting blood glucose (2hFPG), 2-hour fasting insulin (2hFINS), serum total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), LDL-C, body mass index (BMI), and waist-to-hip ratio (WHR). The safety indicator was any adverse event.

2.4. Literature Screening. The identified articles were initially imported into NoteExpress. After reading the title and abstract, initial screening was performed according to the inclusion criteria. The full texts of the qualifying trials were then read to check whether the papers met the aforementioned inclusion criteria. All duplicate trials were excluded. If there was any disagreement between the two researchers, a third researcher was consulted.

2.5. Data Extraction and Management. The following data were collected from each study: (a) Year of publication; (b) Name of first author; (c) Country; (d) Basic characteristics of the included patients; (e) Sample size; (f) Intervention measures; (g) Outcome measures; (h) Adverse reactions; (i) Random allocation methods; and (j) Other relevant information. Microsoft Excel was used for data extraction. If there was any disagreement between the two researchers, a third researcher was consulted.

2.6. Bias Risk Assessment of the Included Studies. Two researchers independently assessed the quality of the literature by using the Cochrane collaborative bias risk assessment tool. The evaluation was conducted according to the standards proposed in the Cochrane Intervention System Evaluation Manual, and the risk of bias was divided into three levels of low, high, and unclear. The bias risk assessment included the following seven aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. If there was any disagreement between the two researchers, a third researcher was consulted.

2.7. Data Synthesis. Data analysis was performed with the RevMan 5.4 software. When the included studies used the same measurement scale, the mean difference (MD) and 95% confidence interval (CI) were used to describe continuous variables. Because the prescriptions used in TCM are different and vary from person to person, there is inevitably heterogeneity in clinical indicators. Therefore, in this meta-analysis, the random-effects model was adopted uniformly.

3. Results

3.1. Literature Search and Screening Flowchart. The selection of the studies is shown in Figure 1. A total of 1277 trials were retrieved in the initial search. After deleting duplicates and filtering through the titles and abstracts, we obtained 25 full texts. Finally, 13 studies were included in the systematic review and meta-analysis [20–32].

3.2. Features of the Included Trials. A total of 825 patients were included in the 13 RCTs. All participants were diagnosed with PCOS according to the Rotterdam criteria, and they only received Chinese herbal medicine treatment or treatment with placebo or metformin. The specific characteristics of these studies are summarized in Table 1, including author, year, country, sample size, age, intervention measures, duration of treatment, and outcome indicators, and the main ingredients and usages of Chinese herbal medicine are summarized in Table 2.

3.3. Evaluation of the Quality of the Trials. Cochrane collaborative bias risk assessment was used to evaluate the quality of the literature. Based on standards outlined in the Cochrane Intervention System Evaluation Manual, two researchers independently assessed the literature. The risk of bias of these 13 trials was divided into three levels of low, high, and unclear. All 13 trials mentioned the word "random" or "randomization," and six studies described the specific randomization methods, of which only three studies described both allocation concealment and randomization methods. For participant and personnel blinding, three trials had a low risk of bias [28, 29, 32], one study did not have enough information to judge the risk level [31], and the remaining studies were at high risk of bias due to the lack of blinding during implementation. Measurements were generally made by third parties other than the researchers, so the blinding of outcome assessment was defined as low risk. In addition, all studies described the missing data and reported both the glucose metabolism and lipid profile indicators.

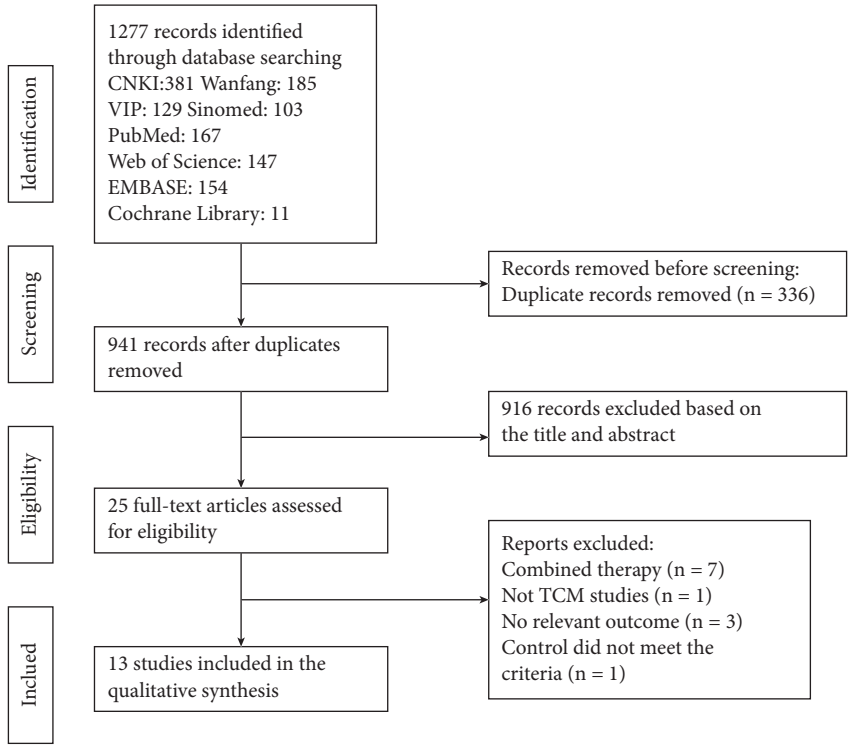

Figure 1: Literature search and screening flowchart.

Detailed information of the quality evaluation is shown in Figure 2. If there was any discrepancy between the two researchers, a third researcher was consulted.

3.4. Results of the Meta-Analysis

3.4.1. Compared with the Placebo Group. (1) General Indicators.

Meta-analysis showed no statistically significant differences for BMI ($P = 0.84$) or WHR ($P = 0.06$) between the Chinese herbal medicine groups and the placebo groups. The results of the meta-analysis are shown in Figure 3 (3.1.1–3.1.2).

(2) Glucose Metabolism Indicators.

The analysis showed that compared with the placebo group, FINS (MD = −2.45, 95% CI = [−4.74, −0.17], $P = 0.04$) and 2hFPG (MD = −0.33, 95% CI = [−0.64, −0.02], $P = 0.04$) were significantly improved in the Chinese herbal medicine group. However, no significant difference was seen in the HOMA-IR ($P = 0.08$), FPG ($P = 0.11$), or 2hFINS ($P = 0.96$) between the two groups. The results of the meta-analysis are shown in Figure 3 (3.2.1–3.2.5).

(3) Lipid Profile Indicators.

The analysis showed that the indicators of TC (MD = −0.38, 95% CI = [−0.58, −0.18], $P = 0.0002$), TG (MD = −0.36, 95% CI = [−0.58, −0.14], $P = 0.001$), and LDL-C (MD = −0.58, 95% CI = [−0.75, −0.41], $P < 0.00001$) were statistically different when compared with the placebo group, but there was no statistical difference in HDL-C ($P = 0.79$) when the control group was placebo. The results of the meta-analysis of blood lipid metabolism are shown in Figure 3 (3.3.1–3.3.4).

3.4.2. Compared with the Metformin Group. (1) General Indicators.

The analysis of BMI and WHR showed that compared with the metformin group, the BMI of the Chinese herbal medicine group was significantly improved (MD = −1.04, 95% CI = [−1.55, −0.53], $P < 0.0001$), while there was no statistical difference in WHR ($P = 0.47$). The results of the meta-analysis are shown in Figure 4 (4.1.1–4.1.2).

(2) Glucose Metabolism Indicators.

Compared with the metformin group, the analysis showed that no significant difference was seen in the HOMA-IR ($P = 0.36$), FPG ($P = 0.65$), FINS ($P = 0.87$), 2hFPG ($P = 0.63$), or 2hFINS ($P = 0.56$). The results of the meta-analysis are shown in Figure 4 (4.2.1–4.2.5).

(3) Lipid Profile Indicators.

The analysis showed that in the Chinese herbal medicine group, TC (MD = −0.27, 95% CI = [−0.46, −0.07], $P = 0.007$) and LDL-C (MD = −0.12, 95% CI = [−0.22, −0.02], $P = 0.02$) were significantly reduced and HDL-C (MD = 0.09, 95% CI = [0.02, 0.17], $P = 0.01$) was significantly improved. However, no significant difference was seen in the TG ($P = 0.17$). The results of the meta-analysis of blood lipid metabolism are shown in Figure 4 (4.3.1–4.3.4).

3.4.3. Reporting Bias Assessment. When more than 10 RCTs were included in the meta-analysis, a funnel plot was used to assess bias. We tried to test the indicators that included 9 RCTs of metformin as the control group in this study. The results showed that there was no obvious asymmetry in FINS and HDL-C, but the funnel plots of BMI, FPG, and TC were

Table1: Features of the included studies.

Study ID	Sample size	Age	Treatment versus control	Duration of treatment (months)	Outcomes
Chen [23] China	T: 47 C: 15	T: 28.43 ± 3.83 C: 27.27 ± 5.75	T: ban-xia-xie-xin-tang, decocted. C: metformin, 500 mg, po, tid	3	BMI, WHR, HOMA-IR, FPG, FINS, TC, TG, LDL-c, HDL-c
Fang et al. [25] China	T: 45 C: 45	T:25.41 ± 3.48 C:25.32 ± 3.37	T: wen-shen-tiao-jing-tang, decocted. C: metformin, 500 mg, po, bid.	2	BMI, HOMA-IR, FPG, 2hFPG, FINS, 2hINS, TC, TG, HDL-c, LDL-c
Hong et al. [22] China	T: 23 C: 22	T: 24.3 ± 5.8 C: 25.1 ± 6.2	T: jian-pi-qu-tan-tong-luo-tang, decocted. C: metformin, 500 mg, po, tid.	3	BMI, WHR, HOMA-IR, FPG, FINS, TC, TG, HDL-c, LDL-c
Li [24] China	T: 45 C: 45	T: 25.33 ± 3.97 C: 25.33 ± 4.32	T: wu-ji-san, powdered medicine. C: metformin, 500 mg, po, tid.	3	BMI, WHR, FPG, 2hFBG, FINS, 2hFINS, TC, TG, HDL-C
Liang et al. [31] China	T: 48 C: 47	24.69 ± 3.55	T: HYKT (Heyan Kuntai capsule) C: placebo tid, 4 capsules each time.	6	BMI, WHR, HOMA-IR, FPG, 2hFBG, FINS, 2hFINS, TC, TG, HDL-C, LDL-c
Liang [28] China	T: 25 C: 25	T: 23.20 ± 3.73 C: 24.20 ± 3.08	T: zi-shen-qing-re-li-shi-hua-yu-fang (granules). C: placebo granules bid, 1 bag each time.	3	HOMA-IR, FPG, FINS, 2hFPG,2hINS, TC, TG, HDL-C, LDL-C
Luo [30] China	T: 19 C: 16	T: 23.1 ± 5.7 C: 24.1 ± 4.3	T: bu-shen-hua-tan-qu-yu-fang, decocted. C: metformin, 500 mg, po, qd.	6	BMI, FPG, FINS, T C, TG, HDL-C, LDL-C
Mahdie [29] Iran	T: 29 C: 30	T: 28.62 ± 5.74 C: 26.53 ± 6.35	T: cinnamon powder capsules C: placebo 500 mg, po, tid.	3	BMI, HOMA-IR, FPG, FINS, 2hFPG, TC, TG, LDL-C, HDL-C
Mehri [32] Iran	T: 24 C: 36	T: 28.6 ± 4.7 C: 27.2 ± 3.4	T: curcumin C: placebo 500 mg/day	3	BMI, HOMA-IR, FPG, FINS, TC, TG, LDL-C, HDL-C
Wang et al. [20] China	T: 40 C: 35	29.43 ± 4.35	T: cang-fu-dao-tan-tang modified, decocted. C: metformin, 500 mg, po, tid.	3	BMI, HOMA-IR, FPG, FINS, TC, TG, HDL-C, LDL-C
Zhao et al. [27] China	T: 36 C: 26	18 ~ 35	T: yang-yin-yi-qi-huo-xue-fang, decocted. C: metformin, 500 mg, po, tid.	3	BMI, HOMA-IR, FPG, FINS, TC, HDL-C, LDL- C
Zhao et al. [26] China	T: 30 C: 22	T: 23.2 ± 3.1 C: 24.1 ± 2.9	T: yang-yin-yi-qi-huo-xue-fang, decocted. C: metformin, 500 mg, po, tid.	6	BMI, HOMA-IR, FPG, FINS, TC, TG, HDL-C, LDL-C
Zheng et al. [21] China	T: 26 C: 24	T: 27.02 ± 4.98 C: 27.45 ± 4.67	T: duo-nang-yin, decoction. C: metformin, 500 mg, po, tid.	6	BMI, WHR, FPG, FINS, TC, TG, HDL-C, LDL-C

T: treatment; C: control; po: peros; qd: quaque die; bid: bis in die; tid: ter in die; BMI: body mass index; WHR: waist-to-hip ratio; HOMA-IR: homeostasis model assessment of insulin resistance; FPG: fasting plasma glucose; 2hFPG: 2 h fasting plasma glucose; FINS: fasting insulin; 2hFINS: 2 h fasting insulin; TC: serum total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 2: Ingredients and usages of Chinese herbal medicine.

Study ID	Chinese medicine decoction	Main ingredients and usage
Chen [23] China	Ban-xia-xie-xin-tang	Banxia 9 g, huangqin 30 g, huanglian 15 g, ganjiang 15 g, dangshen 12 g, shengyimi 30 g, gouqi 30 g. Add or subtract Chinese herbals according to symptoms. Decoction, 150 ml per dose. One dose each time, two times a day, once in the morning and once in the evening.
Fang et al. [25] China	Wen-shen-tiao-jing-tang	Zishiying 15 g, xianlingpi 15 g, chuanduan 10 g, tusizi 15 g, baishao 10 g, heshouwu 15 g, xiangfu 10 g, danggui 15 g, chuanniuxi 10 g, chuanjiao 10 g. Decoction in water, 1 dose a day, 250 ml per dose, orally two times, once in the morning and once in the evening.
Hong et al. [22] China	Jian-pi-qu-tan-tong-Luo-tang	Baizhu 15 g, dangshen 15 g, fuling 15 g, cangzhu 15 g, xiangfu 15 g, xianglingpi 15 g, guizhi 10 g, chaihu 10 g, danggui 15 g, taoren 10 g, shudihuang 15 g, jixueteng 15 g, chenpi 10 g. Decoction, 200 ml, 1 dose/time, 2 times/day.
Li [24] China	Wu-ji-san, powdered medicine.	Baizhi 9 g, chuanxiong 9 g, gancao 9 g, fuling 9 g, danggui 9 g, rougui 9 g, baishao 9 g, jiangbanxia 9 g, shengmahuang 9 g, chenpi 18 g, zhiqiao 18 g, cangzhu 30 g, ganjiang 10 g, jiegeng 12 g, houpo 12 g. Dissolve the medicine powder with 300 ml warm boiled water and take it two times a day, once in the morning and once in the afternoon. Do not stop taking the medicine during menstruation.
Liang et al. [31] China	HYKT (Heyan Kuntai capsule)	Dihuang, huanglian, baishao, huangqin, ejiao, fuling. 3 times a day, 4 capsules each time.
Liang [28] China	Zi-shen-qing-re-li-shi-hua-yu-fang (granules).	Zhimu 10 g, shanzhuyu 10 g, danshen 10 g, taoren 10 g, yiyiren 15 g, baijiezi 10 g, huangbai 10 g, xuanshen 10 g, gancao 6 g. Boil 1 sachet in water two times a day, take in the morning and evening, drink while still warm.
Luo [30] China	Bu-shen-hua-tan-qu-yu-fang	Tusizi 15 g, xianlingpi 15 g, roucongrong 15 g, shengdi 15 g, danggui 12 g, chuanxiong 6 g, zelan 15 g, fuling 15 g, fabanxia 10 g, cubiejia 12 g, zaojiaoci 15 g, gancao 6 g. Take one dose a day, decoct to 300 ml, and take it twice per day, once in the morning and once in the evening. Stop taking during menstruation.
Wanget al. [20] China	Cang-fu-dao-tan-tang modified	Cangzhu 12 g, xiangfu 12 g, chenpi 15 g, fabanxia 15 g, zaojiaoci 15 g, danggui 12 g, chuanxiong 12 g, shichangpu 12 g, fuling 20 g, danshen 30 g, heye 15 g, shanyao 15 g, huangqi 30 g, xianlingpi 15 g, lujiaoshuang 12 g, zishiying 30 g, sharen 6 g, chuanniuxi 15 g. Take 1 dose a day. Decoct in water two times, drink while warm, once in the morning and once in the evening. Start taking it from the 10th day of menstruation and stop taking it during menstruation, and continue for 3 consecutive months.
Zhao et al. [27] China	Yang-yin-yi-qi-huo-xue-fang	Gouqizi 10 g, ejiaozhu 10 g, zhihuangjing 10 g, hanliancao 10 g, buguzhi 10 g, nvzhenzi 10 g, digupi 10 g, maidong 10 g, nanshashen 10 g, beishashen 10 g, ziheche 10 g, shengdi 10 g, shengbaishao 10 g, huangqi 30 g, gancao 6 g. Add or subtract Chinese herbs according to menstrual symptoms.

Table 2: Continued.

Study ID	Chinese medicine decoction	Main ingredients and usage
Zhao et al. [26] China	Yang-yin-yi-qi-huo-xue-fang	Ejiaozhu 10 g, huangqin 10 g, huangbo 10 g, hanliancao 10 g, buguzhi 10 g, nvzhenzi 10 g, digupi 10 g, maidong 10 g, nanshashen 10 g, beishashen 10 g, shengbaishao 10 g, huangqi 30 g, gancao 6 g, ziheche 3 g. Add or subtract Chinese herbs according to menstrual symptoms.
Zheng [21] China	Duo-nang-yin	Tusizi 15 g, bajitian 10 g, chaihu 10 g, yinyanghuo 10 g, baishao 10 g, longdancao 6 g. Add or subtract Chinese herbs according to symptoms. Soak the herbs in 300 ml of water for 30 minutes, then boil with high heat and then simmer for 20 minutes. Take 200 ml of the first decoction, then add 200 ml of water and continue to decoct for 15 minutes. Take 150 ml of the second decoction, mix with the first 200 ml, and divide it into two doses. Take each dose in the morning and evening.

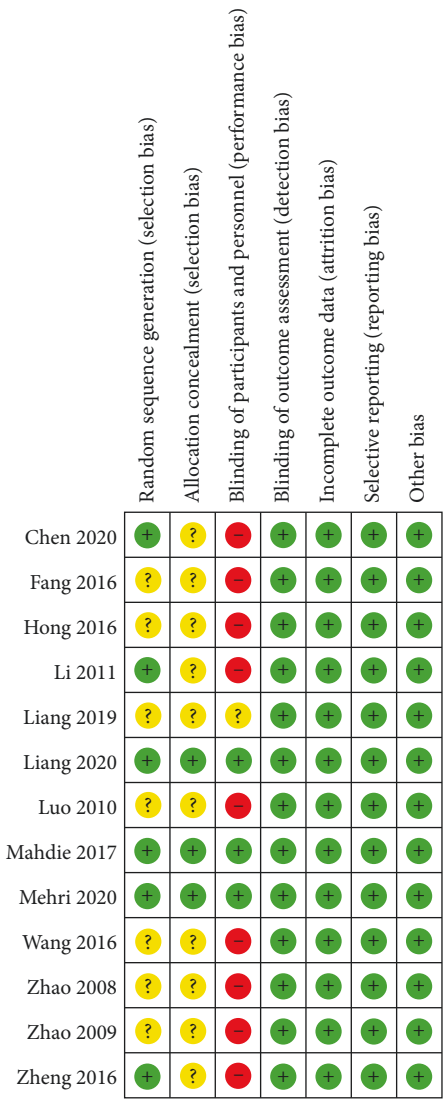

Figure 2: Evaluation of the risk biases of the included studies.

not symmetrical enough, suggesting that there may be publication bias. The reasons for this might be unpublished negative results, low methodological quality, or small sample sizes. They are shown in Figure 5.

3.4.4. Adverse Events. Five studies reported the existence of adverse events. Three studies reported gastrointestinal side effects such as nausea, vomiting, diarrhea, or weakness in the metformin group (control group) and the patients chose to discontinue treatment [24, 26, 27]. Two of the studies reported that there were patients with abdominal distension and anorexia in the Chinese herbal medicine group, but the patients could tolerate these effects and they resolved spontaneously [26, 31]. In another study, a patient in the Chinese herbal medicine group developed skin rash and itching after using cinnamon capsules for 5 days, but the adverse effect disappeared after discontinuation of the treatment without any intervention [32].

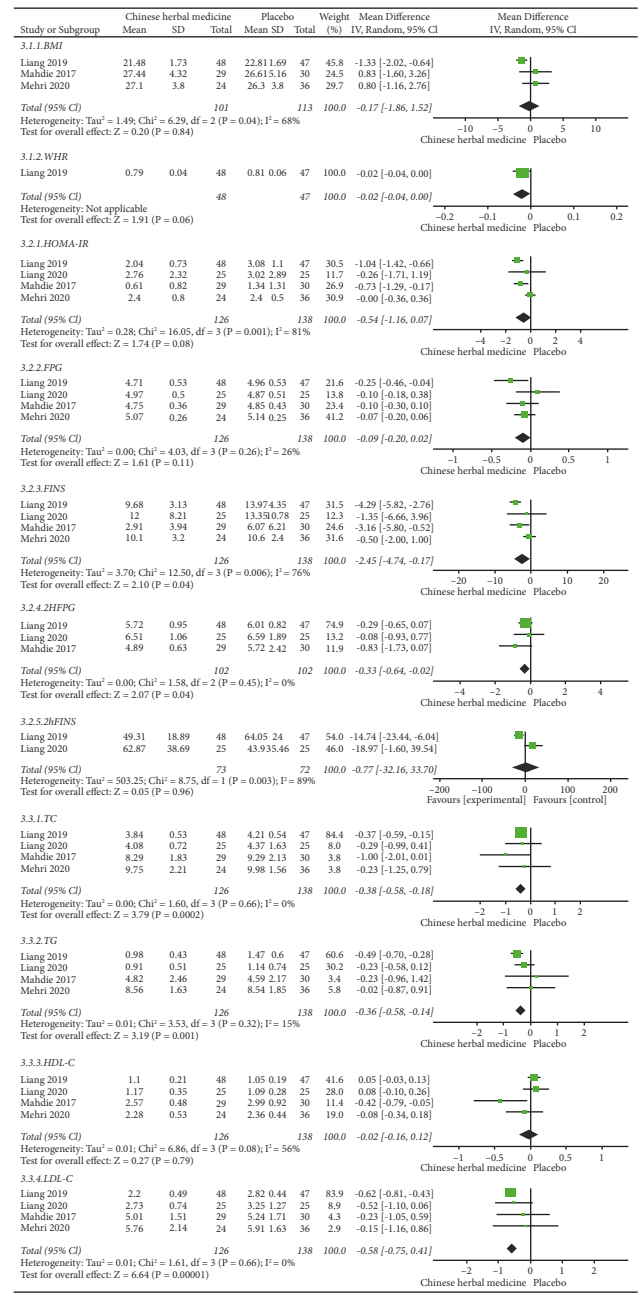

Figure 3: Comparisons of glucolipid metabolism between the Chinese herbal medicine group and placebo group in the treatment of PCOS.

4. Discussion

In this study, a meta-analysis was performed to systematically evaluate the effect of Chinese herbal medicine on glucolipid metabolism in women with PCOS. The results showed that compared with the placebo group, Chinese herbal medicine has a relatively positive effect on glucolipid metabolism in women with PCOS. Chinese herbal medicine has relatively positive effects on lipid metabolism when the control group was metformin, but had no significant effect on glucose metabolism. Overall, the effects of Chinese herbal medicine on glucose metabolism were not as significant as

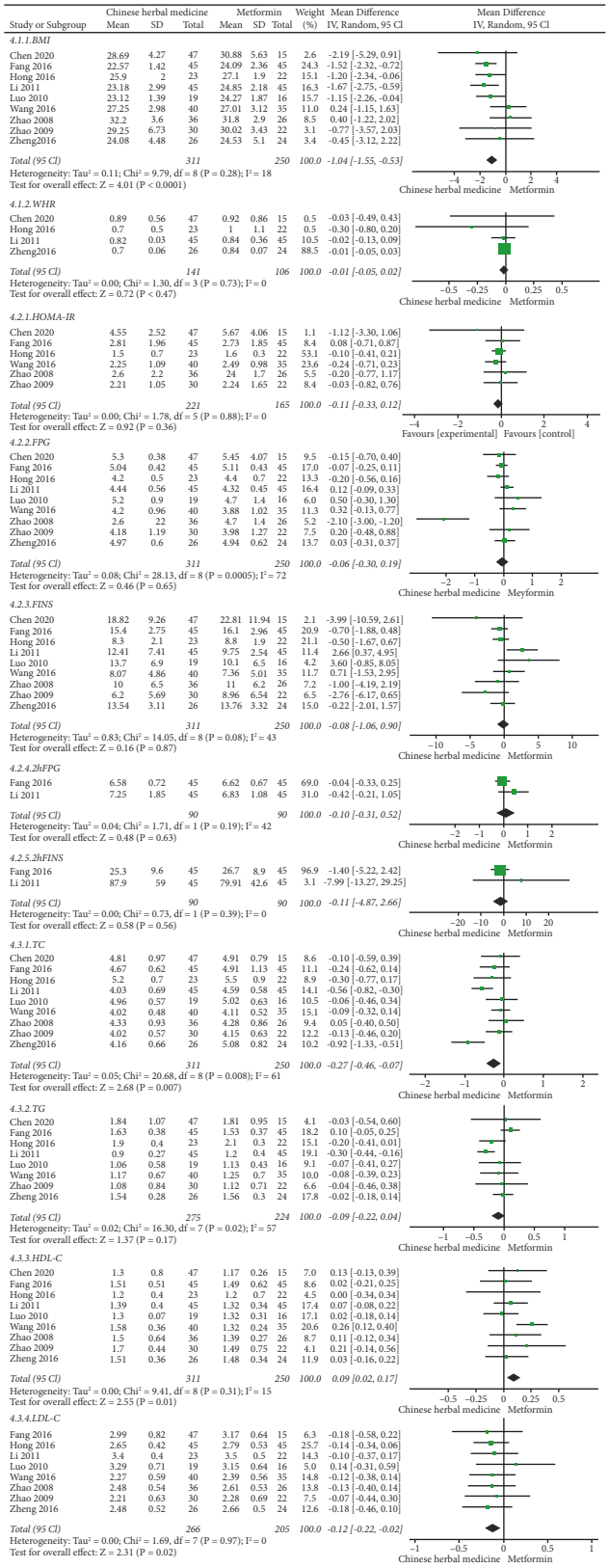

Figure 4: Comparisons of glucolipid metabolism between the Chinese herbal medicine and metformin group in the treatment of PCOS.

metformin, but were more effective than placebo. Although the results of this meta-analysis showed that insulin resistance was not significantly improved, there have still been many related reports of TCM improving insulin resistance [31, 33]. There is also basic research suggesting that Chinese herbal medicine has the potential to modulate the gut microbiota in order to control inflammation and improve insulin resistance in PCOS patients [34]. Chinese herbal medicine rarely caused adverse events, and the reported adverse events, such as abdominal distension, did not have a significant impact, indicating that Chinese herbal medicine is relatively safe and reliable for treating PCOS. Therefore, we conclude that, compared with standard treatments, Chinese herbal medicine is more effective and safer in ameliorating lipid metabolism in patients with PCOS.

However, current meta-analyses of the Chinese herbal medicine treatment of PCOS have mainly focused on infertility and obesity [35, 36], thus there is a lack of research on using Chinese herbal medicine to improve glucolipid metabolism in PCOS patients. At present, the treatments for PCOS are mainly symptomatic treatments, but Chinese herbal medicine treatment focuses on the overall health of the patient, not just their specific symptoms. In the included trials of this meta-analysis, a large percent of the prescriptions were adjusted by physicians based on clinical experience. There are also many classical prescriptions commonly used in clinical practice that were selected, such as Banxia Xiexin decoction, Cangfu Daotan decoction, and Wuji San. The compositions of the prescription are different, but most of them are mainly based on resolving phlegm and removing blood stasis. PCOS patients are often troubled by obesity, and TCM believes that the blockage of "phlegm turbidity" or "static blood" is the main pathological factor behind obesity, and thus the constitution of phlegm dampness and kidney deficiency are likely to be the important pathological basis of obesity and infertility in patients with PCOS.

Modern pharmacology has found that the active polysaccharide in Angelica sinensis has a variety of pharmacological activities, including hematopoietic activity, immune promotion, anti-inflammation, antioxidation, and liver protection. [37]. Citrinin, the active ingredient in tangerine peels, has the effect of inhibiting obesity and can also improve insulin resistance by regulating the inflammatory response caused by obesity in adipose tissue [38]. In addition, some studies have shown that after screening and network pharmacology prediction, a variety of active ingredient monomers in Cangfu Daotan Tang were found to be useful in the treatment of PCOS [39]. Among them, quercetin plays a role in regulating metabolic disorders in the treatment of PCOS [40], and kaempferol can improve insulin resistance by inhibiting the inflammatory response [41].

The results of this study also show that Chinese herbal medicine has a better effect than control interventions. In

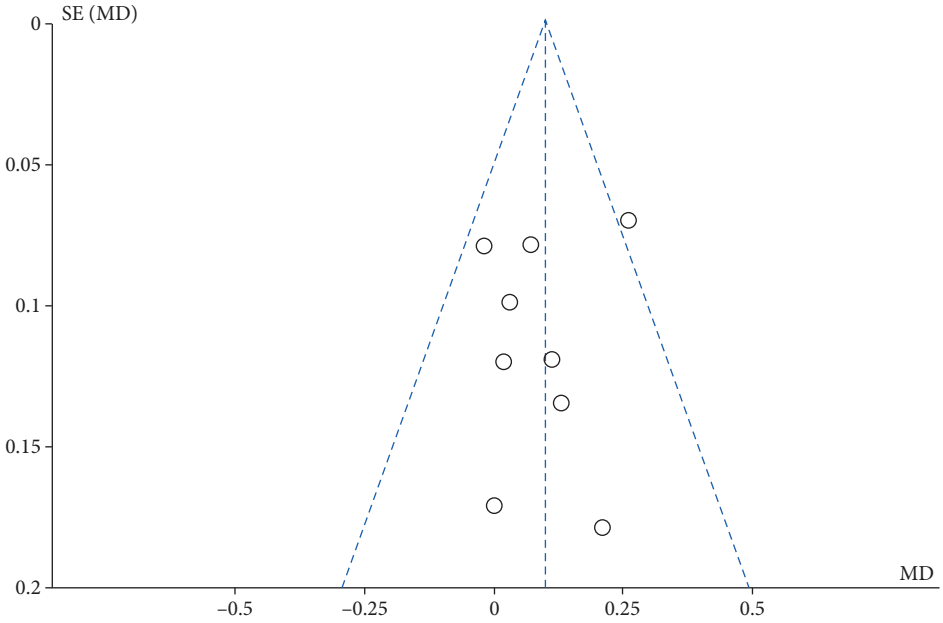

Figure 5: Funnel plot of comparison: HDL-C.

addition to herbal medicine, there are other TCM treatments such as acupuncture, cupping, and massage. Among these, acupuncture greatly improves glucolipid metabolism in patients with PCOS [42]. This meta-analysis demonstrates sufficient evidence for the effectiveness of Chinese herbal medicine in the treatment of PCOS, suggesting that more PCOS patients might choose Chinese medicine as an alternative adjuvant treatment. However, the evidence for the influence of Chinese herbal medicine on glucolipid metabolism in PCOS patients needs more high-quality, large-sample, randomized controlled trials to further confirm these conclusions.

The meta-analysis presented here improves our understanding of the therapeutic effect of Chinese herbal medicine on glucolipid metabolism in women with PCOS. However, this study also has certain limitations. First, the qualities of the included RCTs varied. Only 6 of the 13 RCTs described the specific randomization methods, of which only three studies described both randomization methods and allocation concealment, while the rest only mentioned the use of randomization methods to allocate patients, thus the accuracy and objectivity of the results are reduced. Second, the sample size of the included RCTs was small, and the clinical and physiological characteristics of PCOS were heterogeneous. Third, the choice of intervention in this meta-analysis was Chinese herbal medicine treatment, but the dosage forms, types, quantities, and courses of treatment with Chinese herbal medicine were not the same, and the treatment doses of metformin or placebo in the control groups were also different, resulting in diverse interventions in the included RCTs. Thus, our results can provide a reference for clinical practice, but further accurately designed clinical trials are needed to obtain more rigorous treatment results to overcome these shortcomings.

5. Conclusion

The RCTs analyzed here have shown that, compared with the placebo group, Chinese herbal medicine has relatively positive effects on glucolipid metabolism in women with PCOS. Chinese herbal medicine has a relatively positive effect on lipid metabolism when the control group is metformin, but no positive effect on glucose metabolism. Further large-scale, long-term RCTs that meet strict methodological standards are needed to prove this conclusion.

Data Availability

Disclosure

Jie Li and Ruqun Zheng are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Juan Li and Mei Han contributed equally. Juan Li conceptualized and designed the study. Jie Li and Ruqun Zheng collected and analyzed the data. Jie Li drafted the manuscript. Juan Li and Mei Han reviewed the protocol for important content and revised the manuscript. Juan Li and Huiyan Tan sought funding. All authors contributed to the further editing and final publication of the trial.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant 81804137), the Open laboratory project for college students of Guangzhou Medical University, (Grant 20205016 (Dr. J. Li)), and the Scientific Research Project of Guangdong Provincial Administration of Traditional Chinese Medicine (Grant 20212131 (Dr. H. Tan)).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7012978, 13 pages
https://doi.org/10.1155/2022/7012978

Review Article

Research Progress on the Effect of Traditional Chinese Medicine on Signal Pathway Related to Premature Ovarian Insufficiency

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Received 18 April 2022; Accepted 2 August 2022; Published 14 September 2022

Academic Editor: Tian-Bo Liu

The occurrence and development of premature ovarian insufficiency involves the abnormality of multiple signal pathways. It is a complex disease. Traditional Chinese medicine affects the relevant factors of the occurrence and development of premature ovarian insufficiency (granulosa cell apoptosis, ovarian blood supply, ovarian reserve, ovarian oxidative damage, gap junction, ovarian fibrosis, follicular development, follicular atresia, and other biological processes) by regulating a variety of signal pathways, thus playing the role in antioxidant stress, prevention and treatment of chemotherapy side effects, protection of ovarian function, control of aging, and improvement of ovarian reserve function. The research shows that the research on the related pathways of traditional Chinese medicine in the treatment of premature ovarian insufficiency has been quite extensive. Based on the search of the domestic and foreign literature, it is found that Yulinzhu and Xianziyizhen capsule and ginsenoside Rg_1 can promote the proliferation and differentiation of granulosa cells, inhibit apoptosis, and reduce follicular atresia by affecting the PI3K/Akt/mTOR signal pathway; Guiluo's Anzang decoction, Kuntai capsule, and Yangyin Shugan granule can maintain the balance between pro-apoptotic protein and anti-apoptotic protein through the Bax/cytc/caspase-3 pathway to improve ovarian reserve function; Bushen Jianpi recipe can control cell apoptosis and promote the proliferation and development of ovarian granulosa cells by regulating the MAPK signal pathway; Siwu mixture and Zuo Gui pill can regulate the TGF-β/Smads signaling pathway to promote the recruitment of primordial follicles, promotes follicular development, inhibits follicular atresia, and regulates ovarian function; Erxian decoction, Yiqi Yangrong Fujing formula, and Cistanche deserticola can antagonize the inflammatory symptoms of premature ovarian failure, promote the secretion of relevant vascular growth factors, and enhance the ovarian reserve function through the NF-KB signal pathway; Bushen Culuan decoction can promote damage repair and protect normal ovarian cells through antioxidant stress. The above summary aims at providing reference for the in-depth study of traditional Chinese medicine in the treatment of premature ovarian insufficiency and inspiring new diagnosis and treatment ideas.

1. Introduction

Premature ovarian insufficiency (POI) is defined as a decline in ovarian function that occurs in women before the age of 40 years, resulting in abnormal menstruation, increased FSH levels, decreased estrogen volatility, and decreased fertility. Clinical female patients will have hot flashes, sweating, vaginal dryness, mood changes, and fertility difficulties. POI also increases a woman's risk of osteoporosis, and cardiovascular and cognitive disorders, according to related studies. Thus, the decline of ovarian function seriously reduced the patient's quality of life. At present, the incidence of POI is 1%~65% and has a tendency to increase. Its pathogenesis is also not clear in the existing guidelines, and expert consensus suggests that treatment with hormone replacement therapy is given priority and can effectively relieve the clinical symptoms, but the side effect is more apparent, such as patients with uterine using estrogen therapy may increase

the risk of endometrial cancer. Estrogen and progesterone can increase the risk of ovarian cancer [1]. Therefore, it is important to find nonhormone replacement therapy.

Traditional Chinese medicine treatment is characterized by syndrome differentiation and treatment, which varies from person to person and has few side effects. In traditional Chinese medicine, POI can be classified into "amenorrhea," "infertility," and "blood depletion" [2]. The pathogenesis is mostly due to the deficiency of Yin and blood in the kidney, in combination with liver depression, spleen deficiency, dampness and heat, vein stasis, etc. The treatment of TCM is mainly based on the principles of nourishing Yin and blood, warming the kidney Yang, relieving liver depression, strengthening the spleen, benefiting Qi, activating blood stasis, , and focusing on comprehensive treatment by identification. With the continuous research and excavation of TCM, more and more reports point out that TCM can treat POI through multiple pathways and targets. On this basis, the author collated the domestic and foreign literature in recent years to summarize the pathways of TCM in the treatment of POI and to provide a basis for clinical use.

2. PI3K/Akt Signaling Pathway

Phosphatidylinositol 3-kinase (PI3K) signaling pathway is the basic signaling pathway in animals, playing an indispensable role in cell growth, proliferation, survival, migration, metabolism, and apoptosis [3]. Studies in recent years have revealed that the PI3K/Akt pathway also plays an important role in premature ovarian insufficiency. For example, the PI3K/Akt/mTOR signaling pathway has a regulatory role in the HPG axis [4], and the activated Akt pathway further causes a cascade reaction of signaling pathway, and phosphorylated Akt can phosphorylate a series of downstream target proteins. It includes FOXO3a (antiproliferation and apoptosis), BAD (a pro-regulatory member of the Bcl-2 family, involved in cellular mitochondrial regulatory pathways), mTOR (which controls protein biosynthesis and regulates cell growth), and P27 (which maintains primal follicular reserves).

2.1. PI3K/Akt/mTOR Signaling Pathway. The complex TSC1/2 is both an oncogene and a major inhibitor of mTORC1. Elevated AKT expression promotes the separation of the complex TSC1/2, and the dissociation of the two releases the inhibition of mTORC1 and activates mTORC1, thus allowing the activation of the mTOR signaling pathway. The mTOR activation can inhibit autophagy and thus promote cellular senescence.

A nonrandomized noninferiority controlled study [5] conducted by Teng Xiuxiang et al. found that after treatment with Yulinzhu plus reduction in the treatment group, compared to the control group treated with estradiol valerate tablets plus progesterone capsules, the treatment group had significantly better overall efficacy (61.54%) and TCM evidence efficacy (49.45%), and serum FSH, LH, and E_2 were significantly improved compared to the control group. One study reported [6] that after immune POI mice were given the Chinese herbal medicine Yulinzhu for 6 weeks, Akt protein expression was significantly increased and TSC1 and TSC2 protein expression was significantly decreased in each administration group. RheB, P-s6k1, and P-4E-BP1 protein expressions were elevated, with P-4E-BP1 being the most significantly elevated, ROS and MDA were significantly elevated, and GSH-Px and SOD were significantly decreased, indicating that Yulinzhu inhibits microenvironmental oxidative stress by regulating the TSC/Akt signaling pathway.

A randomized controlled trial [7] conducted by Lin Xiuqin found that the total response rate of Jiajian Guishen pill combined with estradiol valerate tablets and progesterone capsules in the observation group and estradiol valerate tablets and progesterone capsules in the control group was 93.33% and 70%, respectively, with statistically significant differences. In a study by Kong [8], it was found that Jiajian Gui Shen pill was able to increase the number of follicles at all levels in the ovaries of VCD-induced mice, reduce atretic follicles, improve serum sex hormone levels, and also improve ovarian function possibly by upregulating PI3K, Akt, mTOR mRNA, and protein expression levels.

Huang et al.[9] showed that the Xianzi Yizhen capsule was able to increase Ki-67, Akt, and PI3K protein expression and concluded that the mechanism of improving ovarian function might be related to increased PI3K/Akt pathway activity and cell proliferation status in ovarian cells, which increased the content of primordial follicles, secondary follicles, and sinusoidal follicles and decreased the content of atretic follicles.

Ginsenoside Rg1 is an important pharmacologically active component of ginseng, which has the ability to promote the secretion of estrogen and luteinizing hormone (LH). Liu et al.[10] observed that ginsenoside Rg1 can effectively inhibit the expression of PI3K, Akt, mTOR, and S6k proteins compared to the high expression of PI3K, Akt, mTOR, and S6k proteins in aging tissues, indicating that ginsenoside Rg1 can negatively regulate the PI3K/Akt/mTOR signaling pathway and activate autophagy in ovarian tissues, thereby inhibiting the onset of aging and prolong lifespan.

Icariin is a flavonoid extracted from Epimedium, which has phytoestrogen-like effects. Dong [11] found that Epimedium phosphorylated the activation of PI3K/Akt/mTOR signaling pathway-related proteins during the development of chemotherapy-injured POF, inhibited oxidative stress to reduce tissue apoptosis, and improved chemotherapy-injured POF in mice.

Thus, the PI3K signaling pathway not only regulates the growth of oocytes, regulates the survival and development of primordial follicles, and promotes the proliferation and differentiation of granulosa cells, but also inhibits apoptosis, which is crucial for the normal development and physiological function of ovaries.

2.2. Bax/CytC/Caspase-3. Bcl-2 and Bax are a pair of antiapoptotic proteins that are antagonistic to each other. Bcl-2, which is mainly found in the outer mitochondrial membrane and part of the endoplasmic reticulum, can play its role in

inhibiting apoptosis by blocking the release of mitochondrial CytC and then preventing the activation of caspase apoptosis protein. When cells receive apoptotic signals, Bax translocates into the mitochondria and exerts its pro-apoptotic effect by promoting the release of CytC in large amounts and further initiating the caspase cascade reaction. Whether the cells continue to survive or go to death depends to some extent on the ratio of Bcl-2 to Bax.

Yi-Hui observed [12] that Gui Luo's Anzang decoction could increase Bcl-2 protein expression and decrease Bax protein expression in the ovary, reduce granulosa cell apoptosis, and increase granulosa cell secretion.

A meta-analysis of a randomized controlled trial [13] conducted by Liu et al. showed that the total effective treatment rate of Kuntai capsules combined with hormonal therapy for POF was significantly better than that of hormonal therapy alone (OR = 3.76, 95% CI [2.65, 5.35], $P < 0.00001$). In an experiment, Geng [14] observed that the Kuntai capsule could improve ovarian reserve by downregulating the levels of FSH and LH, stimulating E_2 secretion, regulating the Bcl-2/Bax ratio among Bcl-2 family protein members, and upregulating the expression of Bcl-2 and Bcl-xl proteins versus downregulating the expression of Bax and Bad proteins to maintain the balance between pro-apoptotic and apoptosis-inhibiting proteins.

Wang [15] found that Yangyin Shugan granule could regulate the expression levels of apoptosis-related proteins in ovarian tissues, increasing the expression of Bcl-2 protein and decreasing the expression of Bax and caspase-3 proteins, thus inhibiting apoptosis.

A systematic evaluation [16] conducted by Hu et al. showed that the efficacy of Zuo Gui pill combined with western artificial cycles was superior to treatment with western artificial cycles alone for premature ovarian failure, with significant advantages in the combined group in terms of improved menstrual rates and improved values of serum FSH, and Wang [17] and Gao [18] found through experiments that Zuo Gui pill could increase the level of Bcl-2 mRNA and decrease the level of Bax, and Bcl-2>Bax, concluding that the kidney Zuo Gui pill may inhibit chemotherapy-induced granulosa cell apoptosis by affecting the expression levels of apoptotic factors Bcl-2 and Bax, and thus play a role in protecting ovarian function.

2.3. FOXO3 Transcription Factor-P27/Bim. FOXO3 is an important transcription factor downstream of the PI3K/Akt pathway. When the Akt pathway is inhibited, dephosphorylated FOXO3 enters mostly into the nucleus and accelerates cell regulation. FOXO3 together with RUNX3 binds to the Bim gene in order to activate Bim and thus induce cell regulation.

A randomized controlled trial [19] conducted by Xiao et al. found that in POI, the clinical efficacy of Erxian decoction (95.00%) was significantly better than that of the estradiol valerate combined with the progesterone group (77.50%). In an experiment, Zhao [20] observed that Erxian decoction had a protective effect on cisplatin-injured ovarian granulosa cells. On the one hand, it may be that Erxian decoction exerts a pro-proliferative effect by affecting the granulosa cell cycle phase, promoting the transformation of granulosa cells from G_0 to S phase, decreasing the proportion of G_0 phase cells, increasing the ratio of S phase cells, and improving the proliferation index; on the other hand, it may be that Erxian decoction exerts an antiregulatory effect by decreasing the rate of cisplatin-induced granulosa cell apoptosis. And it was further found that the protective effect of Erxian decoction on cisplatin-induced ovarian granulosa cells might be achieved by regulating the expression of FOXO3a and its downstream target proteins. Thus, Erxian decoction can downregulate the expression of FOXO3a in cisplatin-injured granulosa cells, which in turn downregulates the expression of cell cycle blocker protein $P27^{kip1}$ and pro-apoptotic protein Bim, thus promoting granulosa cell proliferation and inhibiting granulosa cell regulation.

Yang [21] found that the intervention of Zuo Gui pill in the experiment may affect the p-Akt-to-Akt ratio by upregulating the p-Akt level on the PI3K/AKT pathway, as well as reducing FOXO3a protein expression, which in turn effectively inhibits granulosa cell damage and prevents premature follicular failure and atresia in order to protect ovarian function.

In addition, Yangyin Shugan granule could regulate the expression levels of PI3K/Akt/FOXO3a pathway-related proteins in ovarian tissues, increasing the expression of PI3K and Akt proteins, and decreasing the expression of FOXO3a proteins.

3. MAPK Signaling Pathway

PTEN is involved in apoptosis through several signaling pathways, among which the activation of MAPK/ERK and PI3K/Akt signaling pathways is more common, and there are also interactions between PI3K/Akt and MAPK/ERK signaling pathways [22]; PTEN proteins can negatively regulate the activation of Raf1 by Ras in the MAPK/ERK pathway by activating Akt in the PI3K/Akt pathway, and can also directly negatively regulate Raf1 in the absence of Ras action, which in turn inhibits the biological effects of MAPK/ERK and promotes apoptosis.

MAPK pathway has an important role in inflammation, stress response, and apoptosis of cells. Among MAPK signaling pathways, extracellular signal-regulated kinase (ERK) regulates the inflammatory response and also regulates the stem cell factor (SCF)/tyrosine kinase receptor (c-kit) signaling pathway [23]. The MAPK pathway regulates apoptosis by a complex mechanism [24], at least through the following pathways: enhancement of c-Myc expression, phosphorylation of P53, involvement in Fas/Fasl-mediated apoptosis, activation of c-jun and c-fos, and induction of Bax translocation. In addition to the above five pathways, p38 MAPK can also induce apoptosis by enhancing TNF expression.

3.1. ER/C-MYC/TERT Signaling Pathway. Estrogen can regulate the expression of the TERT gene. c-Myc is the target gene of the estrogen receptor (ER). Estrogen binding to ER

promotes the expression of transcription factor c-Myc. c-Myc can bind directly to the E-box at the 5′ end of the core promoter of the TERT gene of many cellular telomerases, which promotes TERT gene transcription, thereby activating telomerase and maintaining normal cellular function [25–27].

A randomized controlled trial [28] conducted by Chen found that Bushen Jianpi decoction was 93.30% effective in treating POI. Wang et al.[29, 30] observed that by increasing E_2 levels, promoting ER activation, and stimulating c-Myc expression, Bushen Jianpi decoction can increase ovarian telomerase activity and telomere length, which in turn regulates TERT expression, thereby promoting proliferation and development of ovarian granulosa cells and maturation of oocytes.

3.2. P53. P53, as an oncogene, is located in the nucleus and can bind specifically to DNA, which can be activated when DNA is damaged, causing cells to be blocked in the G_1 phase, and if the damaged DNA cannot be repaired, P53 can contribute to the apoptosis of damaged cells, and high expression of P53 heralds the cessation of cell growth or apoptosis [31].

A randomized controlled trial [32] carried out by Yanjin et al. found that the effective rate of Bushen Tiaochong decoction in treating POI reached 97.90%. Zhang [33] observed that the mechanism of Bushen Tiaochong decoction mainly can significantly improve the sex hormone level in mice, significantly increase the level of E_2 and AMH, and decrease the level of FSH and LH; downregulate the expression of P53, while upregulate the expression of GnRHR, prolong the time of apoptosis, reduce the occurrence of follicular atresia, improve the reserve capacity of ovarian receptors, and then, coordinate ovarian function.

3.3. Ki67/ERK. Ki67 protein is expressed in the nucleus, is closely associated with mitosis, and plays an important role in cell proliferation [34].

A randomized controlled trial [35] conducted by Wu found that the overall effective rate of Bushen Huoxue decoction in treating POI was 93.33%. Zonghui [36] found that Bushen Huoxue decoction increased p-ERK1/2 protein levels in the ovarian tissue of aged mice, activated ERK1/2 phosphorylation, further improved follicular development in mice with decreased ovarian reserve function at advanced age, and increased the proliferation of follicular granulosa cells and granulosa cells of the oocyte complex.

3.4. p-38/Fas/FasL. The Fas/FasL pathway, also known as the death receptor pathway, acts as an apoptotic signaling receptor, forming a death-inducing complex by binding to its ligand FasL and activating its downstream caspase-3 to induce apoptosis [37].

Chunling [38, 39] showed that the Kuntai capsule could repair pathological damage to ovarian function in fertile rats, regulate serum sex hormone levels in mice with POF, downregulate FSH and LH levels, FasL protein, and mRNA expression, upregulate E_2 and AMH levels, and Fas protein mRNA expression, and increase Fas mRNA expression and FasL mRNA expression to increase Fas mRNA expression and decrease FasL mRNA expression, thus protecting and improving ovarian function. In addition, the Kuntai capsule can improve the reproductive function of mice and increase the conception rate and pregnancy rate of mice with POF, and has no significant teratogenicity to the offspring mice.

Dan [40] found that the Gui Zhi Fu Ling capsule could significantly improve sex hormone levels in mice with POF, with the best effect in the middle dose group, which may be related to the inhibition of the Fas/FasL signaling pathway and reduction of ovarian cell apoptosis.

3.5. SCF. Stem cell factor (SCF), also known as Kit ligand, is a granulocyte-derived growth factor that binds to the oocyte c-Kit receptor and its signal is transduced through the PI3K channel.

Sun et al.[41] found that Zuo Gui pill upregulated Cx43 mRNA and protein expression in ovarian tissues of CTX-induced POF mice, increased the distribution of Cx43 between follicles and granulosa cells, and improved gap junction function.

Xinpeng [42] found that Bushen Tiaochong decoction promoted ovulation by increasing the sensitivity of ovaries to gonadotropins; by upregulating the expression of Cx-37 and downregulating the expression of SCF, it promoted the growth and development of early follicles and improved the gap junction function of ovarian tissues in rats and thus repaired ovarian functional damage. Xiang [43] found that Kun Bao pill reduced ovarian fibrosis by decreasing the expression of CTGF, TGF-β1, and SCF, which showed that Kun Bao pill had a positive intervention effect on POF induced by cisplatin injection in mice.

4. TGF-β/Smads Signaling Pathway

TGF-β/Smads signaling is an important transduction pathway that regulates follicular development, and abnormalities in either process of the transduction pathway may cause signaling disorders, leading to impaired follicular recruitment, inhibition of follicular growth and development, accelerated follicular atresia, and premature ovarian failure.

A randomized controlled trial [44] conducted by Liu Jia found that the total effective rate after treatment was 92.68% in the observation group and 81.71% in the control group when the observation group was given the combination of Siwu mixture combined with hexestrol and medroxyprogesterone acetate. Hongbo [45] found that Smad2/3 protein is an upstream regulatory signaling protein of Cyp19a1 through experiments, and by detecting Smad2/3 protein and its phosphorylated protein PSmad2/3, it was found that Siwu mixture upregulated Cyp19a1 expression by promoting the expression of Smad2/3, a Cyp19a1 regulatory protein.

Yameng [46] found that the Kuntai capsule could not only reduce the levels of sex hormones FSH and LH, increase

the levels of E_2 and AMH, repair the damaged ovarian tissue structure, improve ovarian blood supply, promote the development of rats follicles, reduce follicular atresia, and increase the number of mature follicles present, but also increase the expression of GDF-9 and EGR-1 protein and mRNA in rats ovaries. Chen [47] found that the Kuntai capsule may increase the expression of FSHR in rats' ovarian tissue by upregulating the expression of Smad2/3, improve the responsiveness of ovaries to FSH, promote follicle development, and reduce the level of serum FSH; meanwhile, it downregulates the expression of Smad7 in rats ovarian tissue to reduce follicle apoptosis, maintain follicle growth and development, and improve ovarian function in rats.

Zhu [48] found that Zuo Gui pill could upregulate the protein expression levels of ovarian GDF-9 and its signaling protein Smad2 in POF mice and promote the development of growing follicles in Smad2 mice. Compared with the model group, the ovarian GDF-9 and Smad2 protein expression increased and the ratio of ovarian growth follicles increased in the Zuo Gui pill group, suggesting that Zuo Gui pill may regulate ovarian function by improving the regulation of GDF-9/Smad2 signaling, thereby promoting the recruitment of initiating follicles, promoting follicular development, and inhibiting follicular atresia.

In addition, Yu [49] found that TCM tonic kidney and filling essence method could activate the Bmps/Smad signaling pathway, thus initiating primordial follicle development, inhibiting oocyte apoptosis, and improving ovarian function. Huiping [50] found that Bushen Huoxue decoction not only regulated follicle development and promoted granulosa cell proliferation related to the ActA/Smads pathway, but also promoted estradiol E_2 secretion through the TGF-β1/Smads pathway to improve the local microenvironment of the ovary.

5. NF-KB Signaling Pathway

The NF-kB family plays an important role in physiopathological processes such as inflammation and immune response, oxidative stress response, cell proliferation, and apoptosis by regulating the expression of various pro-inflammatory factors, growth factors, and adhesion molecules. It has been reported [51] that NF-kB induces the expression of several pro-inflammatory factors, such as TNF-α, IL-β, FGF, VFGF, CINC1, and ICAM1.

5.1. The TNF-α. TNF-α is a multireactive cytokine that induces apoptosis of granulosa cells or early follicles by autocrine or paracrine means, and also causes vascular endothelial damage and inhibits hormone secretion and follicle growth and development.

In the rats with POF, the protein and gene expression of TNF-α and IFN-γ were increased in ovarian tissues, and IFN-γ could induce enhanced expression of MHC antigens on granulosa cells and activate a series of cytokines such as IL-1, IL-2, TGF-β, TNF-α, and FGF to produce autoimmune responses to cause follicular atresia. TNF-α can cause vascular endothelial damage and affect local blood flow in the ovary, which can block hormone secretion and follicular growth and development at all levels. After the intervention of renal tonic drugs, the expression levels of TNF-α and IFN-γ decreased, the expression of Bcl-2 protein increased, the expression of Bax protein decreased, and the apoptosis of granulosa cells was inhibited.

Zhou [52] observed that Erxian decoction could antagonize inflammatory symptoms in a dose-dependent manner in rats with POF, and could promote the secretion of vascular growth factors associated with POF and enhance ovarian reserve function.

Liu et al.[53] found that Cistanche can reduce follicular atresia and apoptosis by regulating sex hormone levels and inhibiting the expression of TNF-α and IFN-γ in rats, thus slowing down ovarian failure; Cistanche can also upregulate Bcl-2/Bax, suggesting that Cistanche has an inhibitory effect on POF, and the mechanism may be related to ovarian sex hormone levels, TNF-α, IFN-γ, and apoptosis-related protein Bcl-2/Bax expression.

Lan et al. [54] found that the Chinese herbal Fufang Yiqi Yangrong Fujing formula could effectively regulate serum LH, FSH, and E2 levels in CTX-induced POF mice and improve pituitary and ovarian endocrine functions. It could also increase the expression levels of serum IL-2 and TNF-α, inhibit the apoptosis of ovarian granulosa cells, restore ovarian function, and enhance ovarian reserve capacity.

5.2. VEGF and bFGF. Ovarian blood flow is an important factor in maintaining follicular growth, steroid hormone secretion, and follicular sensitivity to gonadotropins. The VEGF and bFGF are vascular endothelial growth factors, which can repair damaged follicles and improve damaged ovarian vascular permeability and can specifically promote cell proliferation survival and chemotaxis in steroid hormone-producing organs and promote neovascularization. The bFGF plays an important role in luteinizing, mainly in promoting the growth and development of follicular corpus luteum, and also in providing nutrition to the corpus luteum entity.

Xu [55] found that Bushen *Tiaochong decoc*tion could prevent the occurrence of premature ovarian failure in rats, and its mechanism of action might be related to the positive effect of certain components in Chinese medicine in the enhancement of gonadotropins, which promotes the repair of ovarian function and improves ovarian function. Serum FSH and LH were decreased, and E_2 was increased in rats with premature ovarian failure by Chinese medicine gavage, and the expression of the VEGF and the bFGF in ovarian tissues was increased [56], and the expression of TNF-α and IFN-γ was downregulated to reduce follicular atresia and slow down the depletion of follicles.

In addition, the expression of VEGF and bFGF was significantly increased in rats with POF after gavage of Kuntai capsules ($p < 0.05$) [57]. This indicates that Kuntai capsules can protect vascular endothelial cells, smooth blood flow in the ovaries, improve ovarian reserve capacity, and prevent POF. By regulating hormone levels and upregulating

Table 1: The regulatory effects of Traditional Chinese medicine on signal pathways related to POI.

Chinese herbal	Sources	Classification	Efficacies	Pathways	Targets	Experimental model	Roles
Ginsenoside Rg1 [10]	Ginseng	Qi tonics	It is a great tonic for the vital energy, restores the pulse, and fixes the detachment, tonifies the spleen, benefits the lung, generates fluid and nourishes the blood, and calms the mind.	PI3K/Akt/mTOR signaling pathways	PI3K↓, Akt↓, mTOR↓, S6k↓	SPF-grade female BALB/*c* mice	Activation of autophagy in ovarian tissues to inhibit the onset of aging.
Epimedium glycoside [11]	Epimedium	Yang tonics	Tonifying the kidney and Yang, strengthening the muscles and bones, dispelling wind and dampness.	PI3K/Akt/mTOR signaling pathways	PI3K/Akt/mTOR↑	Clean-grade female uncrossed Wistar rats	Inhibit oxidative stress and reduces tissue apoptosis.
Cistanche [53]	The fleshy stems of Cistanche or Cistanche, Cistanche fannii, etc., of the family Cistanaceae.	Yang tonics	Tonifying the kidney and Yang, benefiting the essence and blood, moistening the intestines, and opening the bowels.	NF-kB signaling pathways	TNF-α↓, IFN-γ↓, Bcl-2↑, Bax↓	SPF-grade 12-week-old SD female rats	Reduce follicular atresia and apoptosis, which in turn slows ovarian failure.

the expression of VEGF and bFGF mRNA to alleviate the damage to ovarian function caused by Leigongteng polysaccharide tablets, it delayed or blocked POF, promoted follicle development, and improved ovarian function.

6. Keap1 Nrf2/ARE Pathways

Oxidative stress is one of the key factors leading to apoptosis of oocytes and ovarian granulosa cells. The key pathway for cellular resistance to oxidative stress [58] is the Kelch-like epichlorohydrin-associated protein 1 (Keap1)-nuclear factor E2-related factor 2 (Nrf2)/antioxidant response element (ARE), which initiates antioxidant enzymes downstream of the pathway, including superoxide dismutase (SOD), quinone oxidoreductase, glutathione peroxidase (GSH-Px), and catalase (CAT) [59].

A randomized controlled trial [60] conducted by Ma et al. found that in patients with POI infertility, the test group used Bushen Culuan decoction and the control group used "estradiol valerate tablets + clomiphene + progesterone," and the results showed that the ovulation rate, pregnancy rate, sex hormone level, and TCM symptoms score improved significantly better in the test group than the control group. The total effective rate of treatment in the trial group was 95.35%. Bushen Culuan decoction ameliorated the oxidative stress induced by raffinose polysaccharide [61], decreased the content of ovarian DNA oxidative damage product 8-OHdG and lipid peroxidation product MDA, elevated the activity of ovarian CAT, GSH-PX, SOD, and HO-1 antioxidant enzymes, and inhibited Keap1, through the Nrf2/ARE signaling pathway, and Bach1 nuclear protein expression and promoted Nrf2 nuclear translocation, thus activating downstream antioxidant enzyme activities, which in turn attenuated the ovarian oxidative damage induced by raffinose polysaccharide.

Yu-Zhi [62] observed that Yiguan Jian decoction not only could improve ovarian morphology and ovarian quality in a rat model of POF, but also could repair damaged and protect normal ovarian cells by mobilizing T-SOD and CAT, thus reducing the damage to ovarian cells by peroxidation-related substances such as MDA and caspase-3.

7. Ovarian Silencing Regulatory Proteins (SIRTs)

SIRTs are regulatory factors involved in the aging process. Early studies suggested [63] that SIRTl promotes follicle development, improves ovarian reserve function, and prolongs ovarian lifespan. SIRT1 inhibits the NF-KB inflammatory signaling pathway and reduces inflammatory factor release. It increases the expression of the anti-apoptotic protein Bcl-xl and downregulates the pro-apoptotic proteins caspase-3 and Bax, through the FOXO1 signaling pathway, and inhibits apoptosis.

Tong et al. [64] found that Zuo Gui pill and You Gui pill could increase follicle count, SIRT1, and serum AMH and INHB, and decrease serum LH and FSH concentrations in mice with POF, in addition to the fact that the number of primordial follicles in the You Gui pill group was significantly higher than that in the Zuo Gui pill group, indicating that Zuo Gui pill and You Gui pill had significant anti-ovarian aging effects, and their mechanism of action was related to the upregulation of ovarian SIRT1 expression.

Table 2: The regulatory effects of Chinese herbal formula on signal pathways related to POI

Recipe	Compositions	Classification	Efficacies	Pathways	Targets	Experimental model	Roles
Yulinzhu [6]	Radix Angelicae Sinensis, Radix Rehmanniae Praeparata, Semen Cuscutae, ginseng, branched Atractylodes macrocephala, Poria, Radix Paeoniae Alba, Cortex Eucommiae, Zanthoxylum piperitum. Rhizoma Ligustici, Radix Glycyrrhizae	Tonic prescriptions	Warming the kidneys and strengthening the spleen to produce essence	PI3K/Akt/mTOR signaling pathways	TSC1↓, TSC2↓, Rheb↑, P-s6k1↑, P-4e-bp1	SPF BALB/*c* female mice with normal motility cycles at 6 to 8 weeks of age.	It can inhibit microenvironmental oxidative stress.
Jiajian Guishen pill [8]	Semen Cuscutae, semen spleen, Radix Rehmanniae, Radix Rehmanniae, Radix Angelicae Sinensis, Radix Paeoniae Alba, Salviae miltiorrhiza	Tonic prescriptions	Tonifying the kidney, strengthening the spleen, tonifying the liver, and invigorating the blood	PI3K/Akt/mTOR signaling pathway	PI3K↑, Akt↑, mTOR mRNA↑	SPF ICR female mice.	Improve ovarian reserve function and increase the number of follicles.
Xianzi Yizhen capsule [9]	Radix Rehmanniae Praeparata, Radix et Rhizoma Polygonati, Radix et Rhizoma Drynariae, Radix et Rhizoma Continuum, Semen Cuscutae, semen spleen	Tonic prescriptions	Tonifying the kidney and filling the essence	PI3K/Akt/mTOR signaling pathways	Ki67↑, Akt↑, PI3K↑	SPF female C57BL/6J type mice.	Increase the content of primordial follicles, secondary follicles and sinus follicles, and decrease the content of atretic follicles.
Guiluo's Anzang decoction [12]	Radix Rehmanniae Praeparata, Fructus Fritillariae, Semen Cuscutae, Radix et Rhizoma Polygoni, Paeoniae Alba	Tonic prescriptions	Nourishing Yin, tonifying the kidneys, calming the mind, and relieving irritability	Bax/CytC/Caspase-3 signaling pathways	Bcl-2↑, Bax↓	SPF healthy female SD rats	Reduce granulocyte apoptosis and increase granulocyte secretion.

Table 2: Continued.

Recipe	Compositions	Classification	Efficacies	Pathways	Targets	Experimental model	Roles
Kuntai capsule [14, 38, 39, 46, 47, 57]	Radix Rehmanniae Praeparata, Rhizoma Polygonati, Radix Paeoniae Alba, Radix Scutellariae Praeparata, Radix Aconiti, Poria	Tonic prescriptions	Nourishing Yin, clearing heat, calming the mind, and relieving irritability	Bax/CytC/Caspase-3 signaling pathways, p-38/Fas/FasL signaling pathways, TGF-β/Smads signaling pathways, NF-kB signaling pathways	Bcl-2↑, Bcl-xl↑, Bax↓, Bad↓, Fas mRNA↑, FasL mRNA↓, GDF-9↑, EGR-1↑, Smad7↓, VEGF↑, bFGF↑	SPF healthy female SD rats	Maintain the balance between pro-apoptotic and anti-apoptotic proteins and improve ovarian reserve function; improve ovarian blood supply, promote follicle development, reduce follicular atresia and increase the number of mature follicles present in rats.
Yangyin Shugan granule [15]	Radix Bupleurum, Radix Paeoniae Alba, etc.	Mediative formulas	Nourishing Yin and soothing the liver	Bax/CytC/Caspase-3 signaling pathways	Bcl-2↑, Bax↓, Caspase-3↓, FOXO3a↓	SPF healthy female SD rats	It can inhibit apoptosis.
Zuogui pill [17, 18, 21, 41, 48, 64]	Radix Rehmanniae Praeparata, Rhizoma Dioscoreae, Fructus Lycii, Cornu Cervi Pantotrichum, Radix Achyranthes Bidentatae, Semen Cuscutae, antler gum, turtle board gum	Tonic prescriptions	Nourishing Yin, tonifying the kidney, and nourishing the blood	Bax/CytC/Caspase-3 signaling pathways, FOXO3, SCF, TGF-β/Smads signaling pathways	Bcl-2 mRNA↑, Bax↓, FOXO3a↓, CTX↑, Cx43 mRNA↑, Smad2↑, GDF-9↑	Human ovarian granulosa cells; SPF 8-week-old BALB/*c* female mice	It can inhibit chemotherapy-induced granulosa cell apoptosis and protect ovarian function; improve suture junctions; promote recruitment of initiating follicles, promote follicular development, inhibit follicular atresia, and regulate ovarian function.
Erxian decoction [20, 52]	Herba dipteris, Epimedium herba, Phellodendron phellodendron, Euphorbia officinalis, Angelica, Anemone anemone	Tonic prescriptions	Warming kidney Yang, tonifying kidney essence, and dipping kidney fire	FOXO3 transcription factor -P27/Bim	FOXO3↓, P27KiPi↓, Bim↓	Clean-grade SD healthy female rats	Promote granulosa cell proliferation and inhibit granulosa cell regulation; promote the secretion of vascular growth factor associated with premature ovarian failure in rats, and enhance ovarian reserve function.
Bushen Jianpi decoction [29, 30]	Polygonatum officinale, Radix et Rhizoma Polygonatum, Radix et Rhizoma Polygonatum, Fructus Lycii, Atractylodes Macrocephala, Poria	Tonic prescriptions	Tonifying the kidneys and strengthening the spleen	ER/c-myc/TERT signaling pathways	c-Myc↑, TERT↑	B6AF1 female mice	It can promote the proliferation and development of ovarian granulosa cells and the maturation of oocytes.

Table 2: Continued.

Recipe	Compositions	Classification	Efficacies	Pathways	Targets	Experimental model	Roles
Bushen Tiaochong decoction [33, 42, 55]	Cuscuta seed, Euphorbia officinalis, Ripe Rehmannia, Cistanche, yellow essence, amethyst, Schisandra fruit, Angelica, Ligusticum wallichii, etc	Tonic prescriptions	Tonifying the kidneys and regulating the flushing process	P53, SCF, NF-kB signaling pathways	P53↓, GnRHR↑, Cx-37↑, SCF↓, VEGF↑, bFGF↑, TNF-α↓, IFN-γ↓	Clean-grade female SD rats	Prolong the time of apoptosis, reduce the occurrence of follicular atresia, improve the reserve capacity of ovarian receptors and thus coordinate ovarian function; promote the growth and development of early follicles, improve the function of ovarian tissue gap junctions and thus repair ovarian function damage in rats.
Bushen Huoxue decoction [36, 50]	Herba epimedium, Semen Cuscutae, Fructus Lycii, Fructus Lycii, Fructus Morindae, mulberry, raspberry, yam, Radix Codonopsis, Radix Astragali, Radix Paeoniae Alba, Fructus Ulmoides, bark, Salviae Miltiorrhiza, Radix Angelicae Sinensis, Radix Angelicae Sinensis	Tonic prescriptions	Tonifying the kidney and invigorating the blood	Ki67/ERK	p-ERK1/2↑, ERK1/2↑	Mouse intrasinus follicular granulosa cells	It can improve follicular development and increase the proliferation of follicular granulosa cells and granulosa cells of the oocyte complex in mice with reduced ovarian reserve function at an advanced age.
Gui Zhi Fu Ling capsule [40]	Cassia twig, Poria cocos, peony bark, peony root, peach kernel	Blood-regulating formula	Promoting blood circulation, removing blood stasis, and eliminating symptoms	p-38/Fas/FasL	Fas/FasL↓	SPF female Wistar rats	Reduce ovarian apoptosis.
Kun Bao pill [43]	Chasteberry, raspberry, Cuscuta, etc.	Tonic prescriptions	Nourishing the liver and kidneys, calming and tranquilizing the mind, nourishing the blood, and opening the meridians	SCF, TGF-β	CTGF↓, TGF-β1↓, SCF↓	SPF healthy female SD rats	Reduce ovarian fibrosis.

Table 2: Continued.

Recipe	Compositions	Classification	Efficacies	Pathways	Targets	Experimental model	Roles
Siwu mixture [45]	Radix Rehmanniae Praeparata, Radix Angelicae Sinensis, Radix Paeoniae Alba, Ligustici wallichii	Tonic prescriptions	Nourishing Yin and replenishing blood	TGF-β/Smads signaling pathways	Smad2/3↑, Cyp19a1↑	Healthy female SD rats	It can promote follicular growth and development and slow down follicular atresia.
Yiqi Yangrong Fujing formula [54]	Astragalus, Bacopa monnieri, Dendrobium Ferrugineum, Rhizoma Polygonatum	Tonic prescriptions	Benefiting Qi, warming Yang, nourishing Yin, and restoring essence	NF-kB signaling pathways	IL-2↑, TNF-α↑	Healthy female C57BL/6 mice	Inhibit the apoptosis of ovarian granulosa cells, restore ovarian function, and enhance ovarian reserve capacity.
Bushen Culuan formula [61]	Radix chasteberry, Semen Cuscutae, Fructus Lycii, Radix et Rhizoma mulberry, Radix et Rhizoma Sequoiae, Radix Angelicae Sinensis, Radix Paeoniae Alba, Radix Zelenium	Tonic prescriptions	Tonifies the kidneys and enhances the essence	Bax/CytC/Caspase-3 signaling pathways, Keap1/Nrf2/ARE signaling pathways	Akt↓, mTOR mRNA↓, PI3K mRNA↓, Bcl-2↑, Keap1↓, Bach1↓	SPF 8-week-old female BALB/*c* mice	Reverse excessive apoptosis of granulosa cells, reduce follicular atresia, reduce excessive activation of primordial follicles, and protect ovarian reserve; reduce ovarian oxidative damage.
Yiguan Jian decoction [62]	Raw Rehmanniae, Radix Aristophanae, Angelica, Wolfberry, chinaberry	Tonic prescriptions	Nourishing Yin and softening the liver	Keap1/Nrf2/ARE signaling pathways	T-SOD↑, CAT↑, MDA↓, Caspase-3↓	SPF healthy female SD rats	Protect normal ovarian cells.

The effects of single herbs and Chinese herbal formulas on the regulation of the POI signaling pathway are summarized in Tables 1 and 2.

8. Conclusion

Signaling pathways are used as enzymatic response pathways that can transmit molecular signals from outside the cell to the cell in order to function, and the pathogenesis of various diseases involves the participation of related signaling pathways. In recent years, research on TCM interventions in related signaling pathways has been on the rise, especially in TCM to further understand the pathogenesis of POI by studying interventions in the transmission of related signaling pathways. There are numerous pathways related to two major factors of POI: genetics and immunity. PI3K/Akt signaling pathway, TGF-β/Smads signaling pathway, and MAPK signaling pathway also restore ovarian function by regulating apoptosis, oxidative stress response, etc. In conclusion, Chinese herbal medicines alone or in combination can affect the factors related to the development of POI (granulosa cell apoptosis, ovarian blood supply, ovarian reserve, ovarian oxidative damage, gap junctions, ovarian fibrosis, follicular development, follicular atresia, and other biological processes) by regulating one or more signaling pathways, thus playing a role in antioxidative stress, preventing, and controlling the side effects of chemotherapy, protecting ovarian function, controlling the onset of aging, and improving ovarian reserve function. However, most of the current studies on signaling pathways related to TCM interventions are in their infancy, with relatively few studies and uncertain clinical efficacy. Therefore, further in-depth clinical studies are needed in the future, close to the clinic, to provide useful methods for the clinical treatment of POI patients.

Data Availability

The data that support the findings of this study can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare they have no financial interest.

Authors' Contributions

YDW was involved in the design and conduct of the review and drafting of the manuscript. Dr. JL was involved in revising the manuscript. Professor XXT validated the final version for submission and revised the manuscript.

Acknowledgments

This study was funded by Capital Health Development Research Special Project (2022-2-2233).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 4624311, 30 pages
https://doi.org/10.1155/2022/4624311

Review Article

The Treatment of Complementary and Alternative Medicine on Female Infertility Caused by Endometrial Factors

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Received 20 April 2022; Revised 2 July 2022; Accepted 8 August 2022; Published 7 September 2022

Academic Editor: Slim Smaoui

Recently, with the development of the social economy, the incidence of infertility has increased year by year. With its complex etiology and diversified syndromes, infertility has become one of the most important diseases that plague the physical and mental health of women of childbearing age worldwide. Endometrial factors as an important part affecting female reproductive capacity, due to which induced repeated abortion and multiple uterine cavity operations occur, can destruct endometrium, failing to provide a normal implantation environment for zygote, thus resulting in infertility. Many patients failed to achieve expected results after receiving conventional treatments such as hormone therapy, assisted reproductive technology (ART), granulocyte colony-stimulating factor (G-CSF) therapy, and cell therapy, then turn to complementary and alternative medicine (CAM) therapies for help. Aiming at clarifying the effectiveness and mechanisms of CAM therapy in the treatment of infertility caused by endometrial factors, our paper systematically searched and studied present related literature on the PubMed, CNKI, and other databases, focusing on the aspects of clinical application and mechanism explorations and highlighting the therapeutic effects of Chinese herbal medicine (CHM), acupuncture, and moxibustion on such diseases. Moreover, this paper also introduces the CAM treatments of traditional Chinese medicine (TCM) retention enema, neuromuscular electrical stimulation (NMES), photobiomodulation therapy, dietary intervention, and other measures for infertility caused by endometrial factors, in order to provide a reference for subsequent basic research and clinical work.

1. Introduction

Infertility is defined as a multifactorial and complex reproductive system disorder in which a couple fails to establish a clinical pregnancy after 12 months of normal unprotected intercourse [1]. Biological, genetic, reproductive tract infection, lifestyle, and environmental factors are all associated with female reproductive capacity [2–4]. Infertility has affected 8%–12% of couples of childbearing age globally, with the female factors accounting for nearly as much as the male factors [3, 5]. According to the statistics, the incidence of infertility among women of childbearing age is about 14.3% in Western countries, about 25% in developing countries, and even as high as 30% in backward countries [6].

Infertility can be caused by different diseases, such as ovulation dysfunction, fallopian tube diseases, and uterine/peritoneal diseases, among which the uterine/peritoneal diseases account for about 10% of the common causes of infertility [7, 8]. Normal endometrial function is one of the necessary conditions for successful conception of women. However, the abnormalities of endometrium, such as thin endometrium, endometritis, and adenomyosis, can induce the occurrence of infertility, by altering the endometrial glycosylation patterns, adjusting the gene expression level of endometrium-related factors, or enhancing local endometrial estrogen effect, which directly reduce the endometrial receptivity that affects the implantation of zygote [9–12]. For patients with infertility caused by endometrial factors, drug or surgical treatment is often required. Nevertheless, the

gonadotropin-releasing hormone agonists can cause changes in related hormone levels, leading to severe ovarian hyperstimulation syndrome (OHSS) and adversely affecting endometrial receptivity [13, 14]. OHSS is also the most serious complication of ART, which has a high failure rate and even life-threatening effect [15]. G-CSF promotes endometrial thickening and improvement of endometrial receptivity, affects embryo implantation, and fundamentally reduces pregnancy loss [16]. Additionally, cell therapy, such as platelet-rich plasma therapy and stem cell therapy, improves endometrial receptivity and clinical pregnancy rates by inducing the production of endometrial cytokines [17, 18]. G-CSF therapy and cell therapy, as emerging methods for the treatment of infertility in recent years, are costly. And their safety remains to be verified by larger-scale clinical trials. Therefore, many patients resort to CAM for treatment in order to obtain higher efficacy and safety.

Current studies have shown that CAM is widely used for infertile women in the world full of regional and cultural background differences [1]. For instance, CAM was used by approximately 63.5% of infertile patients in South Korea, 51% in Turkey, and 49.6% in Iran [19–21]. Long-term treatment with modern medicine has had a huge negative impact on the patients' psychological status and quality of life. As an important supplement to mainstream medicine, CAM is being increasingly chosen and recognized by growing infertile patients who hope to relieve mental stress and improve fertility outcomes through CAM treatment, for its few side effects and high security [22, 23]. Apart from that, as an important cause of infertility, the treatment of endometrial factors involves many CAM treatment methods, but it is still in the exploratory stage. This article focuses on reviewing the intervention measures and mechanisms of CAM in infertility caused by endometrial factors, in order to provide guidance and reference for further research and treatments.

2. Methods

Literatures related to the effectiveness and mechanism of CAM in the treatment of infertility caused by endometrial factors in the past five years have been systematically searched and studied in the PubMed, CNKI, and other databases. Data from studies on the CAM in the treatment of infertility caused by endometrial factors were gathered, and the efficacy and mechanism of CAM therapy were analyzed.

2.1. Chinese Herbal Medicine. CHM can increase the reproductive capacity of infertile patients in various ways, whose efficacy and safety in treatment have attracted growing attention and recognition [24, 25]. A Korean researcher pointed out that a growing number of infertile couples would choose CHM to treat infertility [26]. CHM can restore the endometrial environment to normal and provide fertile "soil" for zygote by improving endometrial blood circulation, increasing the level of sex hormones in the uterus, promoting endometrial thickening, and affecting the morphology of the uterus [27, 28], thus achieving the goal of treating infertility and normalizing pregnancy.

2.1.1. The Application of Chinese Herbal Medicine in Clinical Practice. In many clinical trials, CHM has been proved to be effective in the treatment of infertility caused by endometrial lesions such as thin endometrium, low endometrial receptivity, adenomyosis, and endometritis.

To investigate the clinical efficacy of Gushen Antai pill (GAP; kidney-tonifying and miscarriage-preventing pill) in the treatment of infertility due to thin endometrium, Wu et al. [29] selected 83 patients with thin endometrial infertility and randomly divided them into two groups, observing the endometrial thickness on the day of mature follicles. The results showed that though the endometrial thickness of the two groups was increased after treatment, the outcome of endometrial thickening in the GAP group was better than that of the control group ($P < 0.05$). Yin et al. [30] divided 60 infertile patients with thin endometrium who had no significant differences in age, weight, duration of infertility, mid-luteal endometrial thickness, and sex hormone levels into two groups to observe the effect of Bushen Huoxue recipe (kidney-tonifying and blood-promoting recipe) on the endometrium. The results showed that the pregnancy rate of the observation group was 43.3%, which was significantly higher than that of the control group ($P < 0.05$), and the endometrial thickness increased from 6.2 ± 2.1 mm to 9.6 ± 1.0 mm, which was significantly higher than that of the control group ($P < 0.05$). Moreover, on the basis of conventional Western medicine, Bushen Yanggong decoction (kidney-tonifying and uterus-nourishing decoction), Yangjing Zhongyu decoction (kidney essence-nourishing and pregnancy-helping decoction), Jinfeng pill (Golden Phoenix Pill), and Bubao decoction (uterus-nourishing decoction) can be used to treat thin endometrial infertility, increase endometrial thickness, and improve pregnancy rate [31–34]. CHM, which has the function of supplementing the kidney, activating blood, and filling essence, can effectively improve the operation and distribution of essence, qi, blood, and body fluid in the uterus, so as to give the endometrium power to grow, which is similar to the promotion of endometrial growth by modern medicine.

In addition to increasing endometrial thickness, CHM can effectively improve endometrial receptivity of infertile patients via various ways [35, 36]. To explore the effect of Danyu decoction (DYD; salvia and Evodia decoction) on endometrial receptivity of infertile patients, Liu et al. [37] randomly divided 50 patients into two groups: the observation group was treated with DYD, while the control group was treated with vitamin E. The final results showed that DYD could promote the endometrium transform to type A, which is easy to conceive and reduce the uterine artery resistance index (RI) and pulsatility index (PI), so as to get better blood perfusion for uterus. The statistical results of pregnancy showed that the pregnancy rate of the observation group was significantly higher than that of the control group ($P < 0.05$). Through clinical observation, Geng et al. [38] concluded that Jieyuyubao pills (JYP; stagnation-relieving and uterus-nourishing pill) could increase the rate of type A endometrium, effectively improve endometrial receptivity, and promote the implantation of zygote, thereby increasing the pregnancy rate. Meanwhile, JYP could also significantly

reduce the liver depression symptoms including chest distension, mental depression, and irritability, and lower the syndrome score. Fang et al. [39] designed a randomized, double-blind, and placebo-controlled trial to investigate the effect of Erzhi Tiangui granule on DNA methyltransferase (DNMT) expression in the endometrium of infertile patients with kidney yin deficiency. The experimental results showed that the expression of DNMT1 in the endometrium of the treated group was significantly higher than that of the placebo control group; besides, the endometrium showed a typical decidual endometrium with normal growth of mesenchyme and glands and normal secretion of glandular epithelium. CHM with kidney-tonifying effect such as Yulin Zhuyun prescription (helping pregnancy and giving birth to fetus prescription) and Kuntai capsule (women's health-promoting pill) can promote the expression of integrin $\alpha v\beta 3$ and GLUT4 on endometrium [40, 41]. As indicators to evaluate endometrial receptivity, the better the endometrial receptivity, the higher the levels of integrin $\alpha v\beta 3$, GLUT4, and DNMT expression. The high expression of these proteins in the endometrium of patients after treatment also indirectly demonstrated the improvement of endometrial receptivity by CHM [42–44]. Xu et al. [45] performed biopsies on the endometrium in 72 patients with infertility due to endometrial lesions and found that the endometrial pinopodes of infertile patients who treated with Wenjing Quyu prescription (essence-warming and blood stasis-removing prescription) were richer in structures, and the area of the endometrial surface covered with pinopodes was more than that of the control group. It is concluded that CHM can effectively increase the growth of pinopodes in the mature stage of endometrium, thus improving endometrial receptivity [46].

Infertility caused by adenomyosis is mostly related to the changes in uterine cavity shape, decreased endometrial receptivity, and local or overall chronic inflammatory response [47, 48]. The treatment of adenomyosis in CHM is mostly based on the direction of qi-blood dialectic, and the methods such as boosting qi, invigorating blood, and dispelling stasis have been widely applied, which can effectively relieve the symptoms of adenomyosis, control local lesions, and improve the pregnancy rate [49]. Shaoyao-Gancao decoction (Paeonia and Licorice decoction), as a classic ancient recipe, is often used to treat gynecological diseases. Clinical experiments have confirmed that both Shaoyao-Gancao decoction and Jiawei Shaoyao-Gancao decoction (supplemented Paeonia and Licorice decoction) can inhibit the migration, proliferation, and differentiation of endometrial cells, prevent the occurrence of adenomyosis, and provide a good intrauterine environment for pregnancy [50, 51]. Pan et al. [52] randomly divided 60 patients with infertility due to adenomyosis into two groups: the control group was treated with urinary follicle-stimulating hormone + chorionic gonadotropin + letrozole, while the observation group was treated with Huayu Xiaozheng prescription (HXP; blood stasis-removing and abdominal mass-resolving prescription) on this basis. After 3 menstrual cycles, the intrauterine spontaneous pregnancy rate in the observation group reached 60%. The uterine volume, endometrial thickness, PI, and RI in the observation group were significantly better than those in the control group ($P < 0.05$). The results suggested that HXP could effectively slow down the rate of uterine enlargement, increase the thickness of endometrium, improve the blood flow of intrauterine artery, and increase the intrauterine pregnancy rate of patients. In addition, Sanjie Zhentong capsule (static blood-resolving and pain-alleviating capsule), Yishen Sanjie Quyu decoction (kidney-invigorating and static blood-resolving decoction), and Wenjing Huoxue decoction (essence-warming and blood-promoting decoction), all of which can improve pregnancy rate and treat infertility caused by adenomyosis via improving TCM syndromes, controlling uterine volume, and helping the recovery of uterine artery blood flow [53–55].

Endometritis means the inflammatory state of the endometrium, which is associated with adverse reproductive outcomes. This status is able to negatively affect the endometrial receptivity and act on detriment to embryonic implantation, thus becoming one of the causes of female infertility [56, 57]. Zhou et al. [58] performed hysteroscopic endometrial biopsies in 58 infertile patients caused by asymptomatic endometritis; they found that the endometrium in the Penning granule (PNG; peaceful pelvic cavity granules) treatment group basically restored the endometrium to normal, vascular proliferation disorder, endometrial edema, and punctate or scattered hemorrhage almost disappeared, the endometrial interstitial lymphocyte infiltration was significantly reduced, and the expression of immunohistochemical index CD38 for the diagnosis of endometritis and the expression of inflammatory factor HIF-α were also significantly decreased. The overall pregnancy rate of 54.55% in the treatment group was significantly higher than that of the control group ($P < 0.05$). The results suggested that PNG could eliminate endometrial inflammation, promote the improvement of endometrial receptivity, and increase the clinical pregnancy rate.

Through searching and reading the relevant literature, we found that the clinical reports of CHM for the treatment of infertility caused by endometrial factors were mainly from Chinese medical institutions, while the related research in Western medical institutions mostly focuses on the mechanism research based on animal experiments, whereas much attention has not paid to clinical research. What's more, the sample size of various clinical studies in China is small and single; most of the clinical studies only focus on the evaluation of clinical efficacy, while less attention has been paid to the study of mechanism (see Table 1).

2.1.2. Therapeutic Mechanisms of Chinese Herbal Medicine. The mechanisms of CHM in the treatment of infertility caused by endometrial factors are relatively complex. The possible mechanisms are summarized in the following four points.

2.1.3. Regulation of Endocrine and Promotion of Endometrial Development. Estrogen and progesterone and their receptors play a key role in the proliferation and transformation of

Table 1: Summary of a randomized clinical trial of CHM.

Prescription	Design	Sample size	Interventions	Main outcomes	References
Gushen Antai pill	RCT	83	Treatment group: estradiol valerate + Gushen Antai pill Control group: estradiol valerate	1. Total efficiency: treatment group—84.85% (28/33); control group—68.00% (34/50) 2. Endometrial thickness after treatment: treatment group—0.73 ± 0.16 mm; control group—0.88 ± 0.18 mm	[29]
Bushen Huoxue recipe	RCT	60	Treatment group: estradiol valerate + Bushen Huoxue recipe Control group: estradiol valerate	1. Pregnancy rate: treatment group—43.3% (13/30); control group—26.7 (8/30) 2. Endometrial thickness after treatment: treatment group—9.6 ± 1.0 mm; control group—8.2 ± 0.4 mm	[30]
Bushen Yanggong decoction	RCT	120	Treatment group: Femoston + Bushen Yanggong decoction Control group: Femoston	1. Pregnancy rate: treatment group—53.33% (32/60); control group—31.67% (19/60) 2. Endometrial thickness after treatment: treatment group—8.19 ± 0.83 mm; control group—7.17 ± 0.82 mm	[31]
Bubao decoction	RCT	115	Treatment group: Bubao decoction + aspirin enteric-coated aspirin group Control group	1. Clinical pregnancy rate: treatment group—47.62%; aspirin group—32.56% 2. Endometrial thickness after treatment: treatment group—10.65 ±3.03 mm; aspirin group—8.87 ± 2.50 mm	[34]
Danyu decoction	RCT	50	Treatment group: Danyu decoction Control group: vitamin *E* capsule	1. Pregnancy rate: treatment group—60%; control group—28% 2. The rate of type A endometrium: treatment group—84%; control group—68% 3. Treatment group: RI—0.55 ± 0.08; PI—1.83 ± 0.61; control group: RI—0.64 ± 0.16; PI—2.19 ± 0.54	[37]
Jieyuyubao pills	RCT	107	Treatment group: Jieyuyubao pills + clomiphene citrate Control group: clomiphene citrate	1. Pregnancy rate: treatment group—24.07%; control group—11.32% 2. The rate of type A endometrium: treatment group—53.7%; control group—35.8% 3. Symptom scores: treatment group—2.1 ± 0.6; control group—5.1 ± 0.6	[38]
Erzhi Tiangui granule	RCT	66	Treatment group: Erzhi Tiangui granule + gonadotropin therapy Control group: gonadotropin therapy + placebo granules	1. Clinical pregnancy rate: treatment group—54.55% (18/33); control group: 30.30% (10/33) 2. Endometrial DNMT1 expression: treatment group—3.31 ± 0.46; control group—2.97 ± 0.49	[39]
Yulin Zhuyun prescription	RCT	150	Combine group: Yulin Zhuyun prescription + clomifene citrate capsules CHM group: Yulin Zhuyun prescription Western medicine group: Clomifene citrate capsules	1. Clinical pregnancy rate: combine group—59.1% (26/44); CHM group—44.2% (19/43); Western medicine group—26.8% (11/41) 2. Integrin $\alpha v\beta 3$: combine group—13.1 ± 2.67; CHM group—13.82 ± 2.04; Western medicine group—7.05 ± 1.37 3. GLUT4: combine group—146.82 ± 21.84; CHM group—113.64 ± 15.62; Western medicine group—112.92 ± 18.54	[40]

Table 1: Continued.

Prescription	Design	Sample size	Interventions	Main outcomes	References
Kuntai capsule	RCT	71	Treatment group: Kuntai capsule + clomiphene Control group: placebo + clomiphene	1. Pregnancy rate: treatment group—25% (9/36); control group—11.4% (4/35) 2. Integrin $\beta 3$: treatment group—1.78 ± 0.226; control group—1.46 ± 0.252	[41]
Wenjing Quyu prescription	RCT	72	Treatment group: Wenjing Quyu prescription + Gn + HCG Control group: Gn + HCG	1. Pregnancy rate: treatment group—59.38% (19/32); control group—12.96% (7/24) 2. Covered pinopode area: treatment group—>50%; control group—<20%	[45]
Huayu Xiaozheng prescription	RCT	60	Treatment group: Huayu Xiaozheng prescription + urofollicle-stimulating hormone + chorionic gonadotropin + letrozole Control group: urofollicle-stimulating hormone + chorionic gonadotropin + letrozole	1. Pregnancy rate: treatment group—60% (18/30); control group—33.33% (10/30) 2. Uterine volume: treatment group—100.27 ± 2.13 cm^3; control group—102.47 ± 3.90 cm^3 3. Endometrial thickness after treatment: treatment group—9.89 ± 044 mm; control group—8.04 ± 1.28 mm	[52]
Yishen Sanjie Quyu decoction	RCT	102	Treatment group: Yishen Sanjie Quyu decoction Control group: Sanjie Zhentong capsule	1. Uterine volume: treatment group—95.46 ± 3.21 cm^3; control group—105.44 ± 4.23 cm^3 2. Symptom scores: treatment group—5.34 ± 1.46; control group—11.21 ± 2.01	[54]
Wenjing Yangxue decoction	RCT	70	Treatment group: Wenjing Yangxue decoction + dydrogesterone tablets + moxibustion Control group: dydrogesterone tablets + moxibustion	1. Uterine volume: treatment group—136.47 ± 23.71 cm^3; control group—152.38 ± 39.67 cm^3 2. RI : treatment group—0.63 ± 0.07; control group—0.66 ± 0.05	[55]
Penning granules	RCT	58	Treatment group: Penning granules Control group: levofloxacin + metronidazole tablets	1. Pregnancy rate: treatment group—54.55% (18/33); control group—20% (1/25) 2. CD38 : treatment group—8.89 ± 7.45; control group—20.12 ± 12.35 3. HIF-1α : treatment group—1.44 ± 0.95; control group—2.55 ± 1.40	[58]

the endometrium and embryo implantation. CHM can promote endometrial development and improve endometrial receptivity by regulating the content of endometrial estrogen and progesterone and the expression of their receptors, providing a good microenvironment for embryo implantation and increasing the success rate of pregnancy in infertile patients [59].

Huang et al. [60] explored the effect of Dingkun pill (DK; women's health-stabling pill) on endometrial receptivity and implantation in mouse by establishing different mouse models, and concluded that DK could promote embryo implantation by promoting the expression of estrogen target genes leukemia inhibitory factor (LIF), lactotransferrin (Ltf), insulin growth factor-1 (IGF-1), and spondin 2 (Spon2), altering the uterine responsiveness to estrogen. In the delayed implantation mouse model, DK could establish endometrial receptivity under the premise of suboptimal endometrial estrogen content and improve embryo implantation rate. The experimental results fully confirmed the value of DK in infertility treatment, especially in infertile patients with poor endometrial response to hormones. Tian et al. [61] studied the effect of Ankun Zhongzi Wan (AZW; women-stabling and pregnancy-promoting pill) on the expression of estrogen receptor (ER) and progesterone receptor (PR) in mice with embryonic implantation disorders. And the experimental results showed that ER and PR were strongly expressed in endometrial epithelial and mesenchymal cells in the AZW group, which were better than the model group ($P < 0.05$). The pregnancy rate, average number of embryos born, and embryo implantation rate in the AZW group were also significantly higher than those in the model group ($P < 0.05$). The experimenters believed that AZW could significantly improve the expression of ER and PR in the endometrium of female mice, promote the implantation and development of blastocysts, and improve the pregnancy rate. In addition, there are other CHM with the functions of

tonifying the kidney, invigorating the blood, and boosting the qi, such as Bushen Yiqi Huoxue recipe (kidney-tonifying, qi-benefiting and blood-promoting recipe), Tiaojing Zhuyun capsule (menstruation-regulating and pregnancy-promoting capsule), and Zhuyun I recipe (pregnancy-promoting I recipe), all of which can increase the serum estrogen and progesterone levels in mice with impaired implantation, enhance the effect of estrogen and progesterone on endometrium, improve the expression of ER and PR, promote the maturation of the endometrium, and provide good conditions for pregnancy [62–64].

2.1.4. Improvement of Endometrial Thickness, Blood Flow, Pinopodes, and Other Endometrial Morphology. TCM holds the belief that "blood is the precondition for women" and emphasizes the application of "blood regulation" in the treatment. CHM can treat infertility caused by endometrial abnormalities from multiple angles and levels by improving blood flow, nourishing the endometrium, and promoting endometrial thickening [65].

In order to investigate the effect of Bushen Tiaoxue granules (BTG; kidney-tonifying and blood-promoting granules) and Kunling Wan (KW; female Poria cocos pill) treatment on vascular function and endometrial receptivity in mice and its mechanism, Lv et al. [66] conducted animal experiments and concluded that BTG and KW could improve endometrial angiogenesis, increase endometrial receptivity, and improve pregnancy rate. The main mechanism of action was that BTG and KW could increase the content of blood collaterals in the uterus, increase blood flow, upregulate the expression of vascular endothelial growth factor A (VEGFA) and CD31 in the endometrium, and promote the increase in the number of mature vessels and neovascularization. It also increased the thickness and area of the endometrium, and improved the number, density, and secretory activity of endometrial glands. Several investigators found that CHM such as Bushen Huoxue recipe (kidney-nourishing and blood-promoting recipe) and Xiaoyao powder (Free Wanderer Powder) could increase the expression of endometrial pro-angiogenic factors such as HIF1α and P38, the signaling pathways of COX-PGE2, JNK MAPK, and PI3K/Akt/eNOS, and the expression of its downstream factors such as MMP2, MMP9, PCNA, and FGF2, and then promote its receptor-mediated endometrial angiogenesis, improve endometrial receptivity, and promote embryo implantation, thereby treating infertility [67, 68]. In addition, some formulas such as Tiaojing Cuyun recipe (regulating menstruation and helping pregnancy recipe) and Wenshen Yangxue decoction (kidney-warming and blood-nourishing decoction) have been shown in animal experiments that they can not only promote the formation of endometrial blood vessels, but also increase the number of well-developed mature pinopodes in the endometrium [69, 70]. Yu et al. [71] used pinopodes as an important morphological indicator of endometrial receptivity to further explore the mechanism of Zhuyun recipe (ZR; pregnancy-promoting recipe) in the treatment of infertility in mice with embryo implantation disorders. The results showed that pinopodes were less in the mouse model group with embryo implantation disorders, and there were only a few microvilli on the surface, which were poorly developed. However in the ZR group, the number of pinopodes increased compared with that in the model group, with prominent shape, clear boundary, and complete development. Therefore, ZR could improve the endometrial environment of mice with embryo implantation disorders and increase the pregnancy rate by improving the morphology of the pinopodes.

2.1.5. Modulation of the Expression of Related Factors and Improvement of Endometrial Receptivity. The normal endometrium allows zygote to implant only during a specific brief period called the "implantation window" [72]. During this period, some molecular markers such as integrin $\alpha v\beta 3$, LIF, and osteopontin (OPN) all play important roles. They have a cycle-specific expression pattern, which are upregulated and highly expressed during the period, and are able to mediate adhesion, migration, and signal transduction between cells, participate in the decidual process of the endometrium, and promote embryo implantation [73–75]. By promoting the expression of these related factors, CHM can improve the receptivity of the endometrium, increase the probability of embryo implantation, and avoid the occurrence of pregnancy failure [76].

Li et al. [77] studied the expression of integrin $\alpha v\beta 3$ in rat endometrium during implantation period and the effect of Yiqixue Buganshen recipe (qi- and blood-tonifying, liver and kidney-nourishing recipe) on the expression of it. They found that the expression of integrin $\alpha v\beta 3$ in rat endometrium was time-dependent and was closely related to the gestation process. Compared with the low expression in the model group, the expression of integrin $\alpha v\beta 3$ in the treatment group was significantly increased ($P < 0.01$). Terawaki believed that LIF played an indispensable role in embryo implantation. According to animal experiments, he found that Tokishakuyakusan, a kind of Japanese Kampo commonly used to treat infertility in Japan, could significantly improve the production of LIF protein and the expression of LIF mRNA in endometrial cells and reduce the decidual dysfunction in rats with implantation disorders, resulting in improving embryo implantation conditions, thus treating infertility [78]. In addition, Zishen Yutai pill (kidney-nourishing and fetus-raising pill), modified Shoutaiwai recipe (modified ex-longevity fetus recipe), and Bushen Huoxue recipe (kidney-nourishing and blood-promoting recipe) have all been proved by animal experiments to upregulate the expression of integrin $\alpha v\beta 3$ and LIF on endometrium to increase endometrial receptivity and embryo implantation rate [79–81].

2.1.6. Inhibition of Inflammatory Response and Restoration of Normal Uterine Environment. Inflammation plays an important role in endometrial diseases, and adenomyosis, endometritis, and other diseases are all related to it [82]. The inflammatory response of the endometrium leads to the disruption of the endometrial microenvironment,

impairment of the embryonic implantation process, and poor placental formation, which ultimately leads to infertility [83].

According to animal experiments, Ying et al. [84] found that Qiu's Neiyi recipe could inhibit the activated MAPK signaling pathway on the endometrium and reduce the expression of inflammatory factors in uterine tissues, thus effectively reducing the inflammatory response in the endometrium. Jing et al. [85] studied the changes in the expression of NF-κB and TGF-β1 in the endometrium of rats with chronic endometritis after the treatment with modified Danggui Shaoyao powder (MDSP; Modified Chinese Angelica and Peony Powder). They found that MDSP could inhibit the endometrial inflammatory response by downregulating the expression levels of NF-κB and TGF-β1 proteins and effectively improve the endometrial receptivity in rats with chronic endometritis. Er-Miao-Fang has been confirmed to exert anti-inflammatory effects by inhibiting NF-κB and MAPK signaling pathway proteins [86]. Moreover, Shaofu Zhuyu decoction (lower abdominal stasis-expelling decoction) has been confirmed to reduce the levels of oxidative stress indicators ROS, malondialdehyde, and inflammatory factors including TNF-α, IL-6, and IL-8, thereby reducing endometrial oxidative stress and inflammatory response in the endometrium [87]. Bushen Tiaochong decoction (kidney-nourishing and Chong channel-harmonizing decoction) has been shown to downregulate the expression of the apoptosis-related factors including caspase-1, IL-1β, and IL-18, and reduce the inflammatory response of the damaged endometrium [88]. Accordingly, it can be seen that CHM plays an important role in treating endometrial inflammation and providing a favorable endometrial environment for embryo implantation.

In general, the mechanism of CHM in the treatment of infertility caused by endometrial factors is rather complicated. In order to provide a better theoretical basis for clinical work, more basic experimental studies are needed to systematically and elaborately clarify the therapeutic mechanism of CHM (see Table 2).

2.2. Acupuncture and Moxibustion Therapy. Acupuncture and moxibustion therapy originated from the "The Yellow Emperor's Inner Classic," having a history of more than 2,000 years. It is guided by the acupoint theory, using filiform needles and mugwort as materials and tools to achieve the purpose of preventing and treating diseases mainly by inserting thin sterile metal needles into specific areas of the body or burning cauterizing mugwort to stimulate certain parts of the body, accompanied by certain therapeutic techniques [89–91].

A retrospective analysis of clinical trials has shown that filiform acupuncture is the most commonly used method in the field of ART, and it is most widely used in clinical practice due to its simplicity and efficiency. Additionally, acupuncture combined with moxibustion and electric acupuncture (EA) are also commonly used methods [92]. As an important branch of CAM, with fewer side effects and higher acceptance, acupuncture has attracted the attention of many clinical practitioners; thus, a series of clinical and basic research has been carried out.

2.2.1. The Application of Acupuncture in Clinical Practice. Acupuncture, as one of the external treatments in TCM, can not only prevent the occurrence of diseases, but also be used as a complementary and alternative treatment after the occurrence of diseases [93]. The history of acupuncture treating infertility can be traced back to 1999. That was the first time researchers found that acupuncture could significantly increase fertility rate, pregnancy rate, and the number of live births [94]. Subsequently, in 2002, Paulus et al. [95] showed that acupuncture could significantly increase the pregnancy rate of patients. As a TCM treatment, acupuncture has the unique advantages of convenience, safety, effectiveness, rapidity, and cheapness, making it attract the attention of many domestic and foreign scholars in the field of reproductive medicine [96]. The normal implantation of embryos is closely related to the endometrium [74]. A meta-analysis showed that acupuncture therapy could safely and effectively improve endometrial receptivity by increasing endometrial thickness, improving endometrial morphology, and improving uterine blood circulation. In addition, acupuncture can also relieve tension and anxiety of infertile patients, thus improving their quality of life and pregnancy outcome [97].

2.2.2. The Curative Effect of Acupuncture. In recent years, acupuncture, as a simple and easy-to-operate CAM, has been gradually accepted by most infertile patients, and its positive effects on endometrium have also been confirmed in many studies. Quantities of studies have shown that acupuncture alone or combined with other treatments is more effective than nonacupuncture in improving endometrial receptivity and increasing endometrial thickness [98–100].

Li et al. [101] selected 60 patients with thin endometrial infertility and randomly divided into the treatment group and the control group, 30 cases in each group. The treatment group was given acupuncture combined with CHM treatment, and the control group was given Western medicine treatment. The results showed that the endometrium in the treatment group was obviously thickening than that in the control group, and the pregnancy rate of the treatment group was also significantly higher than that of the control group ($P < 0.05$). This experiment showed that acupuncture with CHM could effectively treat infertility caused by thin endometrium, promote endometrial growth, improve endometrial receptivity, and increase the clinical pregnancy rate of patients with thin endometrial infertility. In order to observe the effect of acupuncture on the endometrium and pregnancy outcome in patients with ovulatory disorders, Xu et al. [102] randomly divided 60 infertile patients into two groups. The control group received conventional ovulation induction program, and the treatment group was treated with acupoints such as Baihui (DU20), Mingmen (DU4), Geshu (BL17), Guanyuan (RN4), and Qihai (RN6) on the basis of the treatment of the control group. The results

Table 2: Summary of basic studies of CHM.

Prescription	Experimental type	Sample size	Interventions	Main outcomes	References
Dingkun pill	CD1 mice	57	Dingkun pill (DK) group Control group Ovariectomized model group Delayed implantation model group	1. Embryo implantation rate (at 22:00 on D4) : DK-treated group—69% (20/29); control group—28.6% (8/28) 2. Relative mRNA level: estrogen-target epithelial genes (Lif, Ltf) and stromal genes (Igf1, Spon2) were more induced in the DK group compared with the control	[60]
Ankun Zhongzi Wan	Kunming mice	60	Ankun Zhongzi Wan (AZW) group Model group Normal group	1. Pregnancy rate: AZM group—75% (15/20); model group—35% (7/20) 2. ER : AZM group—10.55 ± 5.23; model group—4.31 ± 2.39 3. PR : AZM group—8.73 ± 1.16; model group—2.91 ± 0.78	[61]
Bushen Yiqi Huoxue recipe	Wistar rats	60	Bushen Yiqi Huoxue recipe (BYHR) group Progesterone group Model group	1. Pregnancy rate: BYHR group—66.67%; model group—45.45% 2. P : BYHR group—118.98 ± 10.77; model group—73.62 ± 10.24 3. PR : BYHR group—0.23 ± 0.025; model group—0.14 ± 0.022	[62]
Tiaojing Zhuyun capsule	Kunming mice	48	Tiaojing Zhuyun capsule (TZC) group Model group	1. E2 : TZC group—4.67 ± 1.25; model group—1.33 ± 0.80 2. P : TZC group—102.15 ± 45.74; model group—50.18 ± 31.57 3. ER : TZC group–11.62 ± 3.74; model group—5.26 ± 2.11 4. PR : TZC group—8.07 ± 1.22; model group—2.76 ± 0.84	[63]
Zhuyun I recipe	SD rats	70	Zhuyun I recipe (ZIR) group (7.3 g/kg, 14.6 g/kg) Model group Kidney deficiency and blood stasis group Normal group	1. E2 : ZIR group—21.8 ± 2.73(14.6 g/kg); 19.48 ± 6.05(7.3 g/kg); model group—11.00 ± 3.26 2. P : ZIR group—75.41 ± 19.42(14.6 g/kg); 61.80 ± 14.19(7.3 g/kg); model group—39.88 ± 2.83	[64]
Bushen Tiaoxue granules Kunling Wan	SD rats	113	Controlled ovarian hyperstimulation (COH) model group Bushen Tiaoxue granules (BTG) + COH group (0.82 g/kg, 1.64 g/kg, 3.27 g/kg) Kunling Wan (KW) + COH group (0.46 g/kg, 0.91 g/kg, 1.82 g/kg) Control group	1. Pregnancy rate: COH + BTG (0.82 g/kg)—80% (8/10); COH + BTG (1.64 g/kg)—70% (14/20); COH + BTG (3.27 g/kg)—80% (12/15); COH + KW (0.46 g/kg)—92.9% (13/14); COH + KW (0.91 g/kg)—80% (12/15); COH + KW (1.82 g/kg)—80% (8/15) 2. Markers of blood vessels: the fluorescence intensity and the number of VEGFA and CD31-positive vessels decreased in the COH group, while BTG and KW induced vascularization noticeably compared with the COH group.	[66]
Bushen Huoxue recipe	Kunming mice	146	Control group Bushen Huoxue recipe group Controlled ovarian hyperstimulation (COH) model group Bushen Huoxue recipe (BSHX) group (5.7 g/kg, 11.4 g/kg, 22.8 g/kg) Bushen recipe group (5.7 g/kg) Huoxue recipe group (5.7 g/kg)	1. Pregnancy rate: BSHXR group (5.7 g/kg)—53.33% (10/27); BSHXR group (11.4 g/kg)—57.86% (19/28); BSHXR group (22.8 g/kg)—53.85% (7/13) 2. Markers of blood vessels: HIF1α, VEGFA, and COX2-PGE2 level in the model group was lower than that in the control group, while BSHXR and BSR treatment could improve these levels	[67]

Table 2: Continued.

Prescription	Experimental type	Sample size	Interventions	Main outcomes	References
Xiaoyao powder	Kunming mice	78	Controlled group COH group Xiaoyao (XYP) powder + COH group	Pregnancy rate: XYP + COH groups—65% (13/20); COH group—40% (8/20); controlled group—85% (17/20)	[68]
Tiaojing Cuyun recipe	Kunming mice	120	Control group Embryo implantation dysfunction (EID) model group Progesterone (Prog) + EID group Tiaojing Cuyun recipe (TJCYR) + EID groups (12 g/kg, 24 g/kg, 48 g/kg)	1. The number of implantation sites: Control group—15; EID group—2; TJCYR + EID (12 g/kg)—2; TJCYR + EID (24 g/kg)—10; TJCYR + EID (48 g/kg)—12 2. Pinopodes were well-developed; they were sparse; and they in this group improved significantly following treatment with TJCYR.	[69]
Wenshen Yangxue decoction	Wistar rats	100	Control group Model group Wenshen Yangxue decoction (WSYXD) groups (1.3/100 g, 2.6/100 g, 5.2/100 g)	1. Implantation rate: control group—100%; model group—40%; WSYX D (1.3/100g)—40%; WSYXD (2.6/100 g)—50%; WSYSD (2.6/100 g)—70% 2. Control and high groups: a large number of pinopodes but little short microvilli on the endometrial surface; middle group: pinopodes existed in only parts of endometrium; low groups: no pinopode, but numerous microvilli can be found	[70]
Zhuyun recipe	Kunming mice	139	Control group Ovarian stimulation (OS) model group OS + Zhuyun recipe group (ZYR) Embryo implantation dysfunction (EID) model group EID + Zhuyun recipe group Zhuyun recipe group	1. Pregnancy rate: control group—83.33%; OS model group—6.67%; OS + ZYR group—54.55%; EID model group—18.75%; EID + ZYR group—65.22% 2. OS and EID model group: a moderate number of pinopodes without microvilli; OS + ZYR and EID + ZYR group: abundant fully developed pinopodes	[71]
Yiqixue Buganshen recipe	Kunming mice	180	Control group Model group Treatment group: Yiqixue Buganshen recipe (YQBSR) Model group: 9.10 ± 0.93; Treatment group: 12.60 ± 0.73	1. Blastocyst implantation: control group—13.70 ± 0.67; 2. Integrin $\alpha v \beta 3$ expression in the treatment group was higher than in the model group ($P < 0.05$)	[77]
Tokishakuyakusan	Wistar rats	Not clear	Tokishakuyakusan (TSS) group (1%, 3%) Model group Normal group	1. The number of Implantation: 1% TSS group—11.4 ± 1.4; 3% TSS group—14.7 ± 0.8; model group—9.0 ± 2.1 2. LIF mRNA levels: model group—0.42 ± 0.05; 3%TSS group—2.40 ± 0.93	[78]
Zishen Yutai pills	SD rats	90	Normal group, control group Zishen Yutai pill (ZYP) group	1. Pregnancy rate: control group—40%; ZYP group—70% 2. The mRNA and protein expression levels of LIF in the ZYP group were significantly higher than those in the control group	[79]

Table 2: Continued.

Prescription	Experimental type	Sample size	Interventions	Main outcomes	References
Modified Shoutaiwai recipe	Kunming mice	70	Modified Shoutaiwai recipe (MSTW) group Aspirin group Control group	1. Expression of integrin β3: MSTW group—46.7%; aspirin group—23.3%; control group—0% 2. Expression of LIF mRNA : MSTW group—0.9835 ± 0.0059; aspirin group—0.9793 ± 0.0061; control group—0.9670 ± 0.0103	[80]
Qiu's Neiyi recipe	ICR mice	45	Model group, danazol group Qiu's Neiyi recipe group (5 g/kg, 10 g/kg, 20 g/kg)	1. Qiu suppressed the expression of IL-1β, IL-6, and TNF-α 2. The expression of these proteins was significantly decreased after being treated with qiu and danazol ($P < 0.05$).	[84]
Modified Danggui Shaoyao powder	SD rats	60	Blank group Model group Gynecological Qianjin capsule group Modified Danggui Shaoyao Powder (MDSP) group (6.48 g/kg , 12.96 g/kg, 25.92 g/kg)	1. NF-κB : model group—0.72 ± 0.23; GQJC group—0.59 ± 0.20; MDSP group (6.48 g/kg)—0.60 ± 0.03; MDSP group (12.96 g/kg)—0.56 ± 0.10; MDSP group (6.48 g/kg)—0.46 ± 0.23 2. TGF-β1: model group—2.54 ± 3.88; GQJC group—1.57 ± 1.78; MDSP group (6.48 g/kg)—1.54 ± 1.35; MDSP group (12.96 g/kg)—1.33 ± 1.32; MDSP group (6.48 g/kg)—1.10 ± 1.08	[85]
Shaofu Zhuyu decoction	SD rats	46	Control group Model group Estradiol valerate group Shaofu Zhuyu decoction (SZD) group (144 mg/kg, 288 mg/kg)	Embryo implantation rate: model group—33.33%; estradiol valerate group—55.56%; SZD group (144 mg/kg)—44.44%; SZD group (288 mg/kg)—88.89%	[87]
Bushen Tiaochong decoction	SD rats	60	Normal control group; model group; estradiol valerate group; Bushen Tiaochong decoction group (8.525 g/kg, 17.05 g/kg, 34.10 g/kg)	Compared with the model group, the endometrium of each administration group,caspase-1, IL-1β, IL-18, GSDMD, and their mRNA expression significantly decreased low (from $P < 0.05$ to $P < 0.01$)	[88]

showed that the embryo implantation rate and clinical pregnancy rate of patients in the treatment group were higher than those in the control group ($P < 0.05$), indicating that acupuncture could improve their endometrial receptivity, and increase the embryo implantation rate and clinical pregnancy rate of infertile patients based on the conventional ovulation promotion protocol. Through clinical observation of 60 infertile patients, Wang et al. [103] concluded that acupuncture treatment could effectively reduce the PI and RI of patients, increase endometrial blood perfusion, improve endometrial receptivity, and significantly increase the pregnancy rate. Wang et al. [104] also found that the combination of acupuncture and medicine treatment could significantly increase endometrial thickness, improve uterine blood circulation, and help to improve endometrial receptivity.

In order to observe the clinical efficacy of acupuncture and moxibustion combined with umbilical application with TCM in thin endometrial infertility, Yang et al. [105] randomly divided 126 patients with thin endometrial infertility into a treatment group and a control group: the control group was treated with Western medicine, and the treatment group was treated with acupuncture and moxibustion combined with umbilical application with TCM on the basis of the control group. The acupoints were selected from Guanyuan (RN4), Taixi (KI3), Sanyinjiao (SP6), Shenshu (BL23), Mingmen (DU4), Taichong (LV3), and Xingjian (LV2). The results showed that endometrial thickness, pregnancy rate, and live birth rate in the treatment group were significantly higher than those in the control group ($P < 0.05$). Xu et al. [106] concluded that staged acupuncture with moxibustion treatment could also increase endometrial thickness and promote endometrial growth, thus improving the clinical pregnancy rate.

Studies have confirmed that acupuncture using "Tongyuan acupuncture" at specific acupoints can increase endometrial thickness, improve endometrial receptivity, and improve pregnancy outcome in patients with repeated implantation failure (RIF) of thin endometrium [107]. Xue et al. [108] randomly divided 74 patients with RIF of thin endometrium that are to be underwent frozen-thawed embryo transfer into treatment group and control group. The control group was given oral Western medicine, and the observation group was given acupuncture treatment based on the Tongyuan acupuncture method on the basis of the control group. Acupoints such as Baihui (DU20), Dazhui

(DU14), Qihai (RN6), and Guanyuan (RN4) were selected. After treatment, the clinical pregnancy rate of the treated group was 37.8%, which was higher than the 16.2% in the control group, and the endometrial thickness in the treated group was also higher than that in the control group.

Although a large number of clinical trials have demonstrated the efficacy of acupuncture as a complementary and alternative therapy in the treatment of infertility caused by endometrial factors, most of the clinical experiments have small sample sizes and there is variability in acupuncture manipulation and acupoint selection. Therefore, it is necessary to further expand the sample size, deeply explore the relevant mechanisms of action, and formulate reasonable clinical protocols (see Table 3).

2.2.3. Therapeutic Mechanisms of Acupuncture. Scientific studies have concluded that the insertion of acupuncture into the skin creates a holistic connection with the nervous system, immune system, and endocrine system through meridians [113]. A great deal of studies have shown that the mechanism of acupuncture-assisted treatment for infertility induced by endometrial factors may be related to three aspects of local microcirculation in the uterus, reproductive endocrine, and molecular biology.

2.2.4. Improvement of Endometrial Microcirculation. Studies have shown that acupuncture can inhibit the activity of the central sympathetic nerve, reduce the uterine blood flow resistance, promote local blood circulation and the development of the endometrium, and improve the receptivity of the endometrium by regulating the hypothalamus-pituitary-ovarian (HPO) axis, which has positive significance for embryo implantation and ultimately improves the clinical pregnancy rate and live birth rate [114].

In a clinical trial [109], 120 infertility patients were randomly divided into three groups: acupuncture test group, acupuncture control group, and blank control group. In the acupuncture group, Guanyuan (RN4), Zhongji (RN3), Zigong (EX-CA1), Sanyinjiao (SP6), Guilai (ST29), and Xuehai (SP10) were selected. In the control group, acupoints were selected from Fengshi (GB31), Yanglingquan (GB34),Waiguan (SJ5), and Sidu (SJ9). And the blank control group was treated with nonacupuncture. The results showed that the spiral artery PI, RI, and S/D values in the pregnancy group were significantly lower than those in the nonpregnancy group, and the spiral artery PI, RI, and S/D values in the acupuncture group were lower than those in the blank control group, with a statistically significant difference ($P < 0.05$). Acupuncture can also stimulate the dopamine system in the brain, regulate the entire reproductive system, promote blood circulation in the uterine arteries, and improve endometrial receptivity [115]. Therefore, it is believed that acupuncture can improve endometrial microcirculation mainly by decreasing the uterine spiral artery blood flow index, improving endometrial blood circulation, and increasing endometrial perfusion, thereby increasing endometrial thickness and endometrial tolerance, which increases pregnancy rate in turn [116, 117].

2.2.5. Regulation of Reproductive Endocrine. From the aspect of reproductive endocrine, the endometrium is the target organ of estrogen and progesterone. Sufficient estrogen and progesterone is one important part of endometrium to complete conception, and the functions of estrogen and progesterone are closely related to the expression of their receptors in turn. Liu et al. [118] randomly divided rats into the normal group, the model group, and the acupuncture group in order to observe the effect of acupuncture on embryo implantation in rats with embryo implantation disorders and preliminarily explore its mechanism of action. Acupuncture was performed at acupoints of "Zusanli (ST36)," "Sanyinjiao (SP6)," and "Taichong (LV3)." And the serum levels of estradiol, progesterone, and prolactin, and the expression of PR and prolactin receptors at implantation site were all detected. The results showed that the acupuncture group could significantly increase the serum levels of estradiol and progesterone, as well as the expression of PR on the endometrium, and the implantation rate and the average number of implanted embryos in the acupuncture group were significantly higher than those in the model group ($P < 0.01$), which may be related to acupuncture stimulation that could enrich the expression of ER and PR on the endometrium of rats with embryo implantation disorders at the same time and exert physiological effects on the endometrium.

2.2.6. Regulation of the Expression of Related Proteins and Factors. The normal implantation of zygote is related to specific molecular markers on the endometrial surface [119]. Current studies have found that integrins, VEGF, etc., can be considered as markers of endometrial receptivity [120], and the regulatory effect of endometrium by molecular markers depends on the "hypothalamus-pituitary-ovarian-uterine" reproductive axis. Moreover, studies have also shown that acupuncture can promote the expression of molecular markers by modulating this reproductive axis, thus improving endometrial receptivity and promoting the growth and development of endometrium [121, 122].

Integrins are a class of cell adhesion molecules that widely exist in endometrium, which can be divided into 3 subtypes: $\alpha 1\beta 1$, $\alpha 4\beta 1$, and $\alpha v\beta 3$. The establishment of high endometrium receptivity is based on the simultaneous expression of the three subtypes [122, 123]. Zhang et al. [124] observed the effect of acupuncture on the implantation of blastocysts in rats and found that compared with the clomiphene group, rats in the clomiphene-combined acupuncture group had better endometrium development, and the embryo implantation rate was significantly higher than that of the model group. The investigators suggested that acupuncture may significantly improve the poor endometrial receptivity status caused by clomiphene ovulation treatment by regulating the protein integrin $\alpha v\beta 3$ and its

Table 3: Summary of a randomized clinical trial of acupuncture.

References	Design	Sample size	Interventions	Main outcomes	Acupuncture points
[101]	RCT	60	Treatment group: acupuncture + TCM Control group: estradiol valerate tablets	1. Total efficiency: treatment group—26.7% (8/30); control group—6.7% (2/30) 2. Endometrial thickness after treatment: treatment group—1.071 ± 0.144 mm; control group—1. ± 0.150 mm	Guanyuan (RN4), Sanyinjiao (SP6) Shenshu (BL23), Zigong (EX-CA1)
[102]	RCT	60	Treatment group: acupuncture + letrozole tablets Control group: letrozole tablets	1. Total efficiency: treatment group—66.7% (20/30); control group—40.0% (12/30) 2. Endometrial thickness after treatment: treatment group—10.32 ± 1.77 mm; control group—9.31 ± 1.47 mm	Baihui (DU20), Mingmen (DU4) Geshu (BL17), Ganshu (BL18) Shenshu (BL23), Ciliao (BL32) Guanyuan (RN4), Qihai (RN6) Dahe (KI12), Sanyinjiao (SP6) Gongsun (SP4), Daimai (GB26)
[103]	Single-blind RCT	60	Treatment group: acupuncture + estradiol valerate tablets Control group: estradiol valerate tablets	1. Total efficiency: treatment group—63.33% (19/30); control group—33.33% (10/30) 2. Endometrial thickness after treatment: treatment group—0.98 ± 0.33 mm; control group—0.68 ± 0.22 mm	Zhongwan (RN12), Tianshu (ST25) Daimai (GB26), Guanyuan (RN4) Qihai (RN6), Zhongji (RN3) Zigong (EX-CA1), Xuehai (SP10) Zusanli (ST36), Sanyinjiao (SP6) Taichong (LV3), Mingmen (DU4) Shenshu (BL23), Ganshu (BL18) Yaoyangguan (DU3),Yaoshu (DU2) Guanyuanshu (BL26)
[104]	RCT	90	Treatment group: acupuncture + TCM + aspirin enteric-coated tablets Control group: aspirin enteric-coated tablets	Endometrial thickness after treatment: treatment group—10.59 ± 2.25 mm; control group—5.39 ± 1.00 mm	Pishu (BL20), Shenshu (BL23) Ciliao (BL32), Sanyinjiao (SP6) Shuiquan (KI5)
[105]	RCT	126	Treatment group: acupuncture + moxibustion + TCM + estradiol valerate tablets + progesterone capsules Control group: estradiol valerate tablets + progesterone capsules	1. Total efficiency: treatment group—47.6% (30/63); control group—28.6% (18/63) 2. Endometrial thickness after treatment: treatment group—7.99 ± 1.46 mm; control group—6.21 ± 1.28 mm	Guanyuan (RN4), Taixi (KI3) Sanyinjiao (SP6), Shenshu (BL23) Mingmen (DU4), Taichong (LV3) Xingjian (LV2)

Table 3: Continued.

References	Design	Sample size	Interventions	Main outcomes	Acupuncture points
[106]	RCT	72	Treatment group: acupuncture + warm group acupuncture + EA + estradiol valerate tablets Control group: estradiol valerate tablets	1. Total efficiency: treatment group—50.0% (18/36); control group—33.3% (12/36) 2. Endometrial thickness after treatment: treatment group—9.94 ± 1.04 mm; control group—7.92 ± 1.0 mm	Gongsun (SP4) Neiguan (PC6)
[108]	RCT	74	Treatment group: Tongyuan acupuncture + estradiol valerate tablets Control group: estradiol valerate tablets	1. Total efficiency: treatment group—37.8% (14/37); control group—16.2% (6/37) 2. Endometrial thickness after treatment: treatment group—9.61 ± 0.76 mm; control group—7.72 ± 0.51 mm	Qiangjian (DU18), Naohu (DU17) Dazhui (DU14), Baihui (DU20) Xinshu (BL15), Geshu (BL17) Ganshu (BL18), Shenshu (BL23) Ciliao (BL32), Weizhong (BL40) Yongquan (KI1), Yintang (EX-HN3) Zhongwan (RN12), Tianshu (ST25) Guanyuan (RN4), Qihai (RN6) Luanchao (TF2), Zigong (EX-CA1) Xuehai (SP10), Zusanli (ST36) Sanyinjiao (SP6)
[109]	Double-blind RCT	120	Treatment group 1: acupuncture test Treatment group 2: acupuncture control Control group: no intervention	Total efficiency: treatment group 1—58.69% (27/46) Treatment group 2—38.29% (18/47) Control group—33.33% (9/27)	Treatment group1: Guanyuan (RN4), Zhongji (RN3) Zigong (EX-CA1), Sanyinjiao (SP6) Guilai (ST29), Xuehai (SP10) Treatment group2: Fengshi (GB31), Yinlingquan (SP9) Waiguan (SJ5), Sidu (SJ9)
[110]	RCT	64	Treatment group: EA intervention Control group: no intervention	1. Total efficiency: treatment group—50.00% (15/30); control group—41.94% (13/31) 2. Endometrial thickness after treatment: treatment group—9.03 ± 1.68 mm; control group—9.46 ± 1.67 mm	Baihui (DU20), Zhongwan (RN12) Guanyuan (RN4), Qihai (RN6) Zigong (EX-CA1) Fenglong (ST40) Xuehai (SP10), Sanyinjiao (SP6) Ganshu (BL18), Shenshu (BL23)

Table 3: Continued.

References	Design	Sample size	Interventions	Main outcomes	Acupuncture points
[111]	RCT	80	Treatment group: EA + clomiphene citrate tablets Control group: clomiphene citrate tablets	1. Total efficiency: treatment group—21.1% (8/38); control group—16.2% (6/37) 2. Endometrial thickness after treatment: treatment group—8.21 ± 1.08 mm; control group—6.54 ± 1.12 mm	Qihai (RN6), Guanyuan (RN4) Dahe (KI12), Zigong (EX-CA1) Zhongji (RN3), Diji (SP8) Sanyinjiao (SP6), Shenshu (BL23) Sanjiaoshu (SP6), Ciliao (BL32)
[112]	RCT	80	Treatment group: TCM + EA + aspirin enteric-coated tablets Control group: aspirin enteric-coated tablets	1. Total efficiency: treatment group—40.0% (16/40); control group—27.5% (11/40) 2. Endometrial thickness after treatment: treatment group—8.78 ± 1.67 mm; control group—7.15 ± 1.42 mm	Guanyuan (RN4), Zigong (EX-CA1) Zhongji (RN3), Sanyinjiao (SP6) Xuehai (SP10), Zusanli (ST36) Taixi (KI3), Zhaohai (KI6) Qihai (RN6), Yongquan (KI1)

mRNA expression, a marker molecule protein of endometrial receptivity. VEGF has the ability to stimulate the proliferation and differentiation of endometrial cells, and can affect local angiogenesis in the endometrium directly. He et al. [125] randomly divided early pregnant rats into the normal group (N), model group (M), acupuncture group (A), and nonacupuncture group (C), and acupuncture points of "Zusanli (ST36)" and "Sanyinjiao (SP6)" were taken in the acupuncture group. The results showed that the pregnancy rate and the average number of implanted embryos in group A were significantly higher than those in groups M and C ($P < 0.05$), which may be related to the increase in VEGF expression in the uterus of rats with embryo implantation disorders by acupuncture.

Moreover, a study performed microRNA sequencing on endometrial samples from infertile women who had received acupuncture or not, and then compared the differences in the two DEmiRNAs and predicted their functions. The results showed that DEmiRNAs may be involved in acupuncture treatment through endocytosis, axon guidance, oxytocin signaling pathway, hippopotamus signaling pathway, and estrogen signaling pathway. And hsa-miR-449a, hsa-miR-3135b, hsa-miR-345-3p, and their target genes were also constructed with miRNA-gene network, which jointly affected endometrial receptivity [126]. Additionally, some researchers also conducted high-throughput RNA sequencing and bioinformatics analysis on the samples of patients who had received acupuncture treatment or not, and concluded that acupuncture treatment could play a role in changing endometrial receptivity by regulating the differential expression of circular RNAs in infertility patients [127]. Yuan et al. [128] performed Erbuzhuyu decoction (EBZYD; two-step Evodia decoction) combined with acupuncture on mice, and the results showed that it could promote the expression of endometrial tolerance-related factors and increase blastocyst number and endometrial thickness through activating PI3K/Akt/mTOR signaling pathway, and its treatment effect was superior to using EBZYD or acupuncture alone (see Table 4).

2.2.7. The Application of Electroacupuncture in Clinical Practice. EA is a therapy that applies electric stimulation to the needle to enhance the stimulation effect during the retention of acupuncture after acupoints have received qi. Previous studies have shown that EA stimulation of acupoints such as Zhongji (RN3), Guanyuan (RN4), Sanyinjiao (SP6), and Zigong (EX-CA1) can not only improve endometrial blood flow and endometrial receptivity, but also improve female reproductive capacity by regulating neuroendocrine and immunity [135].

2.2.8. The Curative Effect of Electroacupuncture. Zhong et al. [110] performed EA intervention on in vitro fertilization-embryo transfer (IVF-ET) patients with kidney deficiency and phlegm stasis, and found that EA could improve endometrial blood flow and increase endometrial receptivity, which positively affected pregnancy outcome. Yu et al. [111] randomly divided 80 PCOS patients into EA combined with the Western medicine group and the Western medicine group. In the combined group, Qihai (RN6), Guanyuan (RN4), Zigong (EX-CA1), Dahe (KI12), Sanyinjiao (SP6), Zhongji (RN3), Diji (SP8), Shenshu (BL23), Sanjiao Yu (BL22), and Ciliao (BL32) were used as the main acupoints. The results showed that the endometrial thickness and the rate of type A endometrium were better in the combined group than those in the Western medicine group after treatment. In addition, the serum estrogen and progesterone levels of the patients after treatment were significantly higher than those before treatment. Therefore, it was concluded that

Table 4: Summary of basic studies of the acupuncture and moxibustion.

References	Animal type	Sample size	Interventions	Main outcomes	Acupuncture points
[118]	Wistar rats	60	Normal group Embryo implantation dysfunction model group Acupuncture group	1. Implantation rate: normal group—95% (19/20); model group—45% (9/20); acupuncture group—75% (15/20) 2. PR-positive staining in the acupuncture group was significantly higher than that in the model group.	Zusanli (ST36), Sanyinjiao (SP6) Taichong (LV3)
[124]	SD rats	Not clear	PCOS model group Clomiphene group Clomiphene + acupuncture group Control group	1. Endometrial thickness: model group—$30 \pm 21\,\mu$m; clomiphene group—$20 \pm 27\,\mu$m; clomiphene and acupuncture group—$59 \pm 31\,\mu$m; control group—$85 \pm 23\,\mu$m 2. The expression of ER, PR, HOXA10, LIF mRNA, LIF, and integrin $\alpha v\beta 3$ protein in endometrium of group $C + A$ increased.	Guanyuan (RN4), Zhongji (RN3) Sanyinjiao (SP6), Zigong (EX-CA1)
[125]	Wistar rats	40	Normal group (N) Embryo implantation dysfunction model group (M) Acupuncture group (A) Control group (C)	1. Pregnancy rate: N group—100% (10/10); M group—40% (4/10); A group—70% (7/10); C group—40% (4/10) 2. The expression level of VEGF mRNA in the acupuncture group was significantly higher than that in the model group and the control group.	Zusanli (ST36), Sanyinjiao (SP6)
[128]	C57BL6 mice	50	Blank control group Superovulation model group Erbuzhuyu decoction (EBZYD) group Acupuncture group EBZYD + acupuncture group	1. The endometrial thickness significantly increased in the EBZYD, acupuncture, and EBZYD combined with the acupuncture group compared with the model group. 2. The expression levels of HOXA10 and VEGF significantly increased in the EBZYD + acupuncture group compared with the EBZYD and acupuncture group.	Guanyuan (RN4), Sanyinjiao (SP6) Shenshu (BL23)
[129]	SD rats	40	Control group Thin endometrium model group BMSC group BMSC + EA group	The amount of ER and PR in the BMSC group and the combined group was significantly higher than that in the model group.	Guanyuan (RN4), Zigong (EX-CA1) Sanyinjiao (SP6)
[130]	SD rats	70	Normal group (G) PCOS model group (M) Clomiphene citrate group (CC) Clomiphene citrate + PVG group (CC + PGV) Clomiphene + EA group (CC + A) Clomiphene + Bushen Huoxue recipe group (CC + M) clomiphene citrate + acupuncture + medicine group (CC + M + A)	1. Endometrial thickness: G—$92 \pm 25\,\mu$m; M—$32 \pm 20\,\mu$m; CC—$22 \pm 16\,\mu$m; CC + PGV—$33 \pm 19\,\mu$m; C + M—$32 \pm 20\,\mu$m; CC + A—$52 \pm 23\,\mu$m; CC + M + A—$89 \pm 27\,\mu$m 2. Compared with the model group, the mRNA expression of PR and HOXA10 in each group was higher in CC + A, CC + M, CC + M + A, and CC + PVG.	Guanyuan (RN4) Sanyinjiao (SP6) Zigong (EX-CA1)

Table 4: Continued.

References	Animal type	Sample size	Interventions	Main outcomes	Acupuncture points
[131]	Adult Sprague Dawley rats	60	Control group Thin endometrium model group EA group	1. Pregnancy rate: control group—100% (20/20); thin endometrium model group—20% (4/20); EA group—100% (20/20) 2. A significantly thicker endometrial lining was identified in the EA group than in the model group. 3. The protein and mRNA expression of HBEGF, Itgav, and Itgβ3 was significantly upregulated in the EA group relative to that in the model group	Sanyinjiao (SP6) Zigong (EX-CA1) Guanyuan (RN4)
[132]	SD rats	80	Normal treatment group Controlled ovarian hyperstimulation (COH) model treatment group (Model) Low-frequency EA group (LF-EA) High-frequency EA treatment group (HF-EA)	1. The results showed that the thickness of endometrium in the LF-EA group and the HF-EA group was significantly higher than that in the model group. 2. The expressions of LIF and P-STAT3 in the LF-EA or HF-EA group were evidently higher than those in the model treatment group.	Guanyuan (RN4) Zusanli (ST36)
[133]	Kunming mice	60	Natural cycle group (NC) COH group EA group	After EA treatment, the expression of IGF-1 protein and its mRNA protein in mouse endometrium increased.	Guanyuan (RN4) Zhongji (RN3) Sanyinjiao (SP6)
[134]	SD rats	40	Normal group Endometrial model group Estrogen group Wheat grain moxibustion group	1. Endometrial thickness: normal group > wheat grain moxibustion group > estrogen group > endometrial model group 2. Grain moxibustion can improve endometrial receptivity by upregulating the expression of keratin, vimentin, and VEGF in rats' endometrium, and improving the levels of endometrial receptivity-related factors such as HOXA10 and LIF.	Shenshu (BL23) Guanyuan (RN4)

the combined EA treatment could not only thicken the endometrium and improve its morphology, but also significantly increase the serum levels of estrogen and progesterone, which played a role in improving the endometrial receptivity.

He et al. [112] randomly divided 80 patients with ovulatory dysfunction infertility into the observation group and the control group with 40 patients in each group. The observation group received EA treatment at Guanyuan (RN4), Zhongji (RN3), Zigong (EX-CA1), Qihai (RN6), Sanyinjiao (SP6), and other acupoints until HCG day, while the control group was given aspirin tablets orally from the 7th day of the menstrual cycle until HCG day. The experimental results indicated that the endometrial receptivity-related indicators and pregnancy rate of the observation group were significantly better than those of the control group. Kong et al. [136] performed early intervention of EA on 310 patients, and selected Guanyuan (RN4), Zigong (EX-CA1), Sanyinjiao (SP6), and Taixi (KI3) acupoints for sparse and dense wave therapy. The results showed that endometrial thickness and pregnancy rate all increased, and the curative effect was relatively significant (see Table 3).

2.2.9. Therapeutic Mechanisms of Electroacupuncture. EA has some effects on the endometrium in terms of reproductive endocrinology, genetics, and molecular biology likewise. It can promote the formation of the pineal gland and enhance endometrial receptivity in the thin endometrium model rat through multiple molecular targets [137]. Therefore, infertile patients with thin endometrium can receive complementary and replacement therapy by EA to increase the number of blood vessels and glands in the endometrium, and ultimately achieve the purpose of improving the shape of the endometrium.

Firstly, EA can promote the proliferation of thin endometrial cells and elevate the levels of ER and PR. Meng et al. [129] divided SD rats into a blank group, a model

group, a cell group, and a combined group, with 10 rats in each group. The combined group was given EA on "Guanyuan (RN4)," "Zigong (EX-CA1)," and "Sanyinjiao (SP6)." It was found that the uterine coefficient and the expression of ER and PR in the cell group and the combined group were significantly increased compared with those in the model group, and the expression of PR in the combined group was higher than that in the cell group ($P < 0.05$). According to years of experience in diagnosis and treatment, Dr. Liu often uses low-frequency stimulation during treatment, based on the fact that the main induction site of low-frequency stimulation is in the hypothalamus, which can bidirectionally regulate the function of the HPO axis, thereby increasing the secretion of estrogen, balancing the dynamic relationship between follicle-stimulating hormone and luteinizing hormone, promoting follicle maturation and the release of dominant follicles, improving ovarian reserve function and endometrial receptivity, increasing pregnancy success rate, and improving pregnancy outcome [138].

Secondly, EA can improve endometrial receptivity by affecting the expression of related factors. It was found that the expression of homologous frame gene 10 (HOXA-10) was closely related to endometrial tolerance; namely, a low level of HOXA-10 expression was indicative of low endometrial tolerance [139]. Jiang et al. [130] found that EA combined with Bushen Huoxue recipe could improve the endometrial receptivity by increasing the expression of HOXA-10 on the endometrium through the observation of PCOS rats after ovulation induction. Xi et al. [131] evaluated endometrial regeneration and endometrial receptivity in thin endometrium rats treated with EA, then concluded that EA could increase the formation of pinopodes through multiple molecular targets, improve endometrial receptivity, significantly increase embryo implantation rate, and improve pregnancy outcome. In addition, You et al. [132] suggested that high-frequency EA could enhance endometrial receptivity and promote embryo implantation in ovarian hyperstimulation model rats by enhancing the expression of the LIF/STAT3 signaling pathway. As molecular markers on the surface of the endometrium, IGF-1 and LIF, act on mediating embryo implantation and influencing endometrial morphology and receptivity [140]. Lin et al. [133] found that EA treatment could upregulate the expression of IGF-1 protein in the endometrium of female mice, significantly increase the average number of implantation sites, and improve the pregnancy rate ($P < 0.05$). Fu et al. [141] found that LIF expression was elevated in mice treated with EA, the growth of endometrial glands was promoted, and the pregnancy rate was also improved.

Generally, both conventional acupuncture treatment and EA can effectively treat infertility induced by endometrial factors. In clinical treatment, they have been accepted and recognized by a growing number of doctors and patients. Nevertheless, there is still a need for more clinical and basic experiments to explore more precise curative effects and mechanisms to provide a solid theoretical basis for the better application of acupuncture in the clinical treatment of infertility (see Table 4).

2.2.10. The Application of Moxibustion Therapy in Clinical Practice. Moxibustion therapy refers to a treatment that is commonly applied in clinical practice, using the generated moxa heat mainly by burning the moxa sticks or moxa columns made of moxa leaves to stimulate acupoints or specific parts directly or indirectly, which has a certain effect on diseases with cold pathogens as the main point of syndrome differentiation [142]. It achieves the purpose of disease prevention and treatment by stimulating meridian qi and regulating physiological and biochemical functions of the human body, whose mechanism is similar to that of acupuncture, exists side by side, and plays a part together. Moxibustion has many advantages, such as simple operation, low cost, and remarkable effect. Among them, warm moxibustion, wheat grain moxibustion, medicinal moxibustion, and thunder fire moxibustion are commonly used to improve the endometrium and increase the pregnancy rate in clinical practice.

2.2.11. The Curative Effect of Moxibustion Therapy. (1) Warm Acupuncture. Warm acupuncture is to transmit the heat generated by the burning of the moxa columns to the deep tissue continuously through the needle body on the basis of needle piercing to get qi, which promotes circulation of organs and tissues smoothly and accelerates metabolism. Warm acupuncture can improve the poor endometrial receptivity status to some extent [143]. Liang and Mo [144] selected 92 patients with thin endometrial infertility and divided them into two groups: 46 patients in the control group were treated with Western medicine, while 46 patients in the observation group were treated with warm acupuncture, and their clinical effects were analyzed. The results showed that the curative effect indicators such as endometrial thickness, endometrial type, pregnancy rate, and endometrial blood flow in the observation group were better than those in the control group after treatment, indicating that warm acupuncture could improve the endometrial receptivity and the clinical pregnancy rate effectively in the treatment of infertile patients secondary to thin endometrium. In order to explore the efficacy of treating endometrial infertility with kidney deficiency and blood stasis, Li [145] divided 136 patients who met the inclusion criteria into two groups randomly: the control group was treated with estradiol valerate orally, while the observation group was treated with warm acupuncture combined with Zhenqi decoction on the basis of the treatment of the control group. Then, the endometrial thickness of the two groups was detected. The results showed that the endometrial thickness in the observation group was higher than that in the control group, which further confirmed that warm acupuncture combined with Zhenqi decoction (glossy fruit and wolfberry decoction) could improve the endometrial receptivity, thus increasing the pregnancy rate. Luo et al. [146] used warm acupuncture for pretreatment in order to improve the endometrial receptivity of frozen embryo transfer patients, which found that warm acupuncture may improve these patients' receptivity of endometrium by improving the endometrial morphology and blood flow, improve the

embryo implantation rate and clinical pregnancy rate, and reduce the early miscarriage rate. Su et al. [147] studied the changes of endometrial receptivity of IVF-ET failures who received acupuncture, EA, and warm acupuncture. In the acupuncture group, common acupuncture treatment was applied at the follicular stage after menstruation and stopped at ovulation stage. The EA group was treated with EA on the basis of the acupuncture group. The warm acupuncture group was treated with warm acupuncture on the basis of the acupuncture group. After continuous treatment for 3 menstrual cycles, it was found that the rate of type A endometrium, subendometrial flow type A rate, embryo implantation rate, and clinical pregnancy rate in the warm acupuncture group were significantly higher than those in the other two groups, indicating that warm acupuncture could improve endometrial receptivity of IVF-ET failures more effectively, thereby increasing the success rate of embryo transfer.

(2) Thunder Fire Moxibustion. It is a kind of the moxibustion method that uses moxa and various medicines to compose plant medicine columns according to a certain proportion, which is based on modern anatomy for syndrome differentiation and treatment and supplemented by acupoints. As an integral part of moxibustion, thunder fire moxibustion has been proved to be effective in treating infertility caused by endometrial factors in many clinical experiments.

Thunder fire moxibustion can improve the pregnancy rate of infertile patients with adenomyosis safely and effectively. Chen et al. [148] selected 60 patients with adenomyosis combined with infertility and TCM syndrome differentiation belonged to the diagnosis of cold congealing and blood stasis, and divided them into the treatment group and the control group randomly, with 30 cases in each group. The treatment group was combined with thunder fire moxibustion on the basis of simple ovulation monitoring in the control group. After 6 months, it was found that the TCM syndromes in the treatment group were significantly improved, and the pregnancy rate was statistically significant compared with the control group ($P < 0.05$). Pan et al. [149] applied estradiol valerate combined with thunder fire moxibustion to treat patients with thin endometrium, and conducted a randomized controlled trial (RCT) on 100 patients with thin endometrial infertility. The control group was treated with estradiol valerate, while the experimental group was treated with thunder fire moxibustion on the basis of the control group. The changes in endometrial thickness, as well as the natural pregnancy rate and pregnancy time, were compared between the two groups before treatment and after 1, 2, and 3 months of treatment. And the results showed that after 1, 2, and 3 months of treatment, the endometrial thickness of the experimental group was thicker than that before treatment, and was significantly thicker than that of the control group. The natural pregnancy rate of the experimental group was significantly higher than that of the control group, and the pregnancy time (64.39 ± 11.77) *d* of the experimental group was significantly shorter than that of the control group (96.59 ± 15.34) *d*, suggesting that estradiol valerate combined with thunder fire moxibustion could promote endometrial growth, shorten the pregnancy time, and improve the rate of natural pregnancy.

(3) Wheat Grain Moxibustion. It is to knead moxa velvet into medium wheat grains with two pointed ends, which is glued to the acupoints and lit. When the patient feels unbearable heat, the moxa fire is quickly removed. It has the characteristics of supplementation and purgation, and biphasic regulation [150]. Li et al. [151] divided 80 patients with adenomyosis into two groups randomly. The control group was given levonorgestrel intrauterine contraceptive system treatment, and the observation group was given routine acupuncture and moxibustion with wheat grain. The treatment was performed once a day from 1 week before menstruation until menstrual cramps. The results showed that the uterine volume, endometrial thickness, inflammatory factors, and menstruation of adenomyosis patients were significantly improved after 3 months of treatment with wheat grain moxibustion combined with acupuncture. Xiao et al. [150] conducted a retrospective study on 60 patients with RIF treated with wheat grain moxibustion through data collection and telephone follow-up, and found that the application of wheat grain moxibustion to adjuvant treatment of RIF patients can increase the endometrial thickness on the endometrial transformation day in the hormone replacement cycle, reducing the endometrial preparation time before transplantation and improving the endometrial receptivity, thus increasing the pregnancy rate.

*(4) Sandwiched Moxibustion.*It is to separate moxa columns and skin with medicinal cakes, such as aconite, salt, ginger, and garlic, which have dual effects and mild stimulation on acupoints to produce biological effects of moxibustion and affect tissue and cell metabolism [152]. In the treatment of thin endometrial secondary infertility patients with sandwiched moxibustion combined with acupoint thread embedding, Gao et al. [153] believed that sandwiched moxibustion combined with acupoint thread embedding could not only exert the therapeutic effect of Shenque (RN8), but also prolong the stimulation time of acupoint thread embedding and enhance the therapeutic effect. In addition, Lin et al. [154] found that the use of EA plus ginger-partitioned moxibustion combined with Western medicine could also improve female endometrial receptivity, increase clinical pregnancy rate, and reduce early miscarriage rate.

(5) Other Moxibustion. Yang et al. [155] adopted the method of regulating Chong, boosting qi and invigorating the kidney combined with the Ren Mai moxibustion treatment in 80 patients with thin endometrial infertility, moving slowly between the Shenque (RN8) and Qugu (RN2) acupoints in the middle of the abdomen of the Ren Mai, which has the functions of regulating qi and blood, warming and nourishing the uterus, and promoting the blood circulation of the uterus. When the uterus is full of qi and blood, and the yin and yang are in harmony, pregnancy is achieved through the adjustment of meridians. Tao et al. [156] found that heat-sensitive moxibustion combined with acupoint injection could increase endometrial thickness, reduce uterine artery blood flow resistance, and improve endometrial receptivity. We have listed some RCTs in Table 5.

Table 5: Summary of a randomized clinical trial of moxibustion.

References	Design	Sample size	Interventions	Main outcomes	Acupuncture points
[144]	RCT	92	Treatment group: warm acupuncture Control group: estradiol valerate tablet.	1. Pregnancy rate: treatment group—34.78% (16/50); control group—13.04% (6/50) 2. Endometrial thickness after treatment: treatment group—9.89 ± 2.06 mm; control group—8.02 ± 2.03 mm	Guanyuan (RN4), Zigong (EX-CA1) Zhongji (RN3), Yinjiao (RN7)
[145]	RCT	136	Treatment group: warm acupuncture + Zhen qi decoction Control group: estradiol valerate.	1. Pregnancy rate: treatment group—63.24% (43/68); control group—41.18% (28/68) 2. Endometrial thickness after treatment: treatment group—9.37 ± 1.53 mm; control group—7.49 ± 1.38 mm	Guanyuan (RN4), Zhongji (RN3) Sanyinjiao (SP6), Zusanli (ST36)
[146]	RCT	56	Treatment group: warm acupuncture Control group: antibiotics and flexor progesterone.	1. Clinical pregnancy rate: treatment group—46.3%; control group—20.7% 2. Endometrial thickness after treatment: treatment group—9 ± 2 mm; control group—9 ± 3 mm	Zhongwan (RN12), Tianshu (ST25) Guanyuan (RN4), Zhongji (RN3) Zigong (EX-CA1), Liangqiu (ST34) Zusanli (ST36), Shangjuxu (ST37) Xiajuxu (ST39)
[148]	RCT	60	Treatment group: thunder fire moxibustion Control group: ovulation monitoring	Pregnancy rate: treatment group—50.00% (15/30); control group—23.30% (7/30)	Guanyuan (RN4), Qihai (RN6)
[149]	RCT	100	Treatment group: estradiol valerate and thunder fire moxibustion Control group: estradiol valerate	1. Pregnancy rate: treatment group—40.00% (20/50); control group—20.00% (10/50) 2. Endometrial thickness after treatment: treatment group—10.56 ± 2.88 mm; control group—7.86 ± 2.16 mm	Shenque (RN8), Guanyuan (RN4) Zigong (EX-CA1), Zhongji (RN3) Guilai (ST29), Qihai (RN6)
[150]	Retrospective study	60	Treatment group: wheat moxibustion Control group: hormone replacement cycle intima; corpus luteum.	1. Pregnancy rate: treatment group—33.33% (10/30); control group—23.33% (7/30) 2. Endometrial thickness after treatment: treatment group—9.28 ± 1.15 mm; control group—8.35 ± 1.14 mm	Qihai (RN6), Guanyuan (RN4) Zigong (EX-CA1), Zusanli (ST36)
[151]	RCT	80	Treatment group: conventional acupuncture and wheat grain moxibustion Control group: levonorgestrel	1. Total efficiency: treatment group—97.5% (39/40); control group—82.5% (33/40) 2. Endometrial thickness after treatment: treatment group—6.7 ± 0.7 mm; control group—7.6 ± 0.8 mm	Guanyuan (RN4), Zigong (EX-CA1) Zhongji (RN3), Sanyinjiao (SP6) Diji (SP8), Shiqizhui (EX-B8) Ciliao (BL32)

Table 5: Continued.

References	Design	Sample size	Interventions	Main outcomes	Acupuncture points
[154]	RCT	70	Treatment group: EA + ginger moxibustion isolation Control group: letrozole + HCG	1. Pregnancy rate: treatment group—56.25% (18/32); control group—30.3% (10/32) 2. Endometrial thickness after treatment: treatment group—0.89 ± 0.14 mm; control group—0.78 ± 0.10 mm	Zigong (EX-CA1), Guilai (ST29) Sanyinjiao (SP6), Taichong (LV3) Hegu (LI4), Qihai (RN6) Dahe (KI12), Luanchao (TF2) Zusanli (ST36), Taixi (KI3) Guanyuan (RN4), Shenque (RN8) Wushu (GB27), Zhongji (RN3)
[155]	RCT	80	Treatment group: invigorating qi-tonifying kidney Chinese medicine + Ren Mai moxibustion Control group: estradiol + dydrogesterone	1. Pregnancy rate: treatment group—67.5% (27/40); control group—42.5% (17/40) 2. Total efficiency: treatment group—87.5% (35/40); control group—65.0% (26/40)	Shenque (RN8), Qugu (RN2) Zhongji (RN3), Guanyuan (RN4)
[156]	RCT	210	Treatment group 1: heat-sensitive moxibustion + acupoint injection Treatment group 2: heat-sensitive moxibustion Control group: cefoxitin sodium	Endometrial thickness after treatment: treatment group 1—10.0 ± 0.98 mm; treatment group 2—9.24 ± 0.87 mm; control group—7.89 ± 1.02 mm	Yaoyangguan (DU3) Guanyuan (RN4), Qihai (RN6) Shenshu (BL23), Sanyinjiao (SP6) Yinlingquan (SP9), Zigong (EX-CA1)

2.2.12. Therapeutic Mechanisms of Moxibustion Therapy. Hu et al. [134] found that wheat grain moxibustion and estrogen treatment on "Guanyuan (RN4)" and "Shenshu (BL23)" acupoints could improve the expression levels of keratin, vimentin, VEGF, HOXA-10, LIF, and other related factors, induce angiogenesis, and improve endometrial receptivity by promoting the growth of endometrial epithelial cells and stromal cells; then, the endometrium gets better repaired. Pinopodes are considered to be the morphological markers of endometrial receptivity, whose region and tip are consistent with the implantation position of animal embryos, while the expression level of pinopodes in RIF patients is almost zero [157]. The mechanism of warm acupuncture may be related to its upregulation of endometrial tissue-related proteins and their mRNA levels. By increasing the expression of pinopodes, it can improve hemorheology in RIF patients, thereby improving the poor state of endometrial receptivity and promoting embryo implantation and increasing clinical pregnancy rate [143].

At present, the mechanisms of moxibustion have not been thoroughly studied in domestic and abroad, so more clinical and basic experiments are needed to verify and clarify its exact mechanisms (see Table 4).

2.3. Other Therapies. In addition to the above-mentioned oral CHM and acupuncture therapy, which can be used to treat infertility caused by endometrial factors, there are also some promising therapies, such as TCM retention enema, NMES, and photobiomodulation therapy, which are worthy of being further studied.

2.3.1. Retention Enema of Traditional Chinese Medicine. After suffering from long-term infertility and repeated IVF-ET failures, TCM retention enema therapy has been tried by more and more women due to its unique advantages of trauma, minimal side effects, and simple operation. The special physiological structure of the female rectum adjacent to the uterus can be preserved by enema to promote the drug into the blood circulation and then absorbed through the rectal mucosa, permeating into the pelvic cavity and helping to reduce inflammation [158]. As the basis for implantation of zygote, the thickness and receptivity of endometrium are the key factors for successful implantation of embryos [159].

Pan et al. [160] conducted an RCT of TCM retention enema combined with EA on 60 patients with thin endometrial infertility, and the curative effect is obvious. The result indicated that the combination of the two methods

could improve the local blood flow index of the endometrium, increase the endometrial thickness and receptivity, and then improve the pregnancy rate. As a classical prescription for treating infertility, channel-warming decoction can effectively improve the endometrial morphology of patients and improve the success rate of pregnancy [161], which was validated in a clinical trial of infertile patients with inadequate endometrial receptivity. The control group was given oral estradiol valerate alone, while the treatment group was given channel-warming decoction retention enema on the basis of the control group. After two menstrual cycles, the pregnancy rate in the treatment group reached 63.3%, while that in the control group was 33.3%; the difference was statistically significant ($P < 0.05$) [162]. Clinical enema treatment for infertile patients caused by adenomyosis is based on the theory that rectal administration of drugs can reduce inflammatory infiltration of pelvic tissue and improve the pelvic microenvironment [163]. In addition, TCM retention enema is also effective in treating infertility caused by endometritis. Jia et al. [164] conducted a clinical trial of antibiotics combined with TCM retention enema in patients with IVF failure and endometritis, and found that compared with the control group without any treatment and the experimental group treated only with antibiotics, its clinical pregnancy rate and embryo implantation rate were significantly increased.

2.4. Neuromuscular Electrical Stimulation. NMES is a low-frequency electrical therapy that targets nerve fibers with electrical pulses of different frequencies to activate an electrical potential, and induces nerve or muscle contraction [165, 166]. At present, it is mainly used as one of the means to treat infertility and pelvic floor muscle rehabilitation in female patients, and its application in infertility is mainly aimed at patients with thin endometrium. Zhu [167] explored the efficacy of NMES combined with Kuntai capsule for thin endometrial infertility patients who were treated with long-term estrogen therapy and had poor results. The results showed that the combination therapy could not only effectively increase endometrial thickness, but also regulate sex hormone levels and uterine hemodynamics, thereby improving clinical symptoms. In the treatment of thin endometrial infertility patients, He et al. [168] combined low-intensity focused ultrasound acupoint stimulation on the basis of biomimetic electrical stimulation for treatment. The studies have shown that it could improve endometrial blood perfusion, thus improving the shape and receptivity of the endometrium and increasing the clinical pregnancy rate.

The existing clinical trial results show that NMES is a kind of physiotherapy, which can obtain better curative effect, but its long-term curative effect needs to be further studied.

2.5. Photobiomodulation Therapy. Laser therapy is a photobiomodulation therapy that improves microcirculation by stimulating its own repair mechanisms to promote tissue healing, regeneration, and recovery. After laser treatment of cells, the energy generated is absorbed by it and increases the levels of ATP in cells, triggering and accelerating the rate of cell proliferation and differentiation [169, 170].

Studies have shown that about two thirds of infertile patients with repeated embryo implantation are due to endometrial dysreceptivity [171]. In a prospective randomized trial, Tsai et al. [172] pretreated 29 women with He–Ne laser irradiation before frozen-thawed embryo transfer, and the remaining 31 women did not receive any pretreatment. The results showed that He–Ne laser irradiation could improve endometrial microcirculation and increase endometrial receptivity and pregnancy rate by promoting the release and expression of growth factors and cytokines in the endometrium during implantation. Low-level laser therapy (LLLT) is also a photobiometric therapy that can absorb lasers at the electronic level without producing thermal effects [173]. El Faham et al. [174] conducted in vitro culture of 40 infertile women's endometrium to explore whether LLLT could enhance its proliferative ability. The studies have shown that when the wavelength of LLLT is 635 nm, the expression of endometrial receptivity genes can be induced, and the ability of endometrial cells to differentiate and regenerate is the strongest.

At present, photobiomodulation therapy is still an emerging treatment method in the stage of exploration, which has not been incorporated into mainstream medicine. In addition, the optimal wavelength and duration of laser irradiation are individual, so more comprehensive and systematic studies are needed to clarify the efficacy.

2.6. Improvement of Reproductive Tract Microbiota Disorders. With the rapid development of sequencing technology, more and more studies have been devoted to exploring the microbiota of the female reproductive tract. The female reproductive tract microbiota is mainly composed of bacteria, viruses, and other microorganisms, which distribute in the reproductive tract and participate in the immune and barrier processes of the body. Sequencing of microbial 16S rRNA genes has confirmed that the microbiota colonizes the entire female reproductive tract, which is not limited to the lower reproductive tract [175, 176]. Dysregulation of the microflora can trigger mechanisms such as inflammation and immune responses, which can lead to infertility by affecting embryo implantation [177]. Infertility caused by chronic endometritis is mainly due to the long-term inflammatory state of endometrium caused by the disorders of uterine microflora, which reduces endometrial receptivity and interferes with blastocyst development [178]. Compared with healthy women, infertile patients with chronic endometritis have lower vaginal microbial diversity and abundance, especially Lactobacillus, which determines the embryo implantation rate and pregnancy rate [179–181]. Therefore, many studies have begun to focus on increasing the abundance of Lactobacillus to improve pregnancy outcome in infertile patients, and probiotics have received more and more attention for their anti-inflammatory, immunomodulatory, and maintenance of healthy and safe reproductive system properties [182]. A study conducted by Kyono et al. [183] showed that the simultaneous use of

antibiotics and probiotic supplements could effectively establish Lactobacillus predominance in the endometrium of infertile women to a certain extent, which is of great significance for improving the microbial status of the endometrium. Kadogami et al. [184] found that the probiotic vaginal suppository combined with the antibiotics group had the highest clinical response rate after grouping 329 patients. This prospective study demonstrated that probiotics combined with antibiotic therapy were effective and the Lactobacillus predominance could positively affect pregnancy outcome.

Currently, there are limited data on the mechanism and clinical application of probiotics to improve reproductive tract microbiota disorders, and the problems of treatment standards are still inconsistent. However, with the in-depth research on the microbiota, it is reasonable to believe that probiotics can provide new ideas and methods for the treatment of infertility.

2.6.1. Vitamin D. Vitamin D is a fat-soluble vitamin synthesized by the skin after being irradiated by the solar ultraviolet, including two forms vitamin D_2 and D_3. 25-Hydroxyvitamin D (25(OH)D) is the final product of its cycle, which is also the active form of vitamin D. Vitamin D can not only play an important role in protecting bone and maintaining calcium/phosphorus homeostasis, but has also been found to be expressed in uterus and ovary, as well in more and more studies, indicating that vitamin D is also involved in regulating female reproductive activities [185–189].

In a cross-sectional study, the values of 25(OH)D and endometrial thickness were significantly increased in the pregnancy group after intracytoplasmic sperm injection, suggesting that 25(OH)D deficiency in women could affect endometrial thickness [190]. Ashour et al. [191] supplemented vitamin D to a rat model of vitamin D deficiency and found that vitamin D improved the endometrial receptivity of rats by adjusting the expression of HOXA-10. Additionally, a systematic review has investigated the relationship between vitamin D and the success rate of embryo transfer, and found that women with more adequate vitamin D showed higher clinical pregnancy rate and that higher vitamin D levels could improve the success rate of ART [192]. This will bring more hope to infertile women.

2.7. Dietary Intervention. Undoubtedly, as research progresses deeply, it is found that healthy eating patterns are closely related to female fertility. The Mediterranean diet is favored by women suffering from infertility due to its large intake of dietary fiber, fatty acids, and plant-based proteins [193]. Studies have shown that fatty acids are one of the important substrates in the reproductive process, and linoleic acid belongs to polyunsaturated fatty acids (PUFAs), which mainly affects fertility by improving the receptivity of endometrium and participating in embryo implantation [194–196]. A prospective study showed that North American women with a low intake of omega-3 PUFAs had lower fertility [197]. An RCT found that higher intakes of PUFAs, especially linoleic acid and omega-6 PUFAs, were associated with higher pregnancy rate in infertile women undergoing in vitro fertilization [198]. Appropriate supplementation of B vitamins can also improve endometrial receptivity and affect pregnancy outcome [199]. In addition, phytoestrogens may also have some impacts on female fertility. Soy isoflavones are nonsteroidal compounds present in soy that are similar to endogenous estrogens, which can increase endometrial thickness when given in appropriate doses [200, 201]. Similarly, increasing the intake of whole grains may also increase endometrial thickness, which can help improve pregnancy success [202, 203].

Caffeine intake is also seen as a potential factor affecting female reproductive performance [194]. Qian et al. [204] treated preimplantation mice with caffeine or transplanted normal blastocysts into the uterus of caffeine-treated nonpregnant mice, and both obtained abnormal embryo implantation results. It was suggested that caffeine may lead to impaired endometrial receptivity and pregnancy loss by interfering with the response of the uterine epithelium to steroid hormones. Although the effect and mechanism of caffeine on endometrial receptivity are still unclear at this stage, this study may provide some reference value for it.

2.8. Health Education Intervention. Infertile women often face dual pressure from family and society, and are more prone to tension, anxiety, and even depression. Therefore, it is of great significance to relieve the psychological pressure of patients so as to play an auxiliary role in the treatment of this disease. Health education is mainly to improve patients' cognition of disease and self-behavior management ability and adjust emotions reasonably through social support, empathy, health education, and other ways [205, 206]. In an RCT conducted by Luo et al. [207], 228 infertile women were divided into two groups: the observation group received health education intervention on the basis of clinical treatment, and the control group received general nursing intervention. After 6 months of treatment, it was found that the endometrial thickness of two groups increased, and the curative effect of the observation group was significantly better than that of the control group. The results showed that health education intervention based on clinical treatment could thicken the endometrium better and improve its blood flow indicators. At the same time, it can also improve patients' cognition of the disease, and then improve treatment compliance. Additionally, studies have shown that health education for IVF-ET infertile women and their families can help patients reduce negative emotions, improve their quality of life, and have a positive impact on pregnancy outcome [208]. More and more clinicians realize that health education intervention can help patients establish a good psychological state and improve the pregnancy rate.

In addition, acupoint sticking can stimulate the acupoints and meridians, as well as regulate the qi and blood of Chong and Ren. Combined with the warming effect, it promotes local blood circulation and plays a role in the treatment of infertility caused by adenomyosis [163]. Studies

have shown that the hypoxic microenvironment also affects female reproductive capacity, which can result in failing to provide adequate oxygen to implantation failure after embryo transfer in patients with adenomyosis. However, there are no research reports on the relationship between hypoxic microenvironment and endometrial receptivity in infertile patients with adenomyosis [209, 210].

3. Results

From the data collected so far, CAM has a unique advantage in the treatment of infertility caused by endometrial factors. No matter TCM, acupuncture, or other auxiliary treatment methods, such as TCM retention enema, NMES, photobiomodulation therapy, and dietary intervention, they can all improve endometrial receptivity, increase endometrial thickness, or improve the local or overall inflammatory state of endometrium, then play a better interventional and treatment effect on this disease.

4. Discussion

In conclusion, CAM therapy has certain advantages in the treatment of infertility caused by endometrial factors. Although it is not the main intervention and treatment measure for infertility caused by endometrial factors, it is still being more widely used because it can restore the physiological function of endometrium, improve the pregnancy rate, adjust the psychological state of women, and improve the quality of life. However, CAM also has some limitations and lots of challenges at present.

Firstly, although the effectiveness of CAM in the treatment of infertility caused by endometrial factors has been confirmed by many studies, most of the studies have small sample sizes, which may cause deviations in statistical analysis. Furthermore, high-quality evidence-based evidence is often difficult to obtain, that is why it has not been recognized and promoted by many guidelines.

Secondly, as the main body of CAM in the treatment of this disease, though TCM and acupuncture therapy have obvious advantages, there are some differences in treatment among physicians, such as the composition and dosage of drugs, the choice of acupuncture points, and the frequency and intensity of acupuncture. It lacks high effectiveness from an evidence-based medicine perspective as well. In addition, nondrug CAM therapy also has problems such as inconsistent dosage and lack of standardized guidance, which limit the promotion and application of CAM to a certain extent.

Therefore, in order to solve the problems above better, more high-quality scientific evidence-based studies are urgently needed to confirm the efficacy and safety of CAM in the treatment of infertility caused by endometrial factors. How to explore evidence-based medicine and provide high-quality clinical evidence for it, how to standardize and unify nondrug CAM therapy, and how to accurately and effectively utilize different treatment methods in CAM are still the bottlenecks and challenges faced by CAM in the treatment of infertility caused by endometrial factors at present and for a long time in the future.

Disclosure

Jing Lin, Haoyue Ma, and Hang Li are the cofirst authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant no. 82074259), the Cultivation Project of Outstanding Youth Fund of Heilongjiang University of Traditional Chinese Medicine (Grant no. 2018jc02), and the "Outstanding Young Academic Leaders" Scientific Research Project of Heilongjiang University of Chinese Medicine to Y.Z.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 6350257, 14 pages
https://doi.org/10.1155/2022/6350257

Research Article

Analysis of the Mechanism of GuizhiFuling Wan in Treating Adenomyosis Based on Network Pharmacology Combined with Molecular Docking and Experimental Verification

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Received 6 April 2022; Revised 8 July 2022; Accepted 12 July 2022; Published 26 August 2022

Academic Editor: Yong Wang

Background. The effect of GuizhiFuling Wan (GFW) on adenomyosis (AM) is definite. This study aimed to explore the mechanism and key therapeutic targets of GFW in treating AM through network pharmacology combined with molecular docking and experimental verification. *Materials and Methods*. In network pharmacology, firstly, the active components of GFW, its drug, and disease targets were screened through several related public databases, and GFW-AM common targets were obtained after the intersection. Then, the biological function (Gene Ontology, GO) and pathway (Kyoto Encyclopedia of Genes and Genomes, KEGG) of GFW in treating AM were enriched and analyzed. Finally, the interaction and binding force between key components and key targets of GFW were verified by molecular docking. In the animal part, the effect of GFW on the expression of matrix metallopeptidase 2 (MMP-2), matrix metallopeptidase 9 (MMP-9), and vascular endothelial growth factor (VEGF) in mice with AM was observed by HE staining, ELISA, and immunohistochemistry. *Results*. In this study, 89 active components of GFW, 102 related targets, and 291 targets of AM were collected. After the intersection, 26 common targets were finally obtained. The key active compounds were baicalein, sitosterol, and β-sitosterol, and the key targets were MMP-2, MMP-9, and VEGF. GO and KEGG enrichment analyses showed that biological processes such as the positive regulation of vascular endothelial migration and signaling pathways such as TNF and HIF-1 were involved in regulating angiogenesis, invasion, and metastasis in AM. The molecular docking results showed that baicalein, β-sitosterol, and stigmasterol had better binding potential with MMP-2, MMP-9, and VEGF. The results of in vivo analysis showed that GFW could decrease the serum content and protein expression of MMP-2, MMP-9, and VEGF in mice with AM. *Conclusions*. GFW could reduce the expression of MMP-2, MMP-9, and VEGF, which might be an essential mechanism for GFW to inhibit the invasion and metastasis of ectopic tissues of AM.

1. Introduction

Adenomyosis (AM) refers to a disease in which active endometrial tissue (glands and stroma) appears in the myometrium of the uterus [1]. It is often manifested as abnormal uterine bleeding, dysmenorrhea, subfertility, and other symptoms, seriously affecting the life of patients [2, 3]. Nevertheless, many aspects of AM pathogenesis remain poorly characterized. It is generally believed to be closely related to the invasion and metastasis of the eutopic endometrium to the ectopic lesions caused by the invagination of the endometrial basal layer and the repair of tissue damage [4, 5]. The incidence of AM has gradually increased in recent years, and there is still a lack of effective treatment. The most common treatments in Western medicine are drug therapy and surgical treatment. Drug therapy mainly includes gonadotropin-releasing hormone analogs and oral contraceptives. Although the drugs mentioned above can relieve the symptoms of AM, they are accompanied by a series of side reactions such as irregular vaginal bleeding and a high recurrence rate [6]. Hysterectomy is the primary method for diagnosing and curing AM, but it is difficult for

women of reproductive age to undergo hysterectomy [7, 8]. In recent years, the application of traditional Chinese medicine (TCM) syndrome differentiation therapy and the advantages of multicomponents, multi-targets, negligible side effects, etc., have achieved remarkable results in treating AM, which is an indispensable treatment means [9]. The Chinese expert consensus on the diagnosis and treatment of AM in 2020 clearly stated that TCM could be used to relieve pain caused by AM [10].

GuizhiFuling Wan (GFW), composed of *Cinnamon Twig*, *Poria*, *Red Peony*, *Peach Kernel*, and *Moutan Bark*, is a classic prescription created by Zhang Zhongjing in the Eastern Han Dynasty. With the function of dredging collaterals and relieving stasis, it is often used to treat dysmenorrhea, pelvic pain, menorrhagia, and other blood stasis diseases, and it has obvious therapeutic advantages for AM. Modern pharmacological studies showed that GFW could reduce the expression of vascular endothelial growth factor (VEGF), interleukin-2 (IL-2), and interleukin-8 (IL-8) in serum [11] and was found to be effective in rat models of AM [12, 13]. Wang verified that GFW played a role in treating AM by regulating the estrogen signaling pathway [14]. Our group also performed some research on GFW in treating AM. Through data mining, we found that *Red Peony*, *Poria*, and *Peach Kernel* were used more frequently in treating AM [15]. In vivo experiments in mice showed that GFW could increase the expression levels of caspase-3 and caspase-9, thereby promoting the apoptosis of ectopic intima [16]. Although GFW has become the focus of research, few studies have paid attention to the invasion and migration of AM. Moreover, GFW has the characteristics of multiple compounds and targets, which makes further research difficult. For the past few years, exploring the association between TCM prescriptions and complex diseases using the network pharmacology method to combine TCM prescriptions with molecular biological networks has become a research hotspot; it also provides new ideas for analyzing the compatibility of TCM compounds [17, 18]. However, some deficiencies have gradually emerged with the deepening of network pharmacology research, such as irregular data extraction, false positives in network prediction, and lack of follow-up experimental verification. Referring to the "Guide to Evaluation Methods of Network Pharmacology" [19], on the premise of reliable, standardized, and reasonable data collection and network analysis, this study used the network pharmacology method to predict the complex molecular mechanism of GFW in treating AM. In addition, the AM mouse model was established, which verified the drug substance basis and molecular mechanism of GFW intervention in AM, improved the reliability and rationality of the results, and provided the modern theoretical basis and new research ideas for further digging the target of GFW treatment in AM and subsequent clinical research. The specific process is shown in Figure 1.

2. Materials and Methods

2.1. Network Pharmacology

2.1.1. Active Compounds and Targets of GFW. The chemical constituents of *Cinnamon Twig*, *Poria*, *Red Peony*, *Peach Kernel*, and *Cortex Moutan* in GFW were searched in the TCMSP database (https://tcmspw.com/tcmsp.php) [20] andSymMap database (https://www.symmap.org/) [21]. According to the oral bioavailability (OB) $\geq 30\%$ and druglikeness (DL) ≥ 0.18 [20], the active components and targets of GFW were screened and added to refer to the published literature. Subsequently, the names of the targets were standardized using the UniProt database (https://www.uniprot.org/) [22].

2.1.2. Collection of AM Targets. The keyword "adenomyosis" is entered into the GeneCards database (https://www.genecards.org) [23], the DisGeNET database (https://www.disgenet.org) [24], and the DrugBank database (https://www.drugbank.ca) [25] to obtain AM targets. After removing duplicate targets, AM targets were identified. Then, AM targets were converted to gene symbols using the UniProt database.

2.1.3. Construction of a Network of GFW Active Compounds and a Network of Protein-Protein Interactions (PPI). The active compounds and targets of GFW were uploaded into Cytoscape 3.7.2 (Institute of Systems Biolog, USA) [26] to generate network diagrams. The Venn diagram (https://www.bioinformatics.com.cn) was used to obtain the GFW-AM common targets. Then the common targets were uploaded to the STRING database (https://string-db.org/) [27]. The species selected were "human," and the minimum confidence level was 0.4. The corresponding PPI network files were downloaded, and Cytoscape 3.7.2 was used to analyze the network. Also, the key targets were selected whose nodal degree value was higher than the median.

2.1.4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis. The GFW and AM key targets were imported into the DAVID database (https://david.ncifcrf.gov/) [28] to perform enrichment analysis of GO and KEGG pathways. The species was "human" with $P < 0.05$ as the standard. P values were sorted from small to large, and the results of the GO and KEGG enrichment analyses were selected by P value to determine the top 10 and top 20, respectively.

2.1.5. Molecular Docking Verification. To verify the accuracy of network pharmacology, key components in GFW were selected for molecular docking with key targets of AM. The mol2 structures of the ligands were taken from TCMSP, and the 3D structures of the receptors were taken from UniProt (https://www.uniprot.org/). Ligands and receptors were optimized using ADFRsuite and converted to pdbqt structures. Then, the binding potential of ligands and receptors was evaluated using the molecular docking of Autodock Vina [29]. The smaller the binding energy, the more stable the binding of ligands and receptors. When the binding energy is less than -5.0 kcal/mol, the ligands and receptors are considered to have a better binding capacity [30].

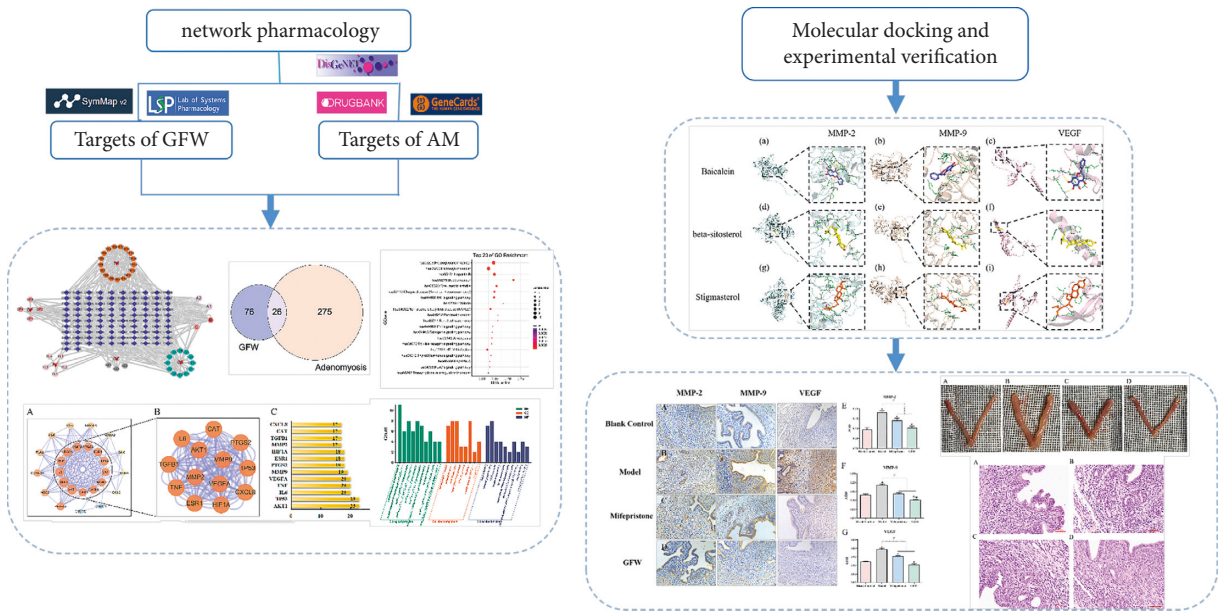


Figure 1: The flow chart of the study.

2.2. Experimental Verification

2.2.1. Animals. The experiment was approved by the Ethics Committee of Affiliated Hospital of Shandong University of Traditional Chinese Medicine (approval number AWE-2019-010). A total of 15 healthy SPF 6- to 7-week-old institute of cancer research (ICR) mice, including 10 female nulliparous mice and 5 male mice, were obtained from Beijing WeitongLihua Laboratory Animal Technology Co., Ltd. (License number: SCXK, Beijing 2016-0006). Raising environment: all mice were raised in the SPF experimental animal center of Shandong University of Traditional Chinese Medicine. They were housed in standard cages with a 12/12-hour light-dark cycle (lights on from 08:00 to 20:00) and had free access to food and water. Indoor temperature was controlled at $24 \pm 2^{\circ}$C and relative humidity was 60%–65%.

2.2.2. Experimental Drugs. Chinese patent medicine GFW (6 g/pill) was provided by Shanxi Tiansheng Pharmaceutical Co., Ltd. (batch number Z14020791); mifepristone (25 mg/tablet) was manufactured by Zhejiang Xianju Pharmaceutical Co., Ltd. (batch number 0950347); tamoxifen citrate (10 mg/tablet) was manufactured by Shandong Health Pharmaceutical Co., Ltd. (batch number H37022925); baicalein reference standards (HPLC $\geq$ 98%, batch number SS8010), β-sitosterol reference standards (HPLC $\geq$ 98%, batch number SS8580), stigmasterol reference standards (HPLC $\geq$ 97.5%, batch number SS8710) were provided by Beijing Solaibao Technology Co., Ltd.

2.2.3. Experimental Chemicals. Enzyme-linked immunosorbent assay (ELISA) kit: matrix metalloproteinase-2 (MMP-2, NO.JYM0019Mo), matrix metalloproteinase-9 (MMP-9, NO.JYM0737Mo), and vascular endothelial growth factor (VEGF, No. JYM0258Mo) were provided by Wuhan Genemei Biotechnology Co, Ltd; immunohistochemical reagents: goat serum blocking solution (EE0008), goat anti-rabbit IgG ($H+L$) HRP (EF0002), endogenous peroxidase blocking solution (EE0007), sodium citrate antigen retrieval solution (50x) (EE0005), and hematoxylin and eosin staining kit (EE0012) were provided by Shandong Sikejie Biotechnology Co, Ltd.; antibody against vascular endothelial growth factor (anti-VEGF, bs-1313R), matrix metalloproteinase-2 antibody (anti-MMP-2, bs-4605R), and matrix metalloproteinase-9 antibody (anti-MMP-9, bs-4593R) were provided by Beijing Boaosen Biotechnology Co., Ltd.

2.2.4. Analysis of Baicalein, β-Sitosterol, and Stigmasterol with High-Performance Liquid Chromatography (HPLC) Method. Baicalein, β-sitosterol, and stigmasterol reference standards were accurately weighed, respectively, and ultrasonically dissolved with the appropriate amount of methanol to yield a reference substance reserve solution. The baicalein, stigmasterol, and β-sitosterol reference solution was measured and diluted with methanol to produce a series of standard solutions. For chromatographic separation, an Agilent Zorbax SB-C18 column (250 mm 4.6 mm, 5 m) was employed. Baicalein was analyzed at a flow rate of 1.0 mL/min using a solvent consisting of 60 : 40 (v/v) methanol and 0.4% phosphoric acid in water solution. The injection volume was set to 10 μL, the column temperature to 30°C, and the detecting wavelength to 270 nm. For the analysis of stigmasterol and β-sitosterol, the following conditions were used: a flow rate of 1.0 mL/min, an injection volume of 10 μL, a column temperature of 30°C, and methanol as the mobile phase. The detecting wavelength was 210 nm. The linear regression of peak area (Y) to concentration (X) showed that the standard curve equation of baicalein was $Y = 46.218X + 0.421$ ($R^2 = 0.9992$), and the linear range of

baicalein was 3.7~370 μg/mL. The standard curve equation of stigmasterol and β-sitosterol was $Y = 81.364X\text{-}0.747$ ($R^2 = 0.9997$) and $Y = 132.027X + 1.245$ ($R^2 = 0.9993$), respectively. The linear range of stigmasterol and β-sitosterol was 5.1~510 μg/mL and 2.4~240 μg/mL.

2.2.5. Construction, Grouping, and Dosing. AM models were induced by tamoxifen citrate [31, 32]. Female mice and male mice were randomly paired in a 2:1 ratio [33]. And 40 female mice were randomly selected on days 2–5 after birth. 5 μl/g peanut oil/lecithin/condensed milk mixture (volume ratio 2 : 0.2 : 3) was fed and 2.7 μmol/kg tamoxifen was added according to body weight. Ten female mice (randomly selected) were simultaneously administered the same amount of the solvent without tamoxifen as the blank control group. After 2 months, 10 model mice were randomly selected to examine uterine tissue. The hematoxylin and eosin (HE) staining showed that the structure of the endometrium was disrupted and the glands and stromal cells had invaded the myometrium, confirming that the modeling was successful [34]. The model mice were randomly divided into the model group ($n = 10$), the mifepristone group ($n = 10$), and the GFW group ($n = 10$). In addition, 10 mice were set as the blank control group. The daily dose of mice in each treatment group was converted according to the formula equivalent dose converted by body surface area between humans and animals. The daily dose of the mifepristone group was 3.2 mg/kg, and the GFW group was 0.8 g/kg, once daily, for 30 consecutive days. After the procedure, mice were anesthetized by an intraperitoneal injection of 1% sodium pentobarbital at a bodyweight of 50 mg/kg, and blood was collected by puncture of the main abdominal vein.

2.2.6. Histomorphological Observation and HE Staining. Firstly, the changes in the uterus of the mice were visually observed, whether the surface is smooth and whether nodules are present. Then, the uteri of the mice were fixed with 4% paraformaldehyde and embedded in paraffin, and the paraffin sections were stained with HE. The changes in the endometrium and myometrium of the mice in each group were observed under the microscope [35].

2.2.7. ELISA Experiments. After blood collection, the blood was to stand at room temperature for 30 minutes and then centrifuged at 3000 rpm/min for 15 minutes. Serum MMP-2, MMP-9, and VEGF were determined using an ELISA kit (operation method refer to instructions manual) [36].

2.2.8. Immunohistochemical Analysis. Uteri were fixed with 4% formaldehyde, embedded in paraffin, and then routinely sectioned, deparaffinized, hydrated, treated with antigen retrieval solution, and blocked with serum. The sections were incubated overnight at 4°C with anti-MMP-2 (rabbit polyclonal, 1 : 200), anti-MMP-9 (rabbit polyclonal; 1 : 200), and anti-VEGF (rabbit polyclonal; 1 : 200) primary antibodies. Then incubated with goat anti-rabbit IgG (1 : 200) secondary antibody, stained, and mounted. Under a 200x microscope, 5 images were randomly selected to observe the positive expression of MMP-2, MMP-9, and VEGF. Image Pro Plus 6.0 image analysis software (Media Cybernetics, USA) was used to analyze the images of 5 different areas in each group. The average integral optical density (AOD) was determined by measuring the covering epithelial area and the integral optical density (IOD) of positive expression in that area. The greater the AOD, the higher the degree of positive expression.

2.2.9. Statistical Analysis. SPSS 21.0 (IBM Corporation, USA) and GraphPad Prism 9.0 (GraphPad Software, USA) statistical software were used for analysis and graphing. Measured data were expressed in terms of mean ± standard deviation ($\overline{x} \pm s$), and comparison between multiple groups according to the normal distribution was performed by one-way analysis of variance (one-way ANOVA). The Kruskal–Wallis H rank-sum test was used to compare multiple groups with nonnormal distribution, and $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Results of Network Pharmacology

3.1.1. Selection of Active Compounds and Targets. After searching the TCMSP and SymMap databases, 494 GFW compounds were tentatively identified. Under the conditions of OB ≥ 30% and DL ≥ 0.18, a total of 89 active compounds were obtained, including 8 compounds from *Cinnamon Twig*, 16 compounds from *Poria*, 11 compounds from *Cortex Moutan*, 25 compounds from *Peach Kernel*, 29 compounds from *Red Peony*. Among them, hederagenin is the common active compound of *Poria* and *Peach Kernel*; (+)-catechin and sitosterol are the common active compounds of *Cinnamon Twig*, *Cortex Moutan*, and *Red Peony*; β-sitosterol is the common active compound of *Cinnamon Twig*, *Peach Kernel*, and *Red Peony*. After removing duplicate targets, 102 related targets were obtained. Combining the results, sitosterol, hederagenin, β-sitosterol, and (+)-catechin were widely distributed and abundant, which could play a better role in treating AM. The study also provides a reference for an in-depth and detailed investigation of the GFW.

3.1.2. Screening of AM Targets and Acquisition of Common Targets of GFW-AM. After searching for AM genes in GeneCards, DisGeNET, and DrugBank databases, 301 targets were identified, and the Venn diagram was constructed based on the intersection of AM and GFW targets. Finally, 26 common targets of GFW in AM were found to be potential targets for therapeutic effects (Figure 2).

3.1.3. Construction of an Active Compound-Target Network of GFW and PPI Network Analysis. Cytoscape 3.7.2 was used to construct the network of the active compounds and targets of GFW (Figure 3). The network showed that 152

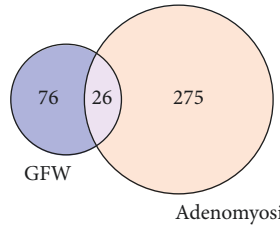


Figure 2: Venn diagram of GFW-AM common targets. The pink circle indicates the AM targets, the blue circle indicates the GFW target, and the intersection of the two circles is the common targets.

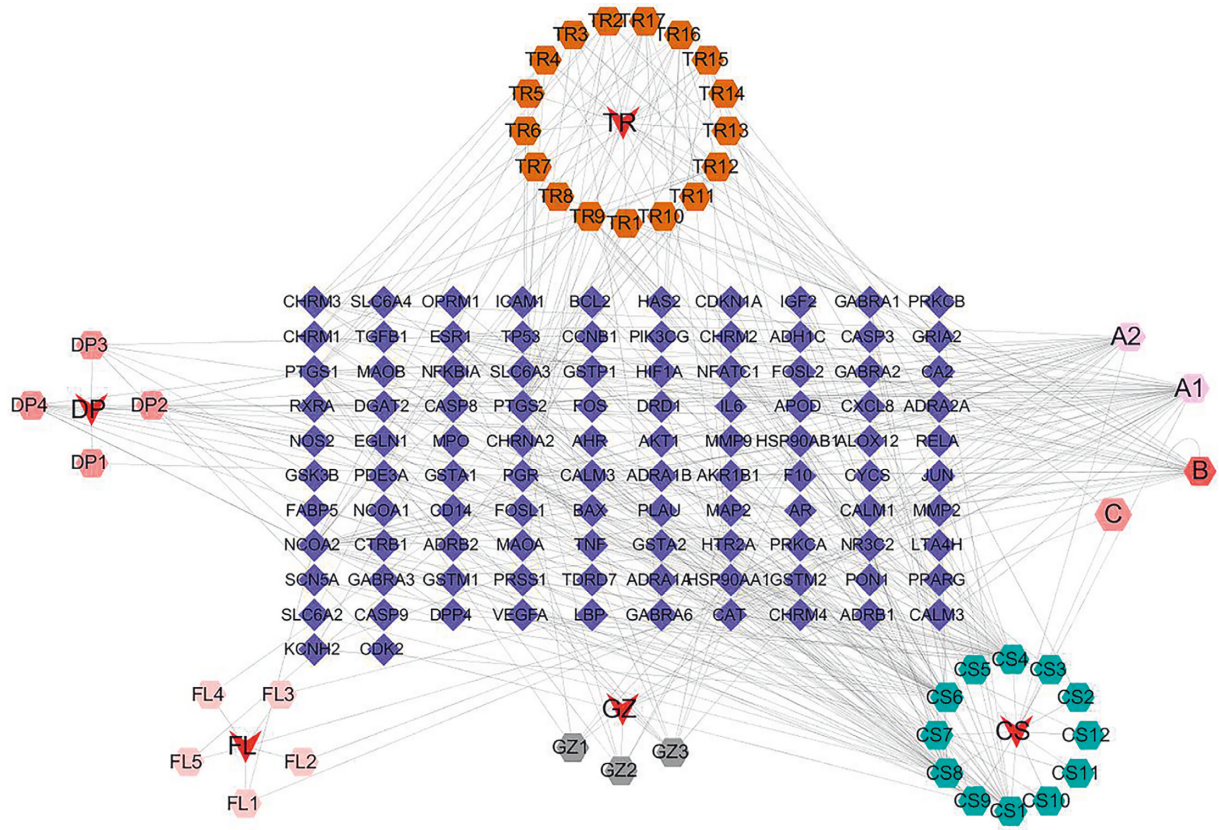
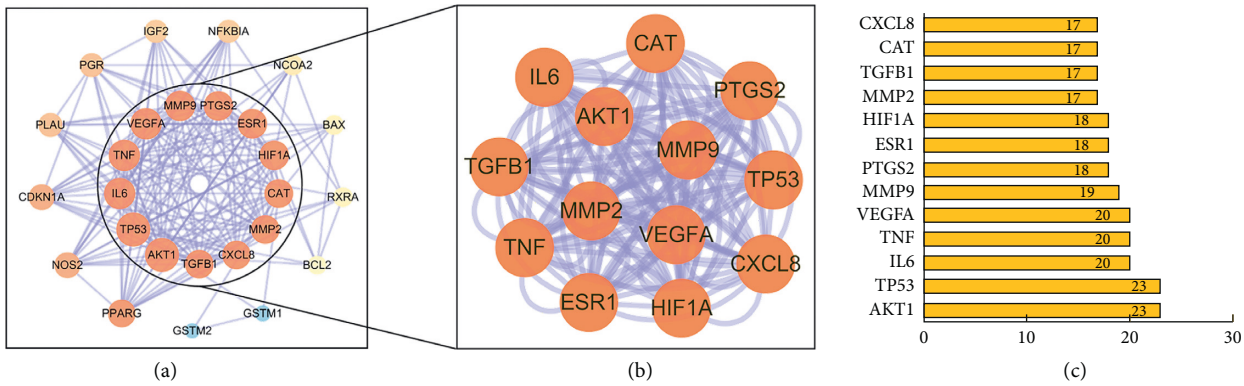


Figure 3: Active component-target network diagram of GFW. The red nodes represent 5 traditional Chinese drugs, the blue nodes represent the targets, and the other colors represent the compound ingredients of different drugs. A1 and A2 are the active compounds shared by *Cinnamon Twig*, *Cortex Moutan*, and *Red Peony*; B is the active compound shared by *Poria* and *Peach Kernel*; and C is the active compound of *Cinnamon Twig*, *Peach Kernel*, and *Red Peony*.

nodes were connected by 390 edges, with the red nodes representing 5 traditional Chinese drugs, the blue nodes representing the targets, and the other colors representing the active compounds of the different drugs. It was found that there was a multilevel and multidimensional correspondence relationship between the active compounds and the targets of GFW, which formed a complex network system. Among them, the active compounds with a high degree of GFW are baicalein (degree = 38), β-sitosterol (degree = 36), stigmasterol (degree = 28), ellagic acid (degree = 21), and (+)-catechin (degree = 15). Possibly via common targets such as AKT1 (degree = 23), VEGFA (degree = 20), MMP-9 (degree = 19), and MMP-2 (degree = 17) play a role.

To investigate the specific mechanism of GFW in treating AM, we constructed a PPI network of common targets using the STRING database and Cytoscape 3.7.2 (Figure 4(a)). Consisting of 26 nodes and 185 edges, its average node degree is 15.5, and its avg. local clustering coefficient is 0.805. Nodes with different colors reflect the degree values of distinct targets by using Cytoscape 3.7.2 to analyze the PPI of common targets. The darker the color, the greater the degree values, and the blue edges represent the interaction between the targets. AKT1, TP53, IL6, TNF, VEGFA, MMP9, PTGS2, ESR1, HIF1A, MMP2, TGFB1, CAT, and CXCL8 were key targets with degree values $\geq$ median (Figure 4(b)-4(c)). The 13 key targets might play a critical role in GFW treating AM.



(a) (b) (c)

Figure 4: (a) PPI network of GFW-AM; (b) key targets of GFW-AM. Nodes size and color are proportional to degree values and combined scores. (c) Degree values of key targets.

3.1.4. Results of GO and KEGG Enrichment Analyses. The DAVID database was utilized to conduct GO enrichment analysis on the key targets to further explore the mechanism of GFW in treating AM. A total of 188 biological functions and 53 signaling pathways were enriched with $P < 0.05$, including 162 items in the biological processes (BP), 18 items in the molecular functions (MF), and 8 items in the cell component (CC). The top 10 GO enrichment showed were chosen for study, and the P values were ranked from small to large (Figure 5). The results revealed that the BP was primarily related to positive regulation of vascular endothelial cell migration, inflammatory response, and immune response during the GFW process in treating AM. The MF is mainly comprised of cytokine activity, enzyme binding, and transcription factor binding, while the CC is mostly made up of the cytoplasm and nucleus.

KEGG enrichment analysis contained 53 signaling pathways. The top 20 signaling pathways were chosen to show the KEGG enrichment analysis through the bubble chart based on the size of the P value (Figure 6). The results revealed that the Toll-like receptor signaling pathway, the tumor necrosis factor (TNF) signaling pathway, and hypoxia-inducible factor-1 (HIF-1) were all related to the GFW in treating AM.

3.1.5. Molecular Docking Verification. Because the pathogenesis of AM is related to ectopic intimal invasion, metastasis, and abnormal angiogenesis, studies indicated that MMP-2, MMP-9, and VEGF were the primary targets involved in ectopic lesion invasion and metastasis [37]. As a result, baicalein (degree = 38), β-sitosterol (degree = 36), and stigmasterol (degree = 28) were chosen as the top 3 components in GFW based on the degree values. MMP-2, MMP-9, and VEGF, involved in invasion and metastasis, were selected for molecular docking to verify the tightness of the binding between the active compounds and the targets. AutoDock Vina performed the molecular docking. The higher the absolute values of the docking results, the greater the probability of playing a role. The absolute values of the binding energies of baicalein, β-sitosterol, and stigmasterol to MMP-2, MMP-9, and VEGF were all greater than 5.0 kcal/mol, according to molecular docking results. Table 1 shows the unique docking binding energies, and Figure 7 depicts the 3D diagrams of the docking. It can be noted that active compounds like baicalein, β-sitosterol, and stigmasterol, and targets like MMP-2, MMP-9, and VEGF may be important to prevent migration and invasion.

3.2. Experimental Results. Despite its benign nature, AM has the ability to migrate and invade in a manner similar to malignant tumors. MMP-2, MMP-9, and VEGF are all involved in AM invasion and migration [37]. The results of network pharmacology indicated that the key targets of GFW in the therapy of AM were MMP-2, MMP-9, and VEGF, all of which were involved in cell invasion, migration, and angiogenesis. GFW had been proven in the study to decrease matrix metalloproteinase (MMP) levels in endometriosis (EM) rats and to limit the invasion and migration of ectopic endometrium [38]. However, no relevant research based on network pharmacology and experimental verification demonstrates that GFW inhibits ectopic endometrial invasion and migration in AM. Additionally, network pharmacology prediction has several drawbacks. To validate the accuracy of network pharmacology, MMP-2, MMP-9, and VEGF were chosen for in vivo verification because they were all associated with invasion and migration among the key targets.

3.2.1. Determination of Baicalein, β-Sitosterol, and Stigmasterol by HPLC. In the chromatogram, baicalein, β-sitosterol, and stigmasterol were all baseline separated from other ingredient. The contents of those components are baicalein 5.103 ± 0.12 mg/g, stigmasterol 1.867 ± 0.09 mg/g, and β-sitosterol 2.014 ± 0.21 mg/g (Figure S1).

3.2.2. Histomorphological Observation. After 30 days of intervention, uteri were taken from the mice (Figure 8). The uteri in the blank control group were light red, smooth, and free of apparent nodules (Figure 8(a)); those in the model group had varying degrees of uterine congestion, thickness, local swelling, and nodules (Figure 8(b)). The uteri in the mifepristone group were somewhat thickened but had no

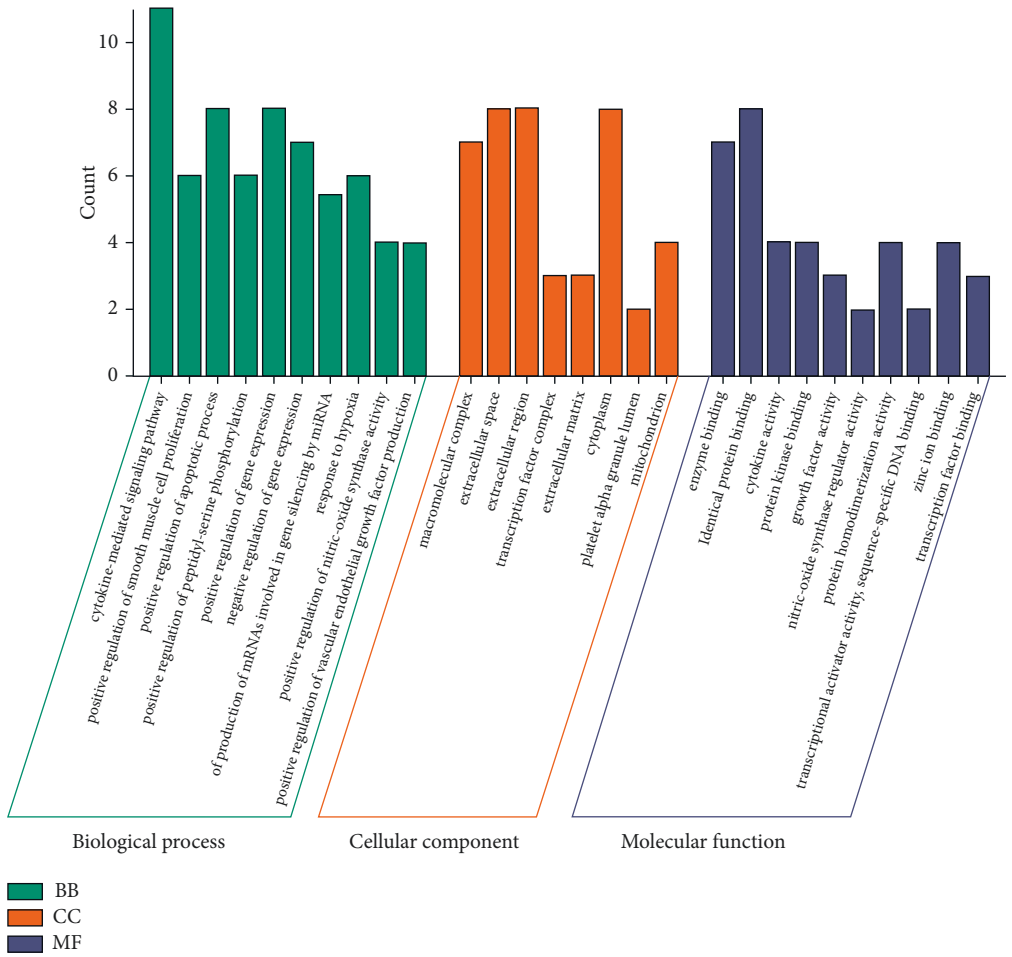


Figure 5: GO enrichment analysis of GFW in treating AM. Green bars represent the BP enrichment process, red bars represent the CC enrichment process, and blue bars represent the MF enrichment process.

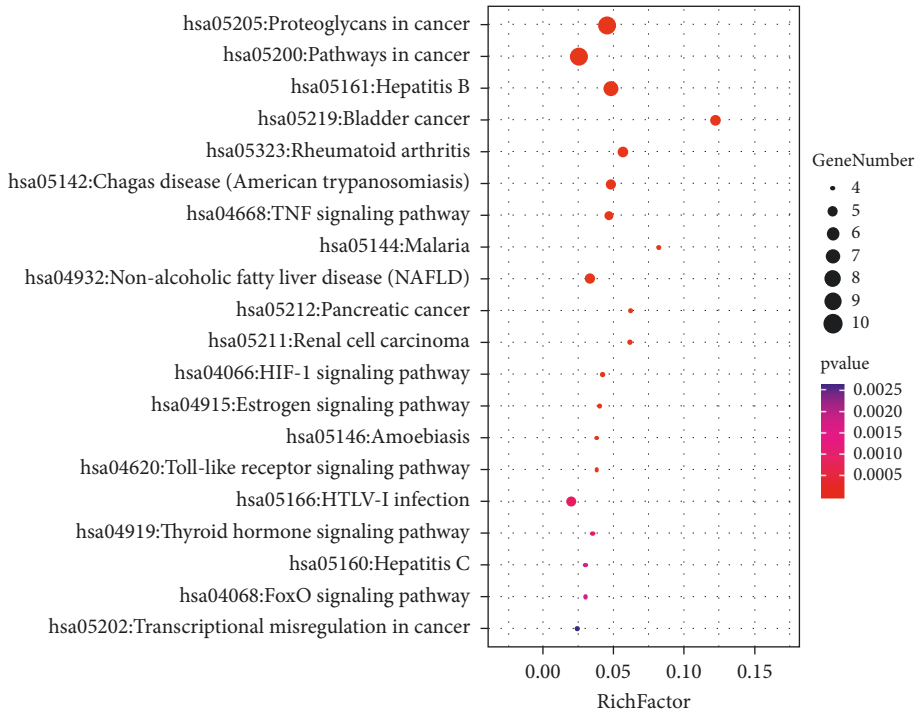


Figure 6: KEGG enrichment analysis of GFW in treating AM. The *x*-axis represents the enrichment factors. The *y*-axis represents the pathway names. The size of the bubbles represents the number of genes; the larger the bubble, the more the number of genes. The color of the bubbles represents the *P* value; the redder the color, the smaller the *P* value.

Table 1: Minimum binding energies for key compounds and key targets.

Key targets	UniProt ID	Key compounds	Binding energy (kcal/mol.)
MMP-2	P08253	Baicalein	−7.9
MMP-9	P14780	Baicalein	−7.9
VEGF	P15692	Baicalein	−6.0
MMP-2	P08253	β-Sitosterol	−7.8
MMP-9	P14780	β-Sitosterol	−7.9
VEGF	P15692	β-Sitosterol	−6.3
MMP-2	P08253	Stigmasterol	−8.5
MMP-9	P14780	Stigmasterol	−8.4
VEGF	P15692	Stigmasterol	−6.6

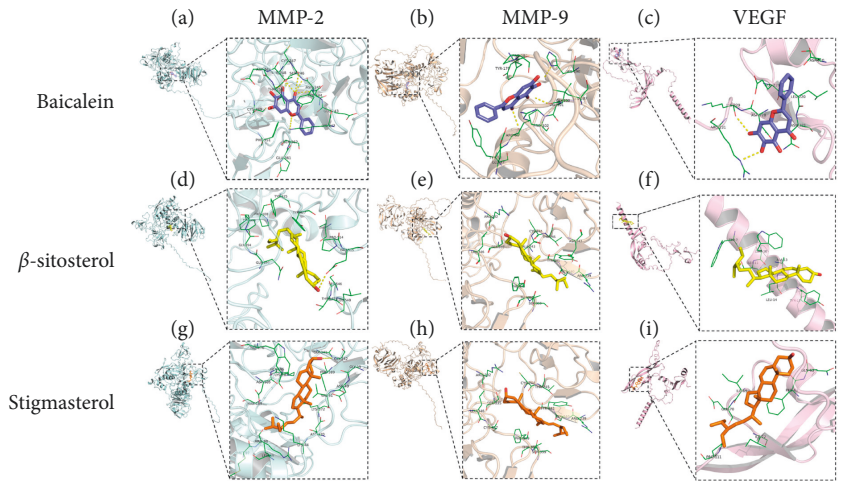


Figure 7: The 3D diagrams of the docking. (a). Baicalein with MMP-2; (b) baicalein with MMP-9; (c) baicalein with VEGF; (d) β-sitosterol with MMP-2; (e) β-sitosterol with MMP-9; (f) β-sitosterol with VEGF; (g) stigmasterol with MMP-2; (h) stigmasterol with MMP-9; and (i) stigmasterol with VEGF.

visible nodules (Figure 8(c)); the uteri in the GFW had no obvious thickening and nodules (Figure 8(d)).

3.2.3. HE Staining. Figure 9 showed the results of HE staining. The endometrial structure of the blank control group was normal, the endometrium-myometrium boundary was visible, although the myometrium had a small number of glands and stromal cells (Figure 9(a)). The disordered myometrium-endometrial structure boundary vanished in the model group, and endometrial glands and interstitial cells invaded the myometrium (Figure 9(b)). There were glands, interstitial cells, and endometrium in the local myometrium of the mifepristone group, but they were less than those in the model group (Figure 9(c)). The GFW group had significantly fewer endometrial and myometrial lesions than the model group, the myometrium had fewer glands, stromal cells, and endometrium than the model group (Figure 9(d)).

3.2.4. Comparison of Serum Levels of MMP-2, MMP-9, and VEGF in Each Group. The treatment of AM with GFW is closely associated with invasion, migration, and angiogenesis, according to the results of network pharmacology in this study. To assess the therapeutic impact, the serum MMP-2, MMP-9, and VEGF levels in each group were examined (Table 2). In the model group, the levels of MMP-2, MMP-9, and VEGF were significantly higher than in the blank control group ($^{*}P < 0.05$). Compared with the model group, mifepristone and GFW groups were reduced to varying degrees ($^{\#}P < 0.05$), and the GFW group was better than the mifepristone group in reducing MMP-2, MMP-9, and VEGF levels ($^{\bigstar}P < 0.05$). GFW appeared to be crucial in preventing the invasion, metastasis, and angiogenesis of AM lesions.

3.2.5. Comparison of MMP-2, MMP-9, and VEGF Protein Expression in Each Group. The immunohistochemistry staining of the uteri in each group is shown in Figures 10(a)–10(d). The positive expression in the model group was higher. In comparison, the expression in the blank control group, mifepristone group, and GFW group was lower, indicating that the model group had higher MMP-2, MMP-9, and VEGF content, whereas the mifepristone and GFW groups had lower content. MMP-2, MMP-9, and VEGF are depicted statistically in Figures 10(e)–10(g). The positive expression of MMP-2, MMP-9, and VEGF in the model group was much higher than in the blank control group ($^{*}P < 0.05$). The GFW group and mifepristone significantly reduced MMP-2, MMP-

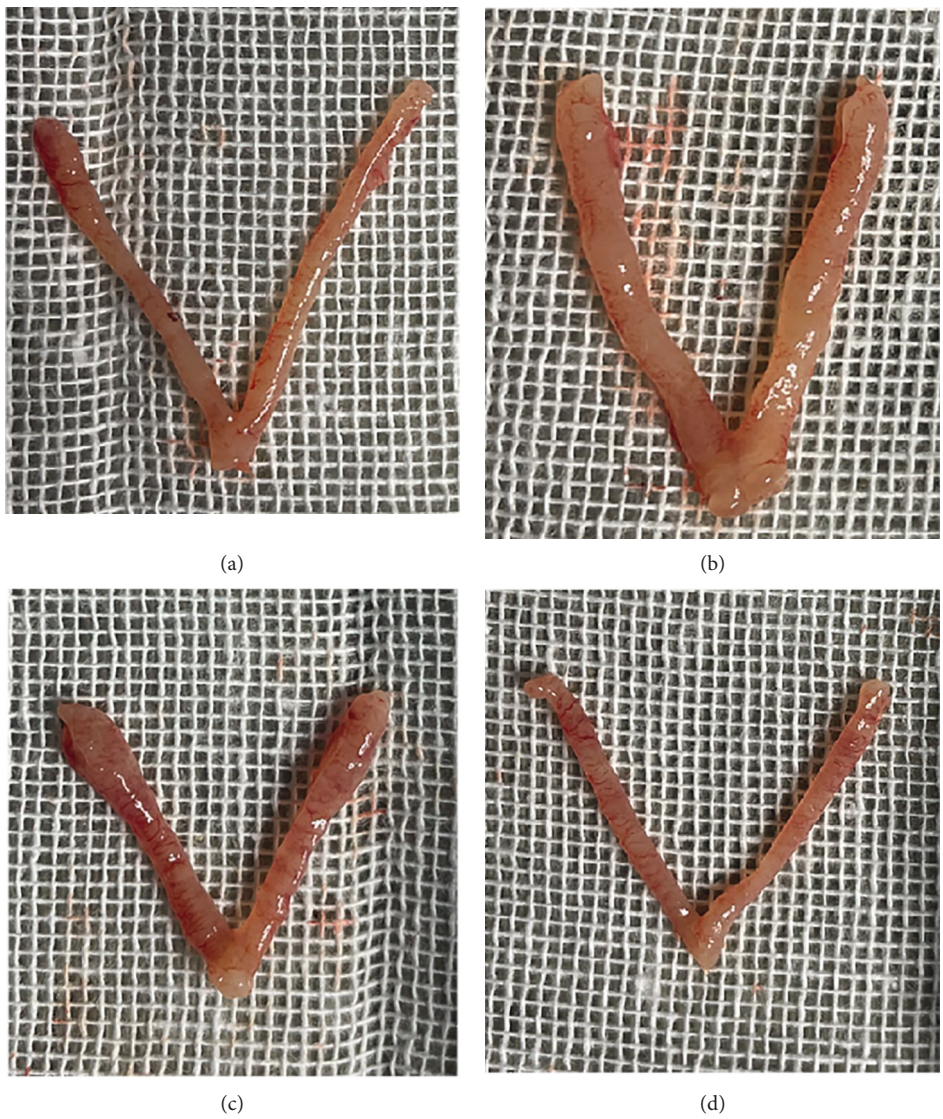


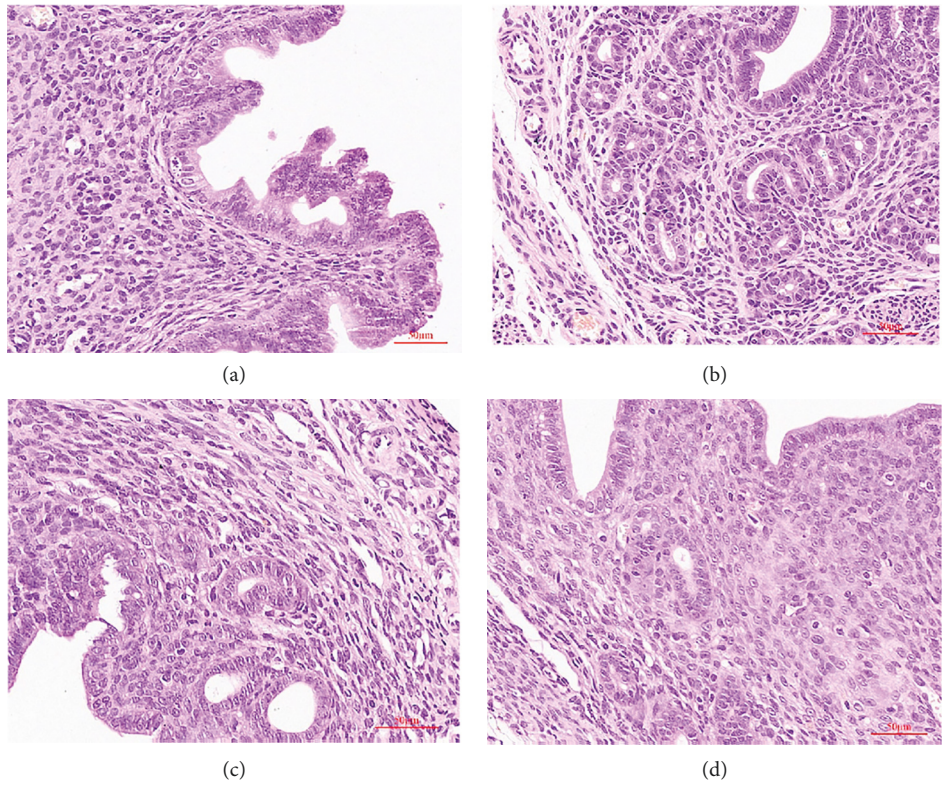
Figure 8: Histomorphological observation of AM mice uteri in each group after 30 days of intervention. (a) Blank control group; (b) model group; (c) mifepristone group; (d) GFW group.

9, and VEGF levels compared to the model group ($^{\#}P < 0.05$). Additionally, the GFW group demonstrated a substantial advantage over the mifepristone group ($^{\star}P < 0.05$).

4. Discussion

The famous Chinese classical prescription GFW created by Zhang Zhongjing in the Eastern Han Dynasty is mainly used to treat blood stasis diseases. Clinical studies found that GFW could reduce the degree of dysmenorrhea in patients with AM and decrease the amount of menstruation [39]. Zhang indicated that GFW reduced the level of VEGF in EM, inhibited the invasion and migration by inhibiting the growth of blood vessels in ectopic lesions [40], and also played anti-inflammatory and endocrine-regulating effects [41]. However, less research has been done on AM. Mori found that GFW decreased the levels of interleukin 6 (IL-6), interleukin 8 (IL-8), and TNF-α in peritoneal fluid; reduced the number of macrophages; and then inhibited the growth of ectopic endometrial neovascularization. The reduction in the formation of new blood vessels also inhibited the levels of MMP-2 and MMP-9 in vivo, further preventing the invasion and adhesion of ectopic cells [36]. Our research demonstrated that GFW decreased the expression of MMP-2, MMP-9, and VEGF and had an inhibitory effect on the invasion and migration of AM, which was consistent with the conclusions of published studies.

AM is a benign gynecological disease, but it has tumor-like invasive and metastatic properties. Multiple uterine cavity surgeries damage the ultrastructure of the endometrium-myometrium and change the morphology of mast cells; furthermore, the myometrium is invaded by active eutopic endometrial glands and stroma. In this process, the degradation of extracellular matrix (ECM) and the formation of new blood vessels exacerbate the progression of AM, in which matrix metalloproteinases (MMPs) are involved.



(a) (b) (c) (d)

Figure 9: HE staining of AM mice uteri in each group after 30 days of intervention (HE 200x). (a) Blank control group; (b) model group; (c) mifepristone group; and (d) GFW group.

Table 2: Comparison of serum levels of MMP-2, MMP-9, and VEGF in each group ($\overline{x} \pm s$, pg·mL^{-1}, $n = 5$).

Group	N	MMP-2 (pg/mL)	MMP-9 (pg/mL)	VEGF (pg/mL)
Blank control	5	158.57 ± 4.20	216.67 ± 14.05	17.42 ± 1.53
Model	5	$196.60 \pm 6.05^{*}$	$345.50 \pm 25.79^{*}$	$21.59 \pm 0.30^{*}$
Mifepristone	5	$159.82 \pm 5.79^{\#}$	$260.44 \pm 6.59^{*\#}$	$18.66 \pm 0.68^{\#}$
GFW	5	$137.64 \pm 5.46^{*\#\bigstar}$	$236.89 \pm 6.70^{\#\bigstar}$	$16.44 \pm 0.92^{\#\bigstar}$

Compared with the blank control group, $^{*}P < 0.05$; compared with the model group, $^{\#}P < 0.05$; compared with the mifepristone group, $^{\bigstar}P < 0.05$.

Although the MMP family has many members, MMP-2 and MMP-9 have great potential to enhance cell migration and invasion ability as important Zn^{2+}-dependent matrix metalloproteinases [42, 43]; in addition, they are highly expressed in patients with AM and EM [44, 45]. MMP-2 and MMP-9 are more critical for angiogenesis than for ECM degradation, which are considered to be the key to promoting tumor angiogenesis. VEGF can promote the growth of vascular endothelial cells so that the ectopic endometrium can form new capillary loops during implantation and transfer [37, 46]. Li et al. showed that the expression of MMP-2 and MMP-9 was enhanced in AM and positively correlated with the production of VEGF, indicating that MMP-2 and MMP-9 aggravated the invasion and metastasis of AM by degrading ECM and promoting angiogenesis [37]. Thus, complex biological processes such as cell invasion and metastasis induced by MMP-2 and MMP-9, as well as angiogenesis and metastasis involving VEGF, are the essential pathological basis for the progression of ectopic AM lesions.

In the network pharmacology study, baicalein, sitosterol, β-sitosterol, (+)-catechin, and other active compounds inhibited the invasion and metastasis of AM ectopic lesions, possibly by acting on MMP-2, MMP-9, and VEGF. Some mechanisms have been confirmed, (+)-catechin decreased VEGF levels in vivo and inhibited the growth of ectopic endometrium [47]. Stigmasterol can inhibit angiogenesis, reduce the invasive ability of tumor cells, promote the apoptosis of abnormal cells, and improve the immunity of the body [48]. GO and KEGG enrichment analyses revealed that biological processes such as vascular endothelial migration, cell proliferation, and inflammatory responses and signaling pathways such as TNF and HIF-1 were closely related to GFW treatment of AM. The TNF signaling pathway induced the production of VEGF by activating nuclear factor kappa-B (NF-κB), thereby promoting the formation of new blood vessels in AM and increasing the invasiveness of ectopic cells [49, 50]. Xue et al. found that HIF-1 promoted the formation of new blood vessels in ectopic tissues under the environment of inflammation and

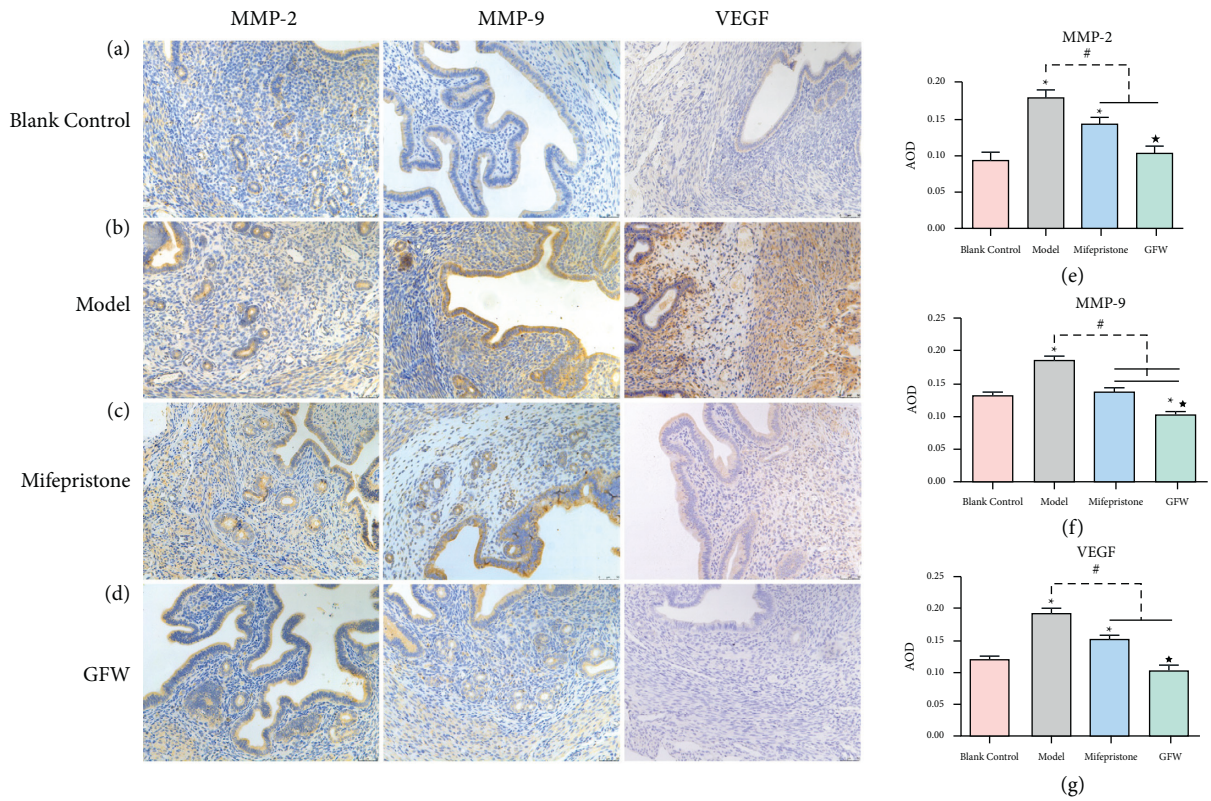


Figure 10: Immunohistochemical staining of MMP-2, MMP-9, and VEGF (IHC 200x). (a) Blank control group; (b) model group; (c) mifepristone group; (d) GFW group; (e) statistical bar chart of MMP-2 expression in each group; (f) statistical bar chart of MMP-9 expression in each group; and (g) statistical bar chart of VEGF expression in each group. Compared with the blank control group, $^{*}P < 0.05$; compared with the model group, $^{\#}P < 0.05$; compared with the mifepristone group, $^{\bigstar}P < 0.05$.

hypoxia, thereby regulating the invasion and metastasis of ectopic endometrium [51, 52]. In addition, GFW reduced the expression of VEGF by downregulating the level of HIF-1α in patients with EM [53]. In summary, it was speculated that GFW reduced the level of VEGF by regulating TNF, HIF-1, and other signaling pathways, thereby inhibiting AM angiogenesis as well as the invasion and metastasis of ectopic cells. Molecular docking was performed to verify the tightness of the binding between the key compounds of GFW and key targets MMP-2, MMP-9, and VEGF. Based on the network pharmacology research and literature verification, we speculated that GFW played a therapeutic role in AM by inhibiting angiogenesis and decreasing cell invasion ability in multiple levels and dimensions.

Network pharmacology might have false-positive results in the prediction process due to the disadvantages of irregular data extraction, a large amount of data, and a lack of unified screening standards. Therefore, high-quality and high-evidence verification results need to be obtained through further experimental research while improving the accuracy of the data.

Based on the mechanisms of AM invasion, metastasis, and angiogenesis, animal experiments were performed to verify the accuracy of network pharmacology. This study used the recognized AM modeling method in ICR mice. The targets MMP-2, MMP-9, and VEGF, which were mainly involved in AM invasion, metastasis, and angiogenesis, were detected by ELISA and immunohistochemistry. The results confirmed that the expression of MMP-2, MMP-9, and VEGF increased during the progression of AM. We believed that this was closely related to the invasion, metastasis, and angiogenesis of AM lesions. GFW drastically reduced the expression of MMP-2, MMP-9, and VEGF, indicating that GFW can inhibit the invasion, metastasis, and angiogenesis of AM lesions, which was consistent with the previous network pharmacology prediction results. However, further experiments with large samples are still needed to verify the accuracy of the results due to the small sample size of this study, different responses of the individual mouse to drugs, and differences in the choice of experimental reagents.

In summary, various compounds in GFW might play a role in treating AM through key targets such as MMP-2, MMP-9, and VEGF. The findings of this study provided a basis for studying the mechanism of action of GFW in treating AM. However, obtaining complete and accurate data by relying on the network was difficult due to the complexity of TCM compounds and the uncertainty of disease targets. Therefore, the study experimentally verified the inhibitory effect of GFW on MMP-2, MMP-9, and VEGF in the invasion and metastasis of AM ectopic cells, to avert the limitations of network pharmacology. The study also provided a molecular basis for further research on the pathogenesis of AM and the therapeutic effect of GFW. Because of the limited number of targets, it was difficult to

explain the complete biological process of GFW inhibiting AM invasion and metastasis. The active compounds and pathways of GFW will be further studied in the future to make up for the insufficiency of this study and provide high-quality and high-evidence results for the mechanism of GFW in treating AM. Table S1 shows the GFW-related compounds and targets. Table S2 shows the AM-related targets. Table S3 shows the GFW-AM common targets. Table S4 shows the GFW-AM common targets' string interactions and key targets.

5. Conclusions

To sum up, the active compounds of GFW in treating AM mainly include baicalein, β-sitosterol, and stigmasterol. The key targets for inhibiting the invasion of ectopic AM are MMP-2, MMP-9, and VEGF. According to the results of animal experiments, GFW can significantly reduce the levels of MMP-2, MMP-9, and VEGF in AM and inhibit the invasion, migration, and angiogenesis of ectopic lesions, so as to achieve the purpose of treating AM. However, due to the limitation of the number of targets, the interactive therapeutic effects of GFW multi-compounds and multi-targets in the treatment process are still not fully clear. The active compounds and pathways of GFW need to be further studied in the future to make up for the insufficiency of this study and provide high-quality and high-evidence results for the mechanism of GFW in treating AM.

Data Availability

All data of this study can be obtained from the provided open platform website. Experimental data and analyses can be provided by the corresponding authors where reasonable.

Conflicts of Interest

All authors declare that this article has no conflicts of interest.

Authors' Contributions

Y. X. Shi, C. Y. Zhang, and X. Wang worked together on manuscript writing, picture drawing, and data analysis. Z. L. Wang and Y. R. Zhang assisted in animal experiments. Z. Y. Liu is in charge of experimental guidance, and X. Wang and W. Shi are in charge of experimental design and manuscript revision. All authors have received the manuscript and original data and approved the final version. Yaxin Shim, Chengyuan Zhang, and Xin Wang are the co-first authors with equal contributions to this work.

Acknowledgments

This project was also supported by the National Nature Science Foundation of China (82104916, 81873330), Taishan Scholar Project of Shandong Province (tsqn201909185), Science and Technology Plan of Jinan (201907009), the Natural Science Foundation of Shandong Province, China(ZR2020QH337). In addition, we appreciate the platform support provided by the Central Laboratory of The Affiliated Hospital of Shandong University of Traditional Chinese Medicine.

Supplementary Materials

Figure S1: HPLC figure of baicalein, β-sitosterol, and stigmasterol. Table S1: GFW-related compounds and targets. Table S2: AM-related targets. Table S3: GFW-AM common targets. Table S4: GFW-AM common targets' string interactions and key targets. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 8698755, 18 pages
https://doi.org/10.1155/2022/8698755

Review Article

The Efficacy and Safety of Dingkun Pill in Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Received 22 April 2022; Accepted 4 July 2022; Published 24 August 2022

Academic Editor: Yong Wang

Objective. Dingkun Pill (DKP) is a proprietary Chinese medicine that has been utilized for patients with gynecological diseases, and its clinical application has been widely accepted in China. However, the effects of DKP on reproduction and metabolism in women with polycystic ovary syndrome (PCOS) have never been systematically evaluated. Our objective was to evaluate the efficacy and safety of DKP in treating reproductive and metabolic abnormalities with PCOS. *Methods*. We searched in PubMed, Embase, Web of Science, Cochrane Library, China National Knowledge Infrastructure, Wanfang Database, VIP Database, and Chinese Biomedical Literature Database up until January 2022 to identify randomized controlled trials (RCTs). The methodological quality of the included RCTs was estimated using the Cochrane collaboration risk-of-bias instrument, and the meta-analysis was performed using RevMan. *Results*. A total of 22 RCTs (including 1994 participants) were identified. DKP, combined with ovulation-inducing drugs (OID) or combined oral contraceptives (COC) was superior to OID or COC alone in improving the pregnancy rate (relative risk (RR) 1.84, 95% CI 1.62 to 2.11 and RR 1.38, 95% CI 1.16 to 1.64, respectively), ovulation rate (RR 1.38, 95% CI 1.03 to 1.84 and RR 1.23, 95% CI 1.11 to 1.37, respectively), endometrial thickness (weighted mean difference (WMD) 2.50, 95% CI 1.91 to 3.09 and WMD 0.62, 95% CI 0.08 to 1.16, respectively), luteinizing hormone (WMD −1.93, 95% CI −2.80 to−.07 and WMD −1.79, 95% CI −2.66 to−0.92, respectively), and testosterone (standardized mean difference (SMD) −2.12, 95% CI −3.01 to−1.24 and SMD −1.21, 95% CI −1.64 to−0.78, respectively). DKP combined with COC led to a greater improvement in homeostasis model assessment-β (WMD 20.42, 95% CI 16.85 to 23.98) when compared with COC alone. There was a significant difference between DKP and COC in terms of decreasing total cholesterol (WMD −0.37, 95% CI −0.72 to−0.02), triacylglycerol (WMD −0.85, 95% CI −1.50 to−0.20), and free fatty acid (WMD −130.00, 95% CI −217.56 to−42.22). However, DKP did not affect the follicle stimulating hormone, fasting blood glucose, fasting insulin, body mass index, waist-to-hip ratio, high-density lipoprotein cholesterol, or low-density lipoprotein cholesterol. Adverse reactions were more common in COC alone compared to DKP and COC in combination (RR 0.22, 95% CI 0.07 to 0.63). *Conclusion*. DKP shows promise in modifying reproductive and metabolic parameters in patients with PCOS and may be used as a primary choice in conventional or complementary therapies for PCOS. The quality of the evidence analyzed was suboptimal, and therefore, our results should be interpreted cautiously. More prospective large-scale and well-designed RCTs, as well as longer intervention durations are required in the future to draw more reliable conclusions.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common gynecologic endocrine disorder that is generally considered to be the leading cause of anovulatory infertility [1], and it affects 6% to 20% of reproductive-age women [2]. PCOS is characterized by menstrual dysfunction, hypo-ovulation/anovulation, hyperandrogenism, and polycystic ovaries [3]. In addition to reproductive disruption, women with PCOS are prone to metabolic disorders, including insulin resistance (IR), impaired glucose tolerance, and dyslipidemia, and they are at an increased risk of developing type 2 diabetes mellitus [4]. In the U.S. alone, the cost of diagnosing, treating, and caring for patients with PCOS was estimated to be $8 billion yearly in 2020, which places an immense financial burden on both the patient's family and society as a whole [5]. Therefore, the effective management and treatment of PCOS can contribute to improving public health.

Western medicine management for PCOS includes ovulation-inducing drugs (OID), insulin sensitizers, combined oral contraceptives (COC), antiandrogens, and/or antiobesity medications aiming at restoring menstruation and improving pregnancy, decreasing androgen levels, lowering IR, and reducing weight [6]. However, they have some potential side effects. Although clomiphene citrate achieves an ovulation rate up to 70% to 80%, the clinical pregnancy rate is only 30% to 40%, and patients are at risk of multiple pregnancies [7]. Letrozole has a short half-life (42 h) and is quickly excreted from the body, thus resulting in the inability to form a dominant follicle [8]. Patients taking Diane-35 or metformin may suffer from abnormal uterine bleeding, gastrointestinal disturbances, and other adverse reactions [9]. Thus, an increasing number of PCOS patients have turned to complementary and alternative therapy to improve their health. According to a recent survey, 70.4% of obstetricians and gynecologists/reproductive doctors in China use traditional Chinese medicine in the treatment of PCOS [10].

Dingkun Pill (DKP) is a traditional Chinese patent herbal medicine originating from the *Golden Mirror of Medicine* written by Wu Qian in the Qing Dynasty and is officially listed in the Chinese Pharmacopoeia [11]. It is composed of 30 Chinese herbals and animal products, including red ginseng (*Radix Ginseng Rubra*), pilose antler (*Cornu Cervi Pantotrichum*), saffron (*Stigma Croci*), debarked peony root (*Radix Paeoniae Alba*), Chinese angelica (*Radix Angelicae Sinensis*), prepared rehmannia root (*Radix Rehmanniae Preparata*), ass hide glue (*Colla Corii Asini*), etc. For centuries, DKP has been used in traditional Chinese medicine to treat gynecological diseases because the combination of these ingredients is thought to nourish the liver and kidney, regulate menstruation, relieve Qi stagnation, benefit Qi, and nourish the blood. Among the traditional Chinese patent herbal medicine used for PCOS, DKP ranks first [10], and an increasing number of animal experiments and clinical studies have demonstrated the reliable efficacy of DKP [12–15]. In experimental studies, DKP and its main active ingredients were found to regulate the reproductive hormone levels in rats with PCOS, decrease the expression of vascular endothelial growth factor in the ovary, and increase the expression of homeobox gene A10 (HOXA10) in the uterus, thereby facilitating uterine receptivity [16, 17]. According to Gao's study, the mechanism of DKP in the treatment of PCOS might be associated with multiple signaling pathways, such as the PI3K-Akt signaling pathway, serotonergic synapses, steroid hormone biosynthesis, and ovarian steroidogenesis, suggesting that DKP can treat PCOS through multiple targets [18]. Regarding the effect of DKP in PCOS, the available clinical data suggest that DKP plays a role in regulating the menstrual cycle, promoting ovulation, increasing the pregnancy rate, and enhancing the function of the hypothalamus-pituitary-ovary axis (HPOA) [19, 20]. Moreover, DKP has also been used in PCOS patients with IR and lipid metabolism abnormalities [21, 22]. As a traditional Chinese patent herbal medicine, DKP has the advantages of easy access, convenient administration, and wide acceptance. Hence, it has great potential for popularization. However, as far as we know, there has been no systematic evaluation of the efficacy and safety of DKP in the treatment of reproductive and metabolic abnormalities in women with PCOS and whether this medicine represents an ideal form of complementary and alternative therapy. Thus, we conducted a systematic review and meta-analysis of available RCTs to provide a reliable basis for the treatment of PCOS.

2. Materials and Methods

This systematic review was conducted and reported according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement guidelines [23] and was registered in PROSPERO (CRD42022298220).

2.1. Search Strategy. The systematic literature search was performed in the following databases: PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database, and the Chinese Biomedical Literature Database (CBM) from their inception to 1 January, 2022. Key words in the literature retrieval included "Dingkun pill," "Dingkun Dan," "polycystic ovary syndrome," "polycystic ovarian syndrome," "PCOS," and related synonyms (the full details of the search strategy are given in Table S1 in the Supplementary Materials). No limits were applied to language or publication status. The references of significant studies were searched manually for possible relevant literature, and conference compilations supplemented the electronic searches.

2.2. Eligibility Criteria. The inclusion criteria was as follows: (a) subjects diagnosed with PCOS regardless of race and age, (b) the intervention group was treated with DKP or DKP combined with the control group's intervention. The control group was treated with Western medicine, placebo, or blank and with an unlimited dose and course of treatment. (c) The study was an RCT. The exclusion criterion was the literature

in which relevant data could not be obtained and data were still not available after contacting the authors.

2.3. Outcome Measures. As improving reproduction is the core in treating PCOS, the primary outcome measure was defined as reproductive indexes, including pregnancy rate, ovulation rate, and endometrial thickness. The secondary outcome measures were defined as hormone parameters—including luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone (T)—metabolic indexes—including fasting blood glucose (FBG), fasting insulin (FINS), and homeostasis model assessment-β (HOMA-β)—lipid profiles—including total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and free fatty acid (FFA)—and anthropometric indices—including body mass index (BMI) and waist-to-hip ratio (WHR). Adverse reactions were also included as outcomes.

2.4. Literature Screening and Data Extraction. Based on the search strategy presented above, the titles and abstracts of the identified articles were read for preliminary screening after eliminating duplicates. The full texts were then read during rescreening in accordance with the inclusion and exclusion criteria established previously to identify the included articles. Data extraction was performed independently by two reviewers, and disagreement was resolved by discussion. The following information was extracted from the included RCTs: (1) the characteristics of the articles, including primary author, publication year, language, and study design, (2) participants' characteristics, including mean age, sample size, and criteria used to define PCOS, (3) the details of interventions and comparison methods, including the type and treatment duration, (4) every outcome measure, and (5) adverse reactions. To ensure that the data were complete and accurate, we contacted the authors via telephone or e-mail regarding missing data.

2.5. Quality Assessment. Two reviewers independently assessed the methodological quality of eligible RCTs using the Cochrane collaboration risk-of-bias instrument [24]. Factors were related to bias risk-included random sequence generation, allocation concealment, the blinding of participants and personnel, the blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. There were three levels used to assess the methodological quality: the low risk of bias, the high risk of bias, and an unclear risk of bias. Then, we used the grading of recommendations, assessments, development, and evaluation (GRADE) [25] system (pro 3.6.1) to evaluate the quality of evidence derived from our systematic review for primary outcomes separately, including the risk of bias, indirectness, inconsistency, imprecision, and publication bias. All discrepancies and disagreements were resolved by consensus or by discussion with the corresponding author.

2.6. Statistical Analysis. All data syntheses were performed using the Review Manager software version 5.3. Dichotomous data were presented as the risk ratio (RR) and continuous data as the weighted mean difference (WMD) or standardized mean difference (SMD), both with a 95% confidence interval (CI). Heterogeneity across the studies was tested by the Cochrane Q-test and I^2 statistic. If $I^2 \leq 50\%$ and $P \geq 0.10$, a fixed effects model was used. Otherwise, a random effects model was used. To determine the stability of the meta-analysis results, a sensitivity analysis was conducted to explore heterogeneity because of extreme data. A funnel plot was used to assess publication biases.

3. Results

3.1. Study Selection. Originally, 185 articles were identified in the database through the search strategy, and it was reduced to 84 records after duplicates were removed. After reviewing the titles, abstracts, and full-text articles, a total of 22 RCTs in 24 publications [10, 19, 21, 22, 26–45], including 1994 women with PCOS, satisfied our inclusion criteria (Figure 1).

3.2. Characteristics of the Included Studies. The summarized characteristics of the 22 RCTs and the 1994 participants are shown in Table 1. All of the RCTs were conducted in China, with the sample size ranging from 60 to 210 participants, and most of them were 20 to 39 years old. Studies were published between 2012 and 2021. Most of the included RCTs used the Rotterdam criteria [46] to define PCOS, while three RCTs [36, 39, 41] used Obstetrics and Gynecology, Chinese Obstetrics and Gynecology Association, and Guidelines for the diagnosis and treatment of PCOS in China, respectively, and six RCTs [29, 31, 34, 35, 37, 40] only reported the diagnosis of PCOS and did not clearly describe the diagnostic criteria. The interventions were DKP alone or in combination with OID or COC, and the controls were OID or COC. Twenty of the 22 RCTs [19, 25–43] were 2-arm studies, and the remaining two RCTs were 3-arm studies. Data from the 3-arm study, divided into DKP vs. COC and DKP + COC vs. COC, were included in the meta-analysis. The duration of treatment was one month in two RCTs [33, 39], three months in 11 RCTs [10, 19, 21, 22, 28–30, 32, 40–44], six months in two RCTs [26, 38], and until pregnancy in the rest. Pregnancy rate and endometrial thickness were the most common outcomes followed by hormone parameters. Ten RCTs [10, 19, 21, 22, 26, 28, 33, 38, 39, 42–44] reported on adverse reactions.

3.3. Risk of Bias of Individual Studies. Figure 2 summarizes the risk of bias of the included trials based on different quality domains using the Cochrane collaboration instrument. Nine trials [10, 21, 22, 25, 29, 30, 33, 37, 41–43] reported random sequence generation using a random number table or software and thus had a low risk, 12 trials [19, 26–28, 31, 32, 34–36, 38–40] only mentioned "random" but were missing details regarding the randomization methods and thus had an unclear risk, and one trial [38] had

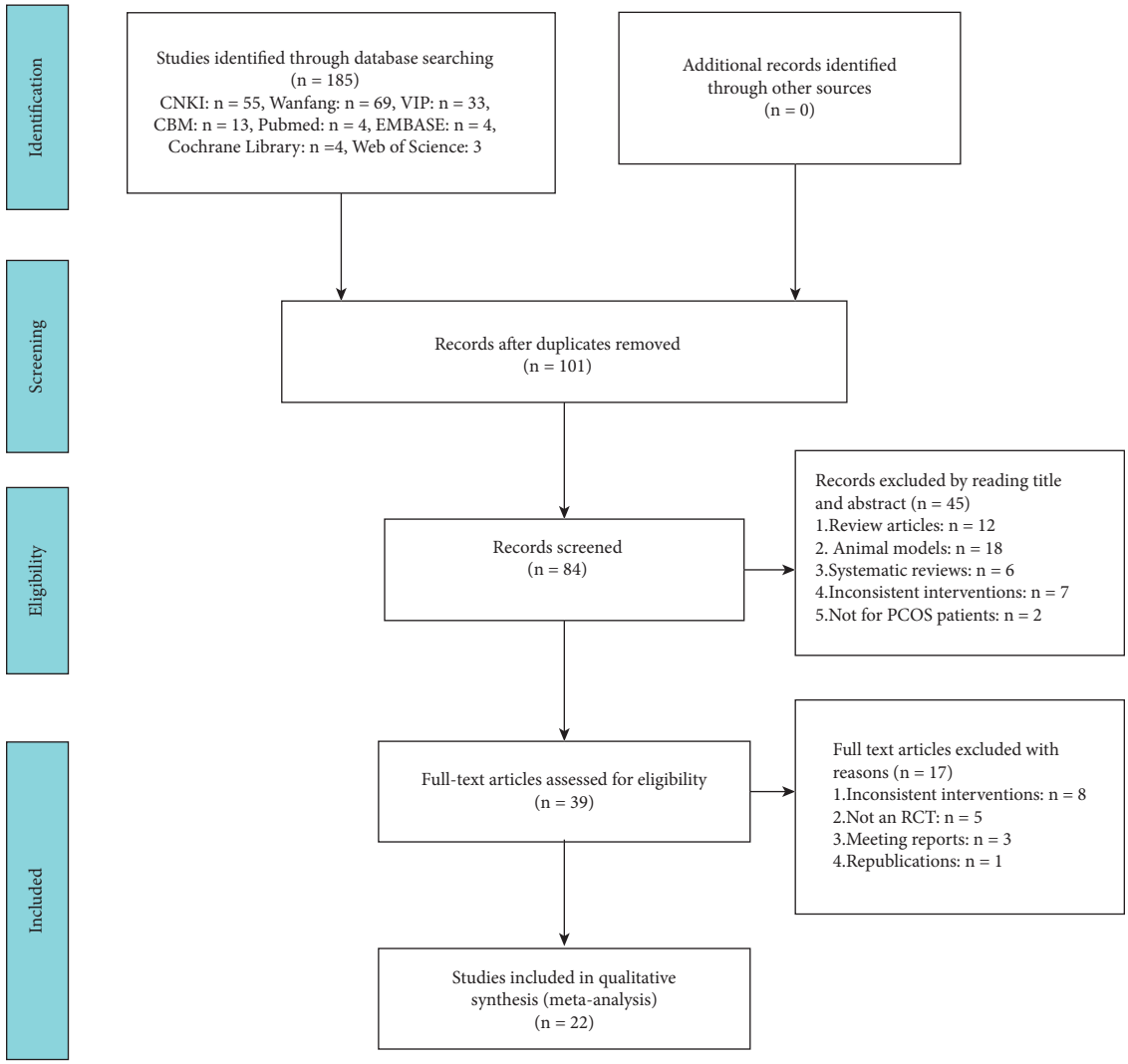

Figure 1: Flow diagram of the study selection process.

a high risk. Only one trial [10, 21, 22] had a low risk when considering allocation concealment. In terms of the blinding of participants or personnel, two trials [10, 19, 21, 22] had a low risk. The outcome assessors were blind in one trial [10, 21, 22] using triple-blinding, and the other 21 trials had an unclear risk. There was no risk of bias for incomplete outcome data or for selective reporting. All trials appeared to have an unclear risk for other biases.

3.4. Findings from the Meta-Analysis

3.4.1. Effects on Reproductive Indexes. The effects of DKP on pregnancy rate was assessed in 18 RCTs (1616 participants). The pooled results showed that the combination of DKP + OID was superior to OID alone in increasing the pregnancy rate (RR: 1.84, 95% CI: 1.62 to 2.11, $P < 0.00001$). Compared with the COC groups, there was a significant improvement in pregnancy rate in the DKP + COC groups (RR: 1.38, 95% CI: 1.16 to 1.64, $P = 0.0004$). However, a comparison of DKP with COC did not show a significant difference in pregnancy rate (RR: 0.82, 95% CI: 0.38 to 1.76, $P = 0.61$) (Figure 3).

In terms of ameliorating the ovulation rate, eight RCTs, including 769 participants, indicated that DKP plus OID or COC for PCOS was better than using OID or COC alone (OID: RR: 1.38, 95% CI: 1.03 to 1.84, $P = 0.03$; COC: RR: 1.23, 95% CI: 1.11 to 1.37, $P = 0.0001$). There was no clear difference between DKP and COC in terms of ovulation rate (RR: 0.96, 95% CI: 0.68 to 1.36, $P = 0.82$) (Figure 4).

There were ten RCTs (772 participants) assessing the effects of DKP on endometrial thickness. Compared with OID alone, the combination DKP + OID significantly improved the endometrial thickness of PCOS patients (WMD: 2.50, 95% CI: 1.91 to 3.09, $P < 0.00001$). Furthermore, the combination of DKP + COC also significantly increased endometrial thickness (WMD: 0.62, 95% CI: 0.08 to 1.16, $P = 0.02$) (Figure 5).

3.4.2. Effects on Hormone Parameters. Thirteen trials totaling 1175 women were used in a meta-analysis of the effects of DKP on LH level. Compared with OID alone, DKP + OID

Table 1: The characteristics of the included studies.

Study ID	Language	Study design	Age (years)	Sample size	Diagnostic criteria	Interventions	Duration	Outcomes	Adverse reaction
Du 2019 [26]	Chinese	RCT	29.7 ± 2.2 29.5 ± 2.1	30 30	Rotterdam	DKP + OID OID	To pregnancy	Pregnancy rate, ovulation rate, endometrial thickness, LH, FSH, T	NR
Hu 2012 [27]	Chinese	RCT	NR	30 30	Rotterdam	DKP + OID OID	6 months	Pregnancy rate, LH, FSH, T	None
Li 2018 [28]	Chinese	RCT	NR	40 40	Rotterdam	DKP + OID OID	To pregnancy	Endometrial thickness, T	NR
Ma 2018 [29]	Chinese	RCT	26.2 ± 4.0 25.4 ± 4.2	40 40	Rotterdam	DKP + OID OID	3 months	Pregnancy rate, endometrial thickness, LH, FSH, T	None
Qin 2021 [30]	Chinese	RCT	30.21 ± 3.81 29.71 ± 3.46	49 49	Not clearly described	DKP + OID OID	3 months	Pregnancy rate, LH, FSH	NR
Ren 2020 [31]	Chinese	RCT	28.7 ± 1.3 28.5 ± 1.2	36 36	Rotterdam	DKP + OID OID	3 months	Pregnancy rate, endometrial thickness, LH, FSH, T	NR
Wang 2019 [32]	Chinese	RCT	28.36 ± 7.92 28.25 ± 6.12	55 55	Not clearly described	DKP + OID OID	To pregnancy	Pregnancy rate	NR
Wei 2012 [33]	Chinese	RCT	28.35 ± 1.25 29.25 ± 1.65	30 30	Rotterdam	DKP + OID OID	3 months	Pregnancy rate, ovulation rate, endometrial thickness	NR
Wei 2018 [19]	Chinese	RCT	29.33 ± 0.96 28.22 ± 0.76	50 50	Rotterdam	DKP + OID OID	1 month	Pregnancy rate, ovulation rate, endometrial thickness	None
Wei 2020 [34]	Chinese	RCT	26.12 ± 3.54 27.35 ± 3.29	45 45	Rotterdam	DKP + OID OID	3 months	Pregnancy rate, endometrial thickness, LH, FSH, T	None
Yu 2020 [35]	Chinese	RCT	30.54 ± 2.34 30.25 ± 2.14	46 45	Not clearly described	DKP + OID OID	To pregnancy	Pregnancy rate	NR
Yuan 2019 [36]	Chinese	RCT	31.12 ± 0.28 30.23 ± 0.62	34 34	Not clearly described	DKP + OID OID	To pregnancy	Pregnancy rate	NR
Yu 2020 [37]	Chinese	RCT	NR	50 50	Obstetrics and gynecology	DKP + OID OID	To pregnancy	Pregnancy rate, ovulation rate	NR
Yu 2021 [38]	Chinese	RCT	27.57 ± 2.25 27.21 ± 2.36	55 55	Not clearly described	DKP + OID OID	To pregnancy	Endometrial thickness, LH, FSH, T	NR
Zhai 2019 [39]	Chinese	RCT	26.15 ± 3.18 25.96 ± 3.33	44 44	Rotterdam	DKP + OID OID	6 months	Pregnancy rate, LH, FSH, T	None
Chu 2020 [40]	Chinese	RCT	29.27 ± 3.59 29.17 ± 3.51	30 30	Chinese obstetrics and gynecology association	DKP + OID + DYD OID + DYD	1 month	Pregnancy rate, ovulation rate, endometrial thickness	None
Xiang 2020 [41]	Chinese	RCT	31.05 ± 3.37 30.25 ± 3.42	105 105	Not clearly described	DKP + COC + MET COC + MET	3 months	Pregnancy rate, ovulation rate, LH, FSH, T, FINS, HOMA-β	NR

Table 1: Continued.

Study ID	Language	Study design	Age (years)	Sample size	Diagnostic criteria	Interventions	Duration	Outcomes	Adverse reaction
Zhong 2021 [42]	Chinese	RCT	28.69 ± 1.75 28.54 ± 1.69	44 43	Guidelines for diagnosis and treatment of PCOS in China	DKP + COC + MET COC + MET	3 months	Pregnancy rate, ovulation rate, LH, FSH, T, FINS, HOMA-β	NR
Chen 2016 [43]	Chinese	RCT	30.3 ± 1.8 30.2 ± 1.7	40 40	Rotterdam	DKP + COC COC	3 months	LH, FSH, T	Yes
Yu 2021 [44]	Chinese	RCT	24.47 ± 4.05 23.83 ± 3.32	30 30	Rotterdam	DKP + COC COC	3 months	Endometrial thickness, LH, FSH, T	Yes
Deng 2020 [10, 21, 22]	English	RCT	27.5 ± 3.4 27.2 ± 3.5 26.7 ± 6.4	35 36 39	Rotterdam	DKP COC DKP + COC	3 months	BMI, WHR, FBG, FINS, TC, TG, HDL-c, LDL-C	None
Zhang 2019 [45]	Chinese	RCT	28.02 ± 3.21 28.18 ± 3.10 27.12 ± 3.30	40 40 40	Rotterdam	DKP COC DKP + COC	3 months	Pregnancy rate, ovulation rate, LH, FSH, T	None

DKP, Dingkun pill; OID, ovulation inducing drugs; COC, combined oral contraceptives; DYD, dydrogesterone; MET, metformin; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone; BMI, body mass index; WHR, waist-to-hip ratio; FBG, fasting blood glucose; FINS, fasting Insulin; HOMA-β, homeostasis model assessment-β, TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NR, not reported.

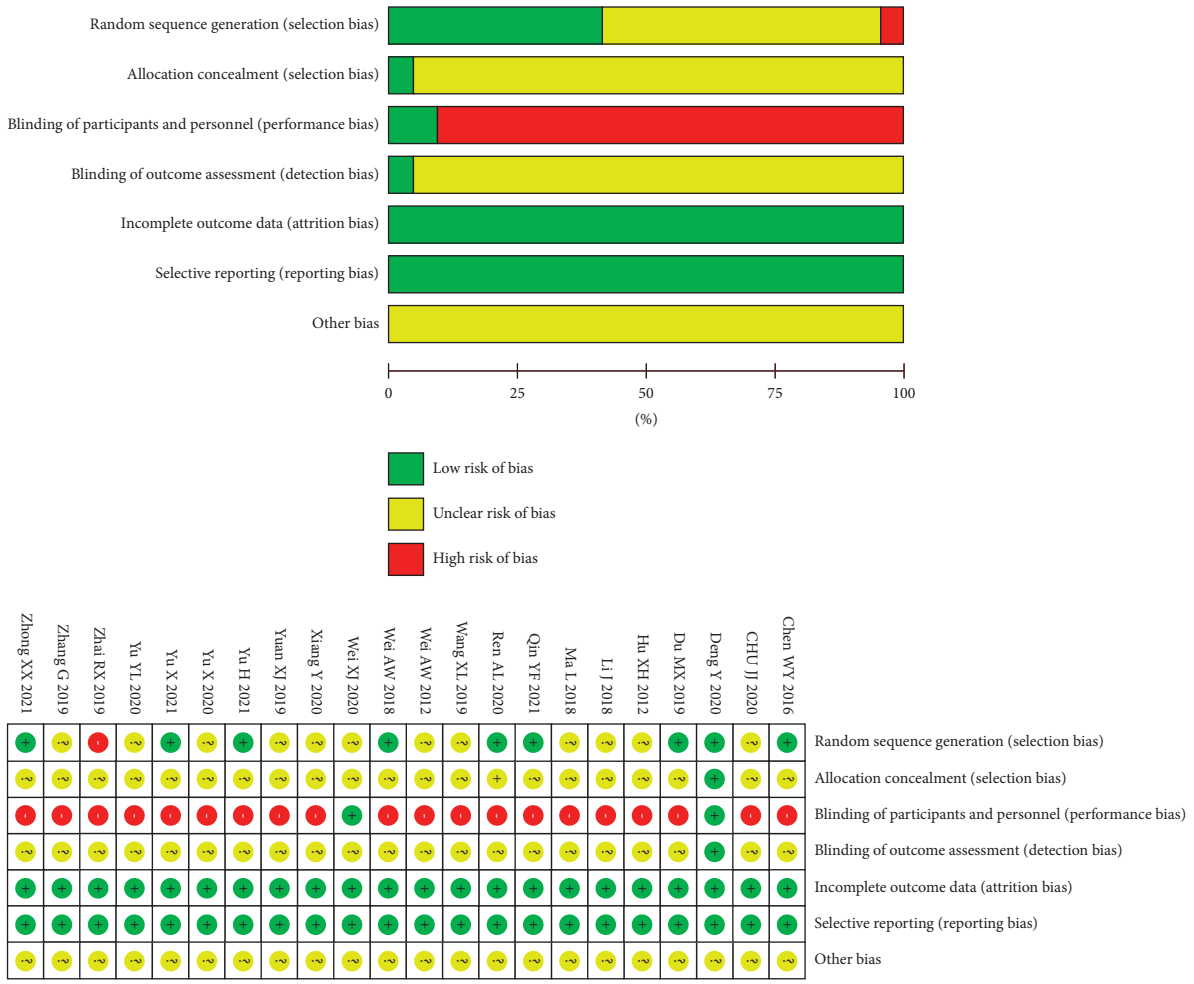

Figure 2: The risk of bias for the included studies shown as low risk of bias (+), high risk of bias (−), and unclear risk of bias (?).

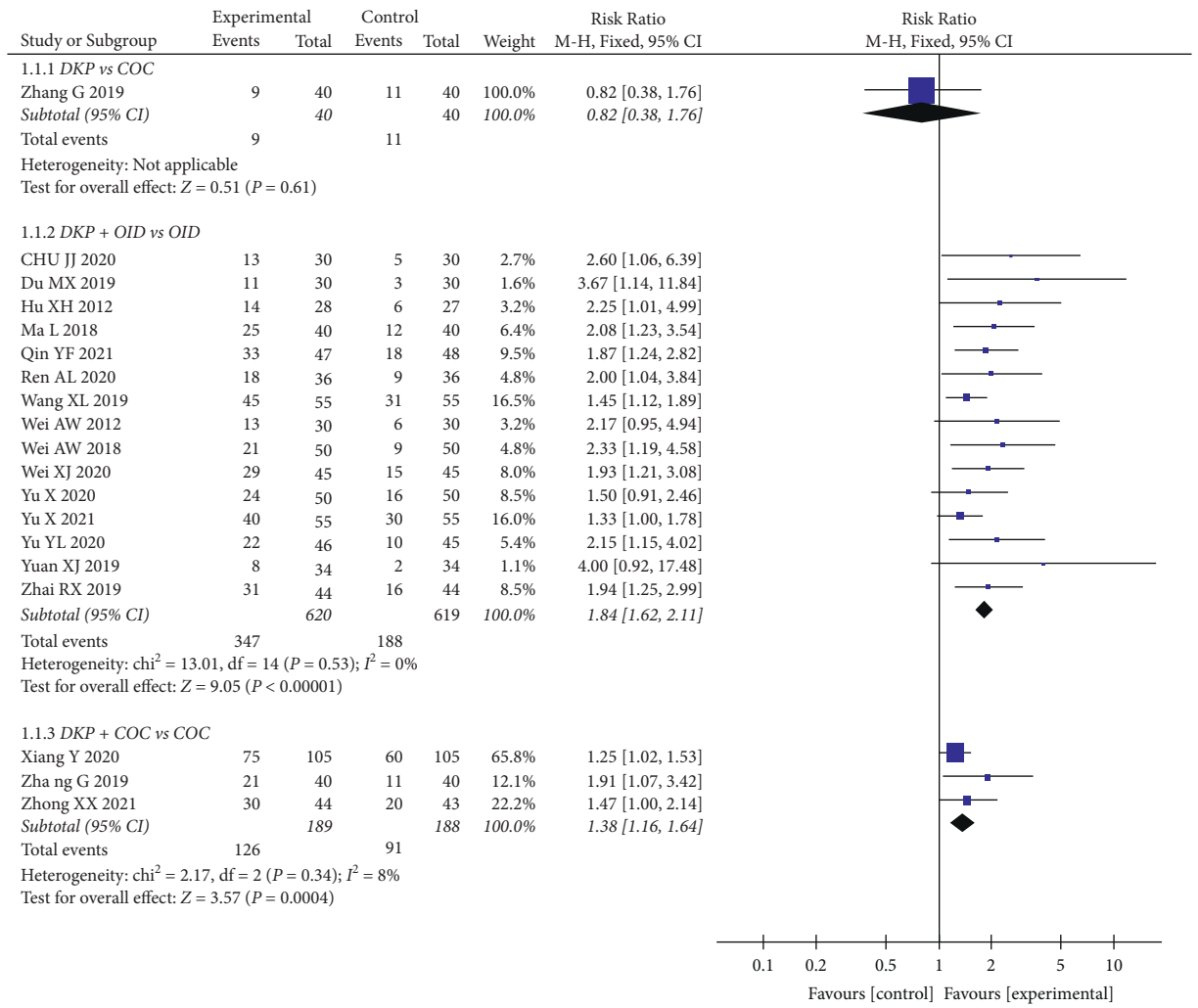

Figure 3: Meta-analyses of the effects of DKP on the pregnancy rate.

significantly decreased LH (WMD: −1.93, 95% CI: −2.80 to−1.07, $P < 0.0001$). Similarly, the combination of DKP and COC was superior in reducing LH compared to COC alone (WMD: −1.79, 95% CI: −2.66 to−0.92, $P < 0.0001$). There was no significant difference between DKP versus COC in reducing LH (WMD: 0.04, 95% CI: −0.29 to−0.37, $P = 0.81$) (Figure 6).

Figure 7 shows the meta-analysis of FSH in 13 RCTs with a total of 1175 patients. DKP did not appear to have a significant effect on improving FSH because no statistically significant differences were seen: DKP + OID versus OID (WMD: −0.37, 95% CI: −0.92 to 0.18, $P = 0.19$), DKP + COC versus COC (WMD: −0.24, 95% CI: −1.03 to 0.55, $P = 0.55$), and DKP versus COC (WMD: 0.04, 95% CI: −0.53 to 0.61, $P = 0.89$). However, a significant difference in the level of FSH was found in the subgroup analysis stratified by the duration of the intervention. In the stratified analysis, interventions lasting three months or more had a significant effect on FSH levels (WMD: −0.40, 95% CI: −0.68 to−0.12, $P = 0.006$). The details of the subgroup analyses are shown in the Supplementary Materials.

A meta-analysis of 13 trials (1157 patients) found that T decreased more after DKP plus OID or COC treatment in comparison with OID or COC alone (OID: SMD: −2.12, 95% CI: −3.01 to−1.24, $P < 0.00001$; COC: SMD: −1.21, 95% CI: −1.64 to−0.78, $P < 0.00001$). On the contrary, the comparison of the DKP groups with the COC groups did not show a significant difference in T (SMD: 0.18, 95% CI: −0.26 to 0.62, $P = 0.42$) (Figure 8).

3.4.3. Effects on Metabolic Indexes. The effects of DKP on FINS were assessed in 3 RCTs (372 patients). There was a significant decline in the FINS level after COC intake in comparison with DKP + COC treatment (WMD: 2.57, 95% CI: 2.22 to 2.91, $P < 0.00001$), while no significant difference was found between DKP and COC on FINS (WMD: −1.52, 95% CI: −6.53 to 3.49, $P = 0.55$) (Figure 9).

One RCT reported FBG as an outcome measure and did not observe any significant change in FBG in PCOS patients after DKP treatment compared to COC treatment (WMD: 0.10, 95% CI: −0.09 to 0.29, $P = 0.31$). In addition, compared with the COC group, there were no significant differences in FBG in the DKP + COC group (WMD: 0.10, 95% CI: −0.15 to 0.35, $P = 0.43$) (Table 2).

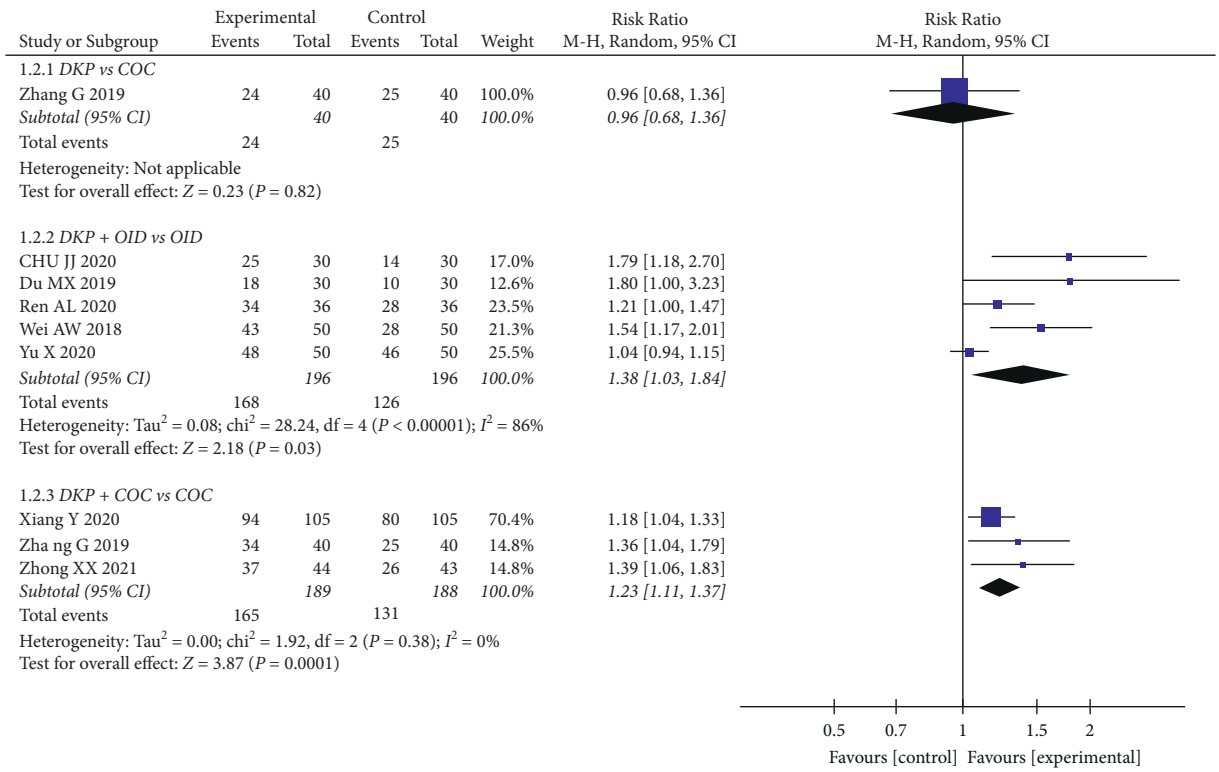

Figure 4: Meta-analyses of the effects of DKP on the ovulation rate.

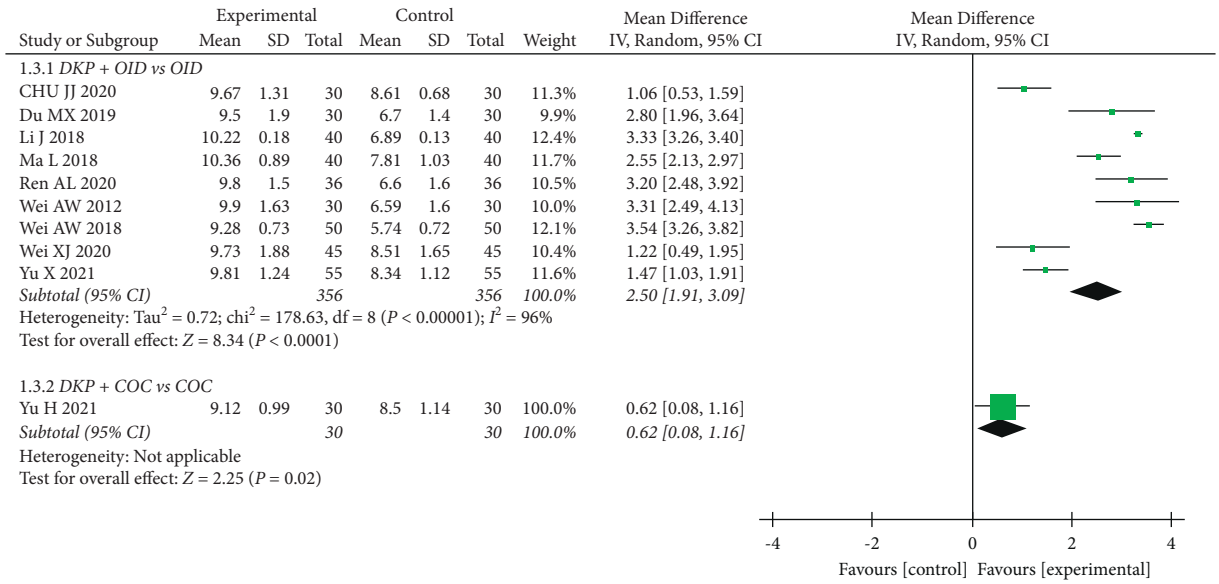

Figure 5: Meta-analyses of the effects of DKP on the endometrial thickness.

When we combined data from two studies (297 patients), a significant increase in HOMA-β was observed in DKP + COC treatment (WMD: 20.42, 95% CI: 16.85 to 23.98, $P < 0.00001$) (Figure 10).

3.4.4. Effects on Lipid Profiles. As illustrated in Table 2, DKP in PCOS patients showed a significant reduction in serum TC (WMD: −0.37, 95% CI: −0.72 to−0.02, $P = 0.04$), TG (WMD: −0.85, 95% CI: −1.50 to−0.20, $P = 0.01$), and FFA (WMD: −130.00, 95% CI: −217.56 to−42.44, $P = 0.004$) levels versus the COC group. However, COC improved the serum concentration of HDL-C compared to the DKP group (WMD: −0.35, 95% CI: −0.55 to−0.15, $P = 0.0008$). There was no significant change in TC (WMD: 0.18, 95% CI:−0.16 to 0.52, $P = 0.31$), TG (WMD: −0.19, 95% CI: −1.02 to 0.64, $P = 0.65$), LDL-C (WMD: 0.21, 95% CI: −0.11 to 0.53, $P = 0.20$), HDL-C (WMD: −0.04, 95% CI: −0.29 to 0.21, $P = 0.75$), or FFA (WMD: −67.00, 95% CI: −157.12 to 23.12, $P = 0.15$) when DKP + COC was administered (Table 2).

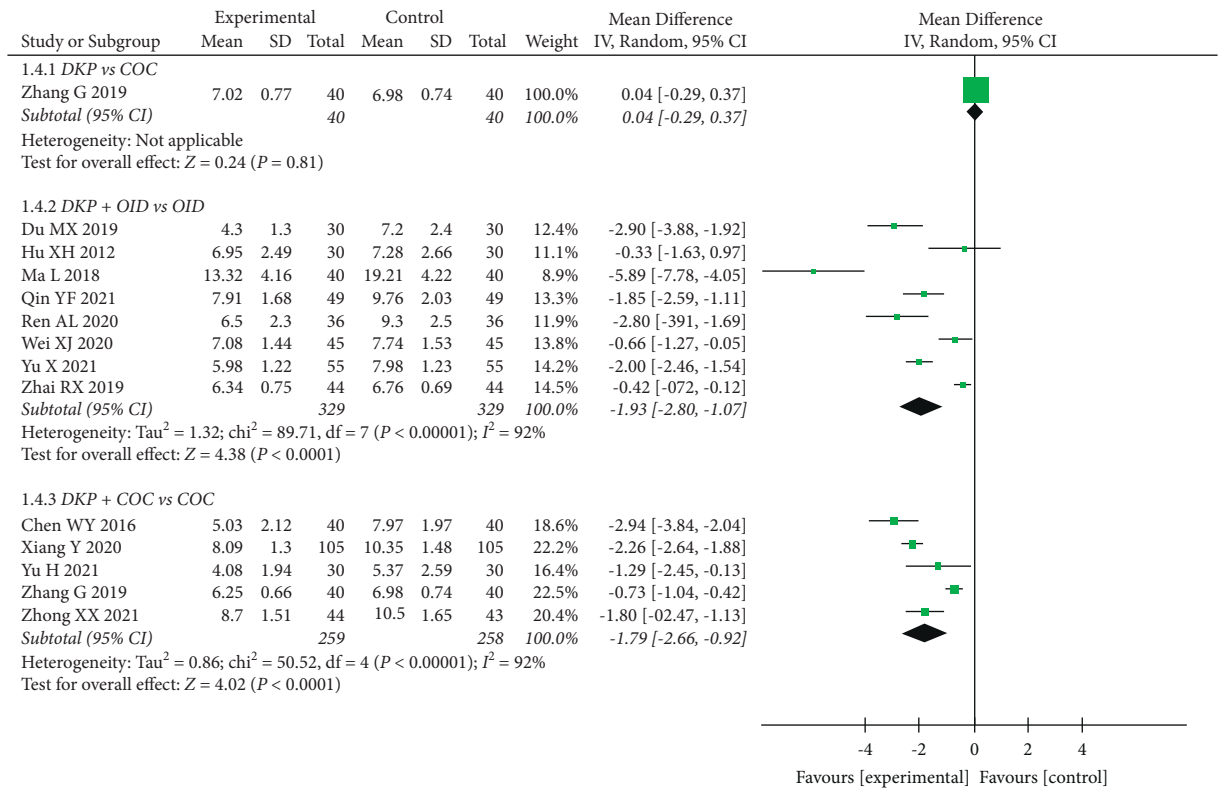

Figure 6: Meta-analyses of the effects of DKP on LH.

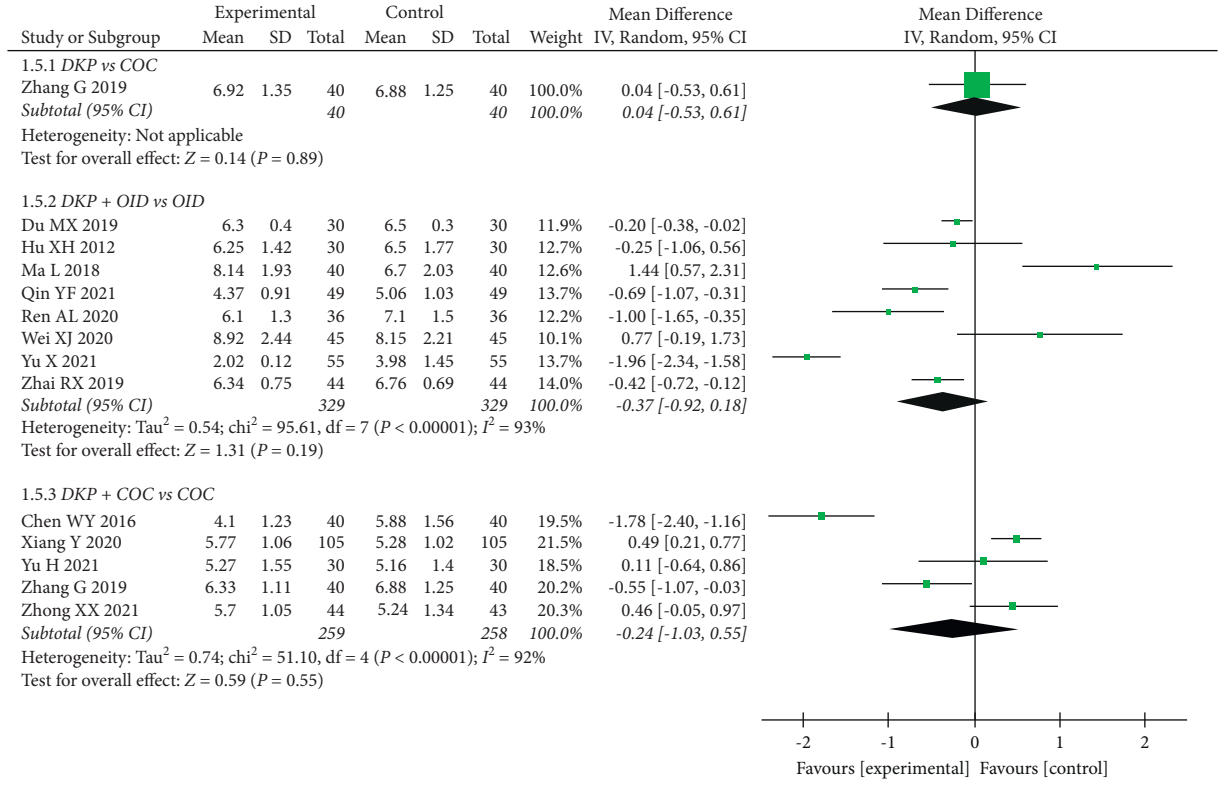

Figure 7: Meta-analyses of the effects of DKP on FSH.

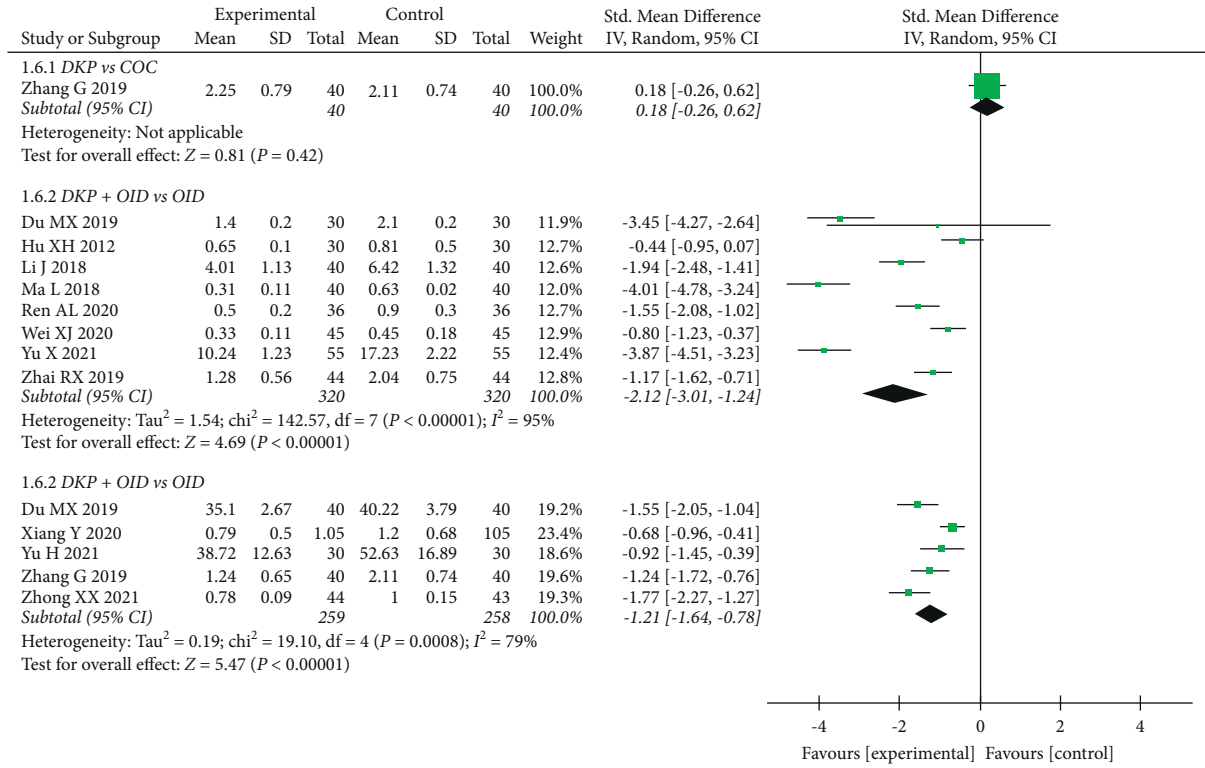

Figure 8: Meta-analyses of the effects of DKP on T.

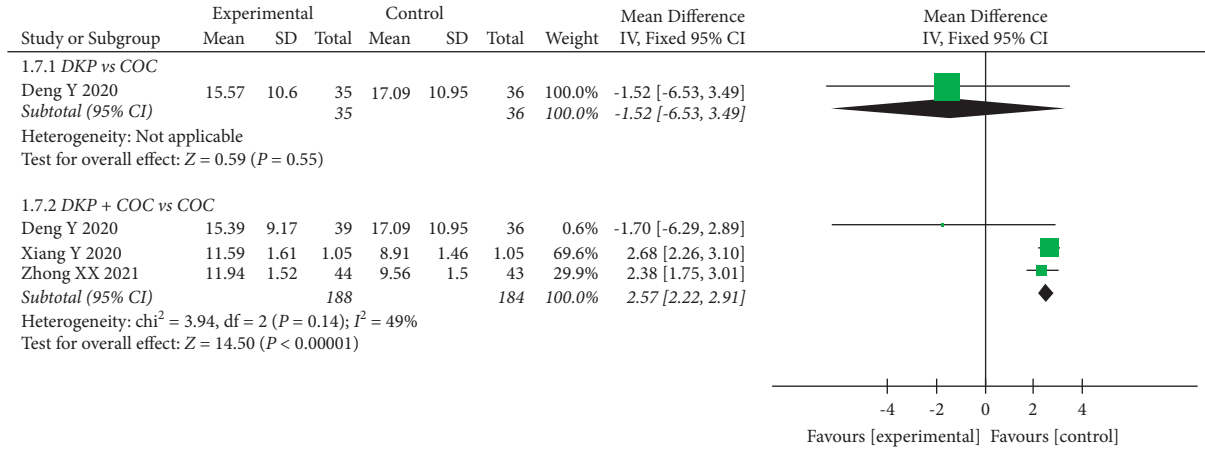

Figure 9: Meta-analyses of the effects of DKP on FINS.

3.4.5. Effects on Anthropometric Indices. BMI and WHR were measured in only one trial, and there were no significant changes in BMI or WHR for DKP alone (BMI: WMD: 0.70, 95% CI: −1.81 to 3.21, $P = 0.59$; WHR: WMD: 0.00, 95% CI: −0.03 to 0.03, $P = 1.00$) or for DKP + COC (BMI: WMD: 0.60, 95% CI: −1.92 to 3.12, $P = 0.64$; WHR: WMD: −0.01, 95% CI: −0.04 to 0.02, $P = 0.47$) (Table 2).

3.5. Adverse Reactions. Ten trials [10, 19, 21, 22, 27, 29, 34, 39, 40, 43–45] recorded adverse events, of which eight trials reported that there were no adverse reactions. In the other two studies [43, 44], statistical analysis showed that DKP + COC was associated with fewer adverse events compared to COC alone (RR = 0.22, 95% CI: 0.07 to 0.63, $P = 0.005$) (Figure 11). The results of Chen et al. [43] showed that adverse reactions occurred in the intervention group (mild nausea in 1 case and mild breast pain in 1 case) and the control group (mild nausea in 3 cases, mild breast pain in 2 cases, interphase hemorrhage in 1 case, and mild headache in 2 cases). In Yu's study [44], three patients had adverse reactions in the DKP + COC group, including TC elevation ($n = 2$) and direct bilirubin elevation ($n = 1$), and ten patients had adverse reactions in the COC group, including direct bilirubin elevation ($n = 3$), glutamic pyruvic transaminase elevation ($n = 1$), apolipoprotein A1 elevation ($n = 1$), TC

Table 2: Data and analyses of RCTs included in this systematic review and meta-analysis.

Outcome or subgroup	Participants	Mean difference	95% CI	*P* Value
FBG				
DKP vs. COC	71	0.10	[−0.09, 0.29]	0.31
DKP + COC vs. COC	75	0.10	[−0.15, 0.35]	0.43
BMI				
DKP vs. COC	71	0.70	[−1.81, 3.21]	0.59
DKP + COC vs. COC	75	0.60	[−1.92, 3.12]	0.64
WHR				
DKP vs. COC	71	0.00	[−0.03, 0.03]	1.00
DKP + COC vs. COC	75	−0.01	[−0.04, 0.02]	0.47
TC				
DKP vs. COC	71	−0.37	[−0.72, −0.02]	0.04
DKP + COC vs. COC	75	0.18	[−0.16, 0.52]	0.31
TG				
DKP vs. COC	71	−0.85	[−1.50, −0.20]	0.01
DKP + COC vs. COC	75	−0.19	[−1.02, 0.64]	0.65
LDL-C				
DKP vs. COC	71	0.09	[−0.23, 0.41]	0.58
DKP + COC vs. COC	75	0.21	[−0.11, 0.53]	0.20
HDL-C				
DKP vs. COC	71	−0.35	[−0.55, −0.15]	0.0008
DKP + COC vs. COC	75	−0.04	[−0.29, 0.21]	0.75
FFA				
DKP vs. COC	71	−130.00	[−217.56, −42.44]	0.004
DKP + COC vs. COC	75	−67.00	[−157.12, 23.12]	0.15

DKP, Dingkun pill; COC, combined oral contraceptives; FPG, fasting blood glucose; BMI, body mass index; WHR, waist-to-hip ratio; TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acid.

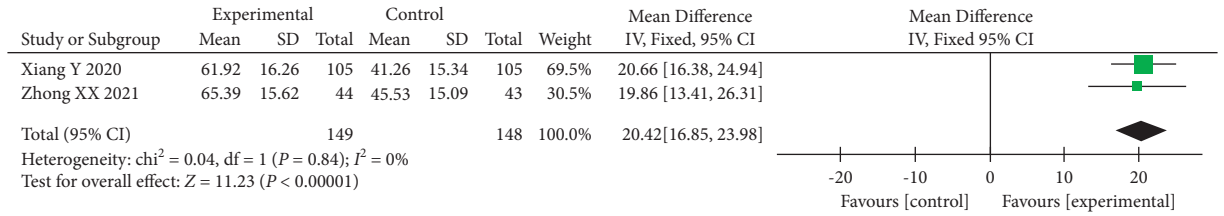

Figure 10: Meta-analyses of the effects of DKP on HOMA-β.

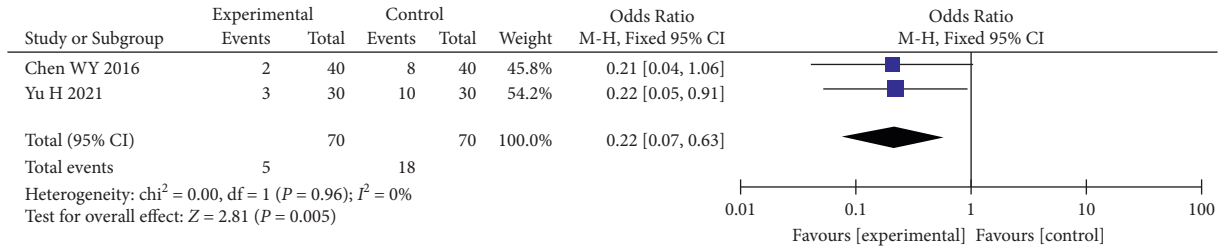

Figure 11: Forest plot for overall adverse reactions.

elevation ($n = 2$), TG elevation ($n = 2$), and breast pain ($n = 1$). All the above adverse reactions were mild, and no serious adverse events were observed in the included trials.

3.6. Sensitivity Analysis. When the heterogeneity was high, we performed a sensitivity analysis. The results indicated that there was no significant change in the effect size of endometrial thickness, LH, or T after the one-by-one exclusion of the included literature, which confirmed the stability and reliability of the meta-analysis. Furthermore, removing Xiang et al. [41], whio investigated the influence of DKP on T, led to a decrease in heterogeneity, while the result remained significant (SMD: −1.37, 95% CI: −1.73 to−1.02, $P = 0.00001$, $I^2 = 49\%$). However, the result of FSH was not robust, and removing Ma et al. [29] resulted in a positive

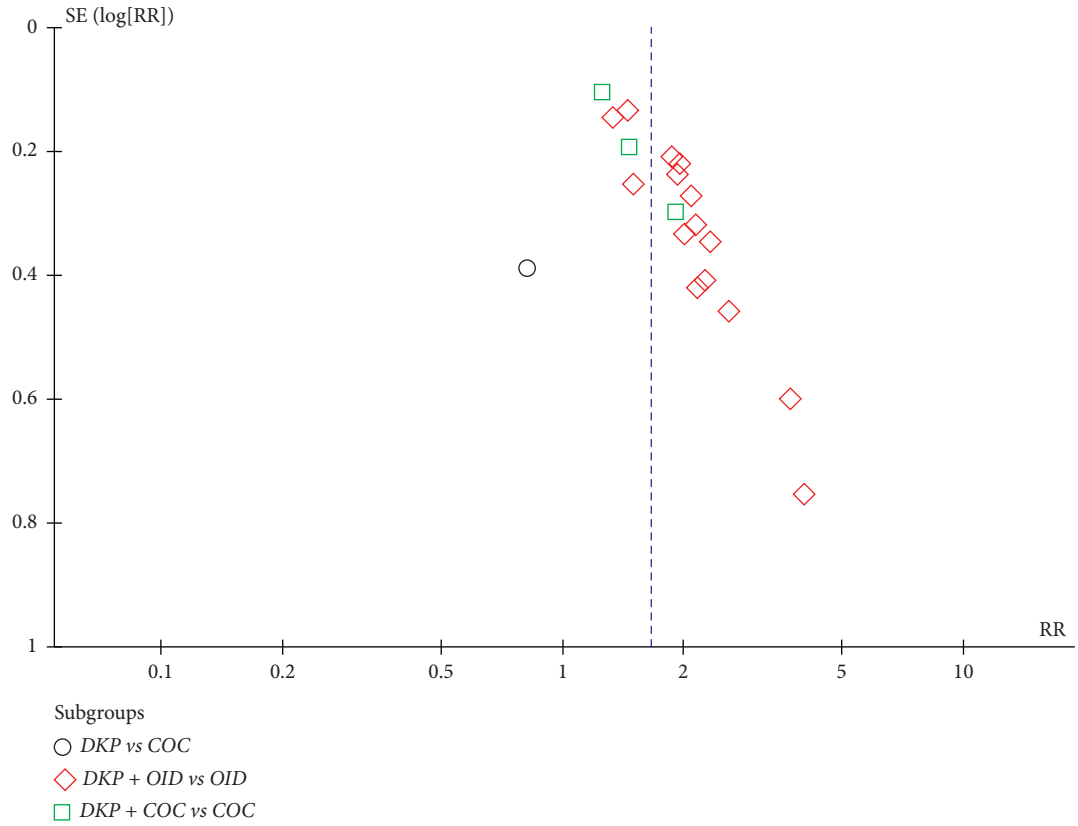

Figure 12: The funnel plot of the literature.

overall effect (WMD: −0.59, 95% CI: −1.13 to−0.05, $P = 0.003$).

3.7. Publication Bias. A funnel plot was used to evaluate publication bias, which showed that the symmetry between different studies was poor and that publication bias existed (Figure 12).

3.8. Quality of the Evidence. Table 3 shows a summary of the quality of evidence grades for selected primary outcomes. The quality of the evidence was downgraded to low or very low certainty according to the GRADE system. The main reason for this degradation was the limitations of the original studies because of the lack of randomization allocation and blinding, the unexplained heterogeneity between studies in the estimates of the treatment effects, the number of patients included being less than 400, and the publication bias.

4. Discussion

4.1. Main Results. The present study is the latest and most comprehensive systematic review of the effects of DKP on reproduction and metabolism in PCOS patients, including 22 RCTs (1994 participants). In this review, DKP was shown to significantly ameliorate 1) reproduction issues as evidenced by increased pregnancy rate, ovulation rate, and endometrial thickness, 2) hormone imbalances as assessed by decreased LH and T, 3) metabolic disorders as assessed by increased HOMA-β, and 4) lipid profile changes as evidenced by decreased TC, TG, and FFA in PCOS patients. However, DKP combined with Western medicine had no significant effects over Western medicine alone on anthropometric indices (BMI and WHR).

According to traditional Chinese medicine, the occurrence of PCOS is closely related to the kidneys, liver, spleen, and the Chong and conception channels, with kidney deficiency being the main cause followed by liver depression and spleen deficiency [47–49]. The combination of stasis blood, phlegm, and fluid retention, as well as water-dampness leads to a series of clinical symptoms. DKP is composed of the ingredients that have an effect on these symptoms, such as pilose antler (*Cornu Cervi Pantotrichum*), barbary wolfberry fruit (*Fructus Lycii*), and degelatined deer-horn (*Cornu Cervi Degelatinatum*), which warm the kidney, reinforce Yang, and nourish the liver and kidney. Chinese thorowax root (*Radix Bupleuri*), nutgrass galingale rhizome (*Rhizoma Cyperi*), Sichuan lovage rhizome (*Rhizoma Ligustici Chuanxiong*), and debarked peony root (*Radix Paeoniae Alba*) are combined to form the representative prescription Chaihu Shugan powder, which is used to disperse stagnated liver qi. Adding safflower (*Flos Carthami*), sanqi (*Radix Notoginseng*), motherwort fruit (*Fructus Leonuri*), and yanhusuo (*Rhizoma Corydalis*) promotes blood flow for regulating menstruation. Red ginseng (*Radix Ginseng Rubra*), largehead atractylodes rhizome (*Rhizoma Atractylodis Macrocephalae*), Indian bread (*Poria*), and licorice root (*Radix Glycyrrhizae*) are the

Table 3: Quality of the evidence of selected primary outcomes according to the GRADE Working Group.

Quality of assessment							Number of patients		Effect			
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Experimental	Control	Relative (95% CI)	Absolute (95% CI)	Quality	Importance
Pregnancy rate: *DKP + OID vs. OID*												
14	RCT	Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	Suspected[d]	325/574 (56.6%)	178/574 (31%)	RR 1.83 (1.6 to 2.09)	257 more per 1000 (from 186 more to 338 more	⨁⨁◯◯ LOW	CRITICAL
Pregnancy rate: *DKP + COC vs. COC*												
3	RCT	Serious[a]	No serious inconsistency	No serious indirectness	Serious[c]	Undetected	126/189 (66.7%)	91/188 (48.4%)	RR 1.38 (1.16 to 1.64)	184 more per 1000 (from 77 more to 310 more	⨁⨁◯◯ LOW	CRITICAL
Ovulation rate: *DKP + OID vs. OID*												
5	RCT	Serious[a]	Serious[b]	No serious indirectness	Serious[c]	Undetected	168/196 (85.7%)	126/196 (64.3%)	RR 1.38 (1.03 to 1.84)	244 more per 1000 (from 19 more to 540 more	⨁◯◯◯ VERY LOW	CRITICAL
Ovulation rate: *DKP + COC vs. COC*												
3	RCT	Serious[a]	No serious inconsistency	No serious indirectness	Serious[c]	Undetected	165/189 (87.3%)	131/188 (69.7%)	RR 1.23 (1.11 to 1.37)	160 more per 1000 (from 77 more to 258 more	⨁⨁◯◯ LOW	CRITICAL
Endometrial thickness: *DKP + OID vs. OID*												
9	RCT	Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Undetected	356	356	—	WMD 2.5 higher (1.91 to 3.09 higher)	⨁⨁◯◯ LOW	CRITICAL

CI, confidence interval; RR, risk ratio; WMD, weighted mean difference; RCT, randomized controlled trial; DKP, Dingkun pill; OID, ovulation inducing drugs; COC, combined oral contraceptives. [a]Randomization allocation and the blinding are unclear. [b]I^2 value was large. [c]Number of patients included was less than 400. [d]Funnel plot indicated a significant asymmetry.

famous prescriptions of Sijunzi decoction used for invigorating the spleen-stomach and replenishing qi, while debarked peony root (*Radix Paeoniae Alba*), prepared rehmannia root (*Radix Rehmanniae Preparata*), Chinese angelica (*Radix Angelicae Sinensis*), and Sichuan lovage rhizome (*Rhizoma Ligustici Chuanxiong*) are combined into a Siwu decoction as the basic prescription for nourishing blood for regulating menstruation, and the two prescriptions are combined to make the Bawu decoction, especially for benefiting qi and nourishing blood. Ass hide glue (*Colla Corii Asini*) is added to nourish Yin and tonify blood, and Baikal skullcap root (*Radix Scutellariae*) is added for clearing heat, removing dampness, and making the mixture tonic but not dry. Finally, the mixture is supplemented with honey (*Mel*) to reconcile the herbs. The whole prescription of DKP is rigorously formulated to harmonize Yin and Yang, coordinate Chong and the conception vessels, reinforce and eliminate in combination, nourish without stagnation and greasiness, and disperse without dispelling, all with the effect of nourishing the liver and the kidney, regulating menstruation and relieving Qi stagnation, and benefiting Qi and nourishing the blood.

Recent studies have also confirmed the efficacy of DKP in the treatment of PCOS. The chemical profiling of DKP by ultra high-performance liquid chromatography Q-exactive Orbitrap high-resolution mass spectrometry characterized over one hundred components and isomers, including amino acids, phenolic acids, lactones, terpenoids, alkaloids, saponins, flavonoids, and other compounds, among which paeoniflorin, ginsenosides, and notoginsenosides were present at high levels [18]. Modern pharmacological analysis suggests that paeoniflorin from debarked peony root (*Radix Paeoniae Alba*), rehmannia glutinosa polysaccharides from prepared rehmannia root (*Radix Rehmanniae Preparata*), and amino acids and proteins from ass hide glue (*Colla Corii Asini*) can enhance the hematopoietic function of the body [50–52]. Ginsenosides and notoginsenosides have been shown to be beneficial for insulin sensitivity and metabolic functions [18, 53]. Velvet antler polypeptides are one of the base components of the medicinal substances in pilose antler (*Cornu Cervi Pantotrichum*) and have fertility-enhancing effects [54]. The volatile oil components of largehead atractylodes rhizome (*Rhizoma Atractylodis Macrocephalae*) have both excitatory and inhibitory effects on the uterine smooth muscle to improve reproductive outcomes [55]. Ferulic acid in Chinese angelica (*Radix Angelicae Sinensis*) and Sichuan lovage rhizome (*Rhizoma Ligustici Chuanxiong*) and safflower yellow pigment in safflower (*Flos Carthami*) have shown inhibitory effects on platelet aggregation and release [56]. The lycium barbarum polysaccharide in the barbary wolfberry fruit (*Fructus Lycii*) can lower blood lipids and glucose levels [57]. Previous studies have shown that Chaihu Shugan powder, which is included in DKP, can regulate HPOA in women, thereby affecting serum hormone levels [58]. Sijunzi decoction has both hypoglycemic and hypolipidemic effects [59], and Siwu decoction has been proven to be effective in reversing infertility [60]. Thus, it can be seen that any of the Chinese herbal medicines or prescriptions contained in DKP exert their respective efficacies through their complex composition, which reflects the synergistic efficacy of Chinese herbal medicines among the composition of prescriptions.

Based on our analyses, DKP appears to have a positive effect on increasing the pregnancy rate, ovulation rate, and endometrial thickness in PCOS patients. Moreover, DKP also has effects on decreasing serum LH and T levels in patients with PCOS, and DKP may improve fertility through multiple possible mechanisms. PCOS is closely associated with HPOA functional disorders [2], including accelerated gonadotropin releasing-hormone pulsatile activity, increased secretion of pituitary LH, and increased ovarian secretion of T and estrogen, which can inhibit the development of follicles and oocytes, eventually contributing to ovulatory dysfunction [61]. A correlation exists between hyperandrogenemia and the development of IR and hyperinsulinemia [62], and excessive androgen also results in elevated levels of LH and FSH in PCOS patients [63]. DKP has a positive effect on restoring the feedback inhibition of HPOA, thus reducing the level of reproductive hormones, including T, LH, and FSH [16]. Furthermore, the implantation of fertilized eggs is impaired in PCOS patients because of changes in endometrial receptivity or to endometrial dysplasia because of inadequate exposure to progesterone [64]. HOXA10 is a characteristic marker of endometrial receptivity and is affected by the level of hormones [65]. An animal trial showed that DKP may play a role in improving endometrial receptivity by enhancing the expression of uterine HOXA10 [53]. In addition, ovarian fibrosis, which is characterized by the excessive proliferation of ovarian fibroblasts and deposition of extracellular matrix, is one of the pathophysiological causes of follicular dysplasia and ovulatory dysfunction in patients with PCOS [66]. DKP could be a promising approach to treating PCOS by downregulating the expression of transforming growth factor-beta 1 and connective tissue growth factor to interfere with extracellular matrix deposition [17].

IR occurs in 50% to 70% of women with PCOS [67], and the pathophysiology of type 2 diabetes mellitus is influenced by IR and abnormal glucose metabolism [68]. Most women with a family history of type 2 diabetes mellitus demonstrate impaired β-cell function or a subnormal disposition index [69]. In this meta-analysis, compared with COC alone, it was observed that DKP combined with COC significantly increased HOMA-β levels in PCOS patients, which suggested that DKP might have an effect on improving insulin sensitivity. We also assessed the effects of DKP on FBG and FINS in PCOS patients. However, we were unable to find any statistical difference between the intervention and control group. It is worth mentioning that Deng et al.'s study [21] found that FBG was significantly decreased in PCOS patients after taking DKP for three months. Ginsenosides, one of the bioactive components of DKP, play a role in inhibiting the increase in blood glucose seen in PCOS patients with IR. The hypoglycemic effect of ginsenosides is mainly achieved by inhibiting hepatic gluconeogenesis, activating the AMPK signaling pathway, and stimulating glucose uptake [70]. Taken together, DKP may have a greater effect on insulin sensitivity than on IR, and DKP administration decreases glucose levels by increasing insulin sensitivity.

Clinical evidence shows a close association between cardiovascular disease and atherogenic dyslipidemia, which is characterized by elevated TC, TG, and LDL-C, and reduced HDL-C [71]. About 70% of women with PCOS in the U.S. suffer from dyslipidemia and possibly an increased risk of developing cardiovascular diseases [72, 73]. Notably, our observations provide a novel dimension to present evidence for the beneficial effects of DKP in mitigating dyslipidemia in women with PCOS. DKP significantly altered TC, TG, and FFA levels compared with the COC group in this meta-analysis. While there was inadequate evidence that DKP had a favorable influence on LDL-C and HDL-C in the present meta-analysis, some findings provided support for further investigation. One RCT reported a reduction in LDL-C and no increase in HDL-C in subjects with PCOS after the intake of DKP [22]. Overweight or obesity increases the risk for metabolic syndrome and cardiovascular disease in women with PCOS [74], and there is evidence that obesity may exacerbate IR, hyperandrogenism, or ovulatory dysfunction in PCOS, which can lead to infertility [75]. Although there was a lack of significant effect of DKP on BMI and WHR in our meta-analysis, some findings provided evidence for further exploration. The notoginsenosides found in DKP are potential active ingredients for the treatment of obesity by reducing lipid synthesis, inhibiting adipogenesis, increasing energy expenditure, and improving insulin sensitivity [76]. It can thus be seen that the results of this analysis were negative because of the insufficient survey of this critically important, yet largely ignored area, however, we cannot exclude a possible regulatory effect of DKP on anthropometric indices in PCOS patients.

Of the included trials, only two trials reported adverse reactions [43, 44]. One study [43] reported gastrointestinal or breast discomfort in the control group and the intervention group, however, the numbers of events were small and the symptoms were mild. In the other trial [44], there were three patients with abnormal serum biochemical indexes in the intervention group, however, there were ten patients presenting with abnormal indexes in the control group, and there were significant differences between the two groups. It should be mentioned that eight trials [10, 19, 21, 22, 27, 29, 34, 39, 40, 45] indicated that no adverse reactions occurred during the treatment. In addition, it is mentioned in the instructions of DKP that patients should stop taking it if they have a cold or flu. According to the current evidence, we believe that DKP is a relatively safe treatment. However, the long-term safety and efficacy of DKP in PCOS patients remains to be further explored.

4.2. Strengths and Limitations. This review has several strengths. We used metabolic indexes, lipid profiles, and anthropometric indexes as new evaluation indicators to discuss the efficacy of DKP on PCOS for the first time, consequently providing more possible therapies for PCOS. A previous meta-analysis [77], which only involved seven studies (with 658 participants), focused on the effectiveness of the combination of DKP and western medicine in ameliorating reproductive issues (ovulation rate, pregnancy rate, endometrial thickness, and hormone parameters), however, it did not perform an analysis of the effectiveness in regulating metabolic function in patients with PCOS. Moreover, our research has been registered on PROSPERO, and all procedures were faithfully executed accordingly, thus increasing the credibility of our results.

However, a few limitations exist in our study. Firstly, the follow-up time of these trials was inadequate, and the majority patients involved in the studies accepted about three months of treatment, and there was no evaluation of long-term outcomes. The live birth rate bears a great role in infertility clinical trials and is recognized as the major outcome [78]. Because of the lack of live birth rate, the trials included in this meta-analysis were insufficient to comprehensively address the role of DKP on reproductive health. Secondly, the heterogeneity between studies may stem from limitations in the methodological quality of the 22 RCTs included, such as the inappropriate use of blinding and differences in allocation concealment. Thirdly, although there were no language restrictions in the search, all trials were conducted in China, and 21 studies were published in domestic journals, which may lead to publication bias. It has been shown that publication bias in Chinese medical journals exists objectively because of the fact that negative results from clinical studies are not easily published and trials with small sample sizes are published [79]. The limitations mentioned above may affect the reliability of this meta-analysis, and thus the results in this review need to be interpreted with caution.

4.3. Implications for the Future. Clinical studies on interventions using DKP for PCOS are gradually increasing. In our meta-analysis, 18 RCTs were published between 2012 and 2020, followed by 4 RCTs published in 2021, indicating that DKP is a relatively new treatment for PCOS and that this intervention has great research value. Furthermore, we hope that modern clinical research on Chinese patent herbal medicines (such as DKP) can be spread to other countries to obtain more high-quality evidence for the use of such medicines in the clinic. At present, there is no single drug that is capable of treating infertility and metabolic complications associated with PCOS. As a multicomponent drug compound, DKP may have a role in the treatment of PCOS through multitarget synergistic actions. Therefore, future research on DKP for PCOS should focus on metabolic outcomes to identify more therapeutic methods for treating PCOS.

5. Conclusion

In summary, our results indicate that DKP has a promising application in modifying the reproductive and metabolic abnormalities in patients with PCOS and may be used as a primary choice in conventional or complementary therapies for PCOS. However, considering the inherent limitations and heterogeneity among the studies analyzed here, our results should be interpreted with caution. We expect more prospective large-scale and well-designed RCTs with longer

intervention durations to further determine the clinical efficacy and safety of DKP in treating PCOS.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Bao Jin and Yang Zhang are the co-first authors.

Acknowledgments

This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (81803945), the Scientific Research Project of Traditional Chinese Medicine in Heilongjiang Province (ZHY19024), and the Project of Young Innovative Talents in Colleges and Universities in Heilongjiang Province (UNPYSCT-2016216).

Supplementary Materials

Table S1. Search strategy. Figures S1–S6. Results of subgroup analyses. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1790041, 15 pages
https://doi.org/10.1155/2022/1790041

Research Article

Acupuncture Improves Endometrial Angiogenesis by Activating PI3K/AKT Pathway in a Rat Model with PCOS

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Received 20 April 2022; Revised 20 July 2022; Accepted 9 August 2022; Published 24 August 2022

Academic Editor: Min Hu

Background. Acupuncture, a treatment derived from traditional Chinese medicine, can effectively relieve the symptoms and improve pregnancy outcome in patients with polycystic ovary syndrome (PCOS); however, its mechanism remains unclear. This study aimed at investigating whether acupuncture could improve endometrial angiogenesis and thus endometrial receptivity via activating PI3K/AKT pathway in PCOS rats. *Methods*. We established a rat model with PCOS, which was induced by DHEA. Acupuncture was performed every other day for 15 days, and the PI3K inhibitor (LY294002) was intraperitoneal injected 30 mins before acupuncture treatment. Females rats were mated with male SPF SD rats in a ratio of 2 : 1 after treatment and sacrificed on the 5th day when the vaginal plug was identified. The number of implantation sites was observed, followed by ovarian and endometrial morphology detected with hematoxylin-eosin staining and a scanning electron microscope, estrous cycle detected with vaginal smear analysis, and sex hormones and angiogenesis-related PI3K/AKT gene/protein expression detected with enzyme-linked immunosorbent assay, western blotting, immune histochemistry, and quantitative real-time PCR. *Results*. Acupuncture notably improved implantation sites' number, endometrial receptivity factors including endometrial morphology, pinopodes, HOX-10, and LIF protein expression, as well as angiogenesis and PI3K/AKT pathway factors such as VEGF, VEGFR2, Ang-1, PI3K, AKT, and P-AKT gene/protein expression and the level of eNOS and NO in the endometrium of rats with PCOS; PCOS-like symptoms were alleviated as well. The efficacy of acupuncture on a rat model with PCOS was counteracted by the combination with the PI3K inhibitor. *Conclusion*. Acupuncture improves endometrial angiogenesis by activating the PI3K/AKT pathway, thus promoting endometrial receptivity and the number of implantation sites in rats with PCOS.

1. Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine and metabolic disorder in gynecology and mainly characterized by hyperandrogenism and insulin resistance [1]. Up to 72% of patients with PCOS experience infertility, with the prevalence among women of reproductive age being 5–20% [2]. Infertility due to PCOS will exacerbate patients' psychological illnesses including anxiety, depression, and other mental health issues, which will eventually result in metabolic syndrome (obesity), type 2 diabetes, and cerebrovascular disorders [3]. Gonadotropin-releasing hormone agonist (GnRHa) or clomiphene (CC) is currently being used for PCOS, with ovulation rates of 50%, pregnancy rates of only 23.9%, and abortion rates of 25.8% [4]. Therefore, it is crucial to explore the effective methods alleviating a high ovulation rate and a low pregnancy rate in patients with PCOS.

Impaired endometrial receptivity (ER), which was induced by the dysfunction of cytokines, proteomics, and gene expression, is the major reason for low fertility as it prevents embryo implantation development in patients with PCOS [5]. The endometrial epithelium permits implantation during a brief period known as the implantation window (WOI) due to the endometrial receptivity [6]. Recently, studies have focused on angiogenesis together with increased vascular permeability, which were considered to be the key factors in endometrial decidualization, embryo implantation, and placentation. Vascular endothelial growth factor (VEGF) is a representative regulator of angiogenesis and vascular permeability [7]. Kodaman et al. reported that the increased expression of VEGF in the middle and late menstrual luteal periods was spatially synchronized with the WOI, which could be considered a molecular marker of ER [8–10]. Zhao et al. reported that VEGF mRNA and protein expression in PCOS endometrium decreased significantly at the WOI, which indicated impaired endometrium angiogenesis existed in patients with PCOS [11]. VEGF activates through the PI3K/AKT signaling pathway, which is the regulatory center for angiogenesis. Wang et al. reported that the expression of PI3K/AKT signaling pathway-related proteins was impaired during the WOI period of patients with PCOS [12]. Above all, we speculated the decreased endometrial receptivity induced by PI3K/AKT signaling pathway-mediated angiogenesis disorder was involved in the occurrence of infertility in PCOS.

Acupuncture is a traditional Chinese medicine without obvious side effects. A great deal of studies proved that acupuncture could effectively increase the pregnancy rate and relieve the symptoms of PCOS. Cheng's research suggested that acupuncture might increase the pregnancy rate of patients undergoing IVF-ET by the regulation of ncRNAs [13]. Xu's research suggested that acupuncture can break the vicious cycle initiated by excessive androgen secretion and regulate the androgen receptor as well as connexin 43 in PCOS to improve the reproductive function [14]. Studies reported that acupuncture can improve endometrial receptivity in superovulation mice via miR-494-3p/HOXA10 axis [15]. Our previous study revealed that angiogenesis disorder may be related to ER disorder during the WOI period in patients with PCOS due to the decreasing VEGF expression in the endometrium, while acupuncture can notably increase VEGF expression and improve endometrial thickness, morphology, blood perfusion, and endometrial receptivity, and ultimately pregnancy rates. Although the mechanism is yet unknown, few studies have examined the effectiveness of acupuncture in improving ER at the WOI stage; in addition, the study focused on the endometrial PI3K/AKT pathway during the WOI period in patients with PCOS. The majority of previous studies mostly focused on follicular growth and the proliferation of ovarian granulosa cells in PI3K/AKT and angiogenesis pathways. We hypothesized that acupuncture can improve endometrial angiogenesis by activating the PI3K/AKT pathway, thus promoting endometrial receptivity in rats with PCOS.

In this study, a PCOS-like rat model was established by dehydroepiandrosterone (DHEA) injection. Acupuncture-mediated modulation of the PI3K/AKT signaling pathway and its involvement in PCOS-like symptoms, endometrial receptivity, implantation sites' number, and angiogenesis were further investigated. Our findings shed light on the novel protective mechanisms of acupuncture in treating PCOS.

2. Materials and Methods

2.1. Animal Modeling and Grouping. Forty-five three-week-old female Sprague-Dawley (SD) rats were purchased from Hunan SJA Laboratory Animal Co, Ltd. (Hunan, China). Thirty-five female SPF SD rats were randomly selected to establish the PCOS model. Of these rats, 30 established PCOS models verified from the body weight, ovarian pathology, estrous cycle, and sex hormone levels were randomly divided into the model group (model), the acupuncture group (Acu), and the PI3K inhibitor + acupuncture group (PI + Acu) ($n = 10$ per group). In addition, 10 female SPF SD rats were selected as the normal control group (control). The PCOS model was induced by daily subcutaneous injection with DHEA (Biotopped, China) at a dose of 6 mg/100 g body weight and sesame oil (Liaoning Shinsun Pharmaceutical Co., Ltd, China) at a dose of 0.2 ml/d for 20 consecutive days [16]. After verifying the establishment of the PCOS model, appropriate treatment was initiated in each group. Acupuncture treatment was performed every other day for 15 days. According to our previous study [17–19], the acupuncture points SP4, PC6, EX-CA1, and CV4 will be selected. Huatuo milli-needles (0.19 mm × 10.00 mm, Suzhou Medical Equipment Co., Ltd.) were inserted perpendicularly or obliquely into the above-mentioned acupuncture points, the depth of which was 3 mm. The acupuncture duration was 20 min every time. The rats in the PI + Acu group received intraperitoneal injection with 0.3 mg/kg LY294002 (Abcam, UK) at 30 min before each acupuncture treatment [20]. The rats in the control and model groups were received intragastric administration with saline. Saline treatment was performed every other day for 15 days.

2.2. Sample Collection. After 15 days' treatment, the female rats were mated with male SPF SD rats in a ratio of 2 : 1. Rats were sacrificed during the implantation window time on the 5th day when the vaginal plug was identified. Rats were anesthetized with 3% pentobarbital sodium (0.1 ml/100 g of body weight), and then, blood samples were obtained from the abdominal aorta after overnight fasting. Blood samples were then centrifuged at 3000g for 15 min at 4°C for ELISA. The whole uteri from 5 random rats of each group were collected for implantation sites' number and SEM examination. The collection of endometria from the other 5 random rats of each group was divided into five parts: one for HE and four for gene and protein expression analysis using immunohistochemistry, ELISA, qRT-PCR, and western blot, respectively. The ovarian tissue will also be collected for HE detection.

2.3. Weighing. Body weight was measured every 2 days from the first day of DHEA subcutaneous injection. The last weighing was carried out at the time of sample collection.

The ovary wet weight was measured, and the ovarian index was calculated after the rats were sacrificed. Body weight gain = (weight of rats before sacrifice-initial weight)/initial weight × 100%. Ovarian index = ovarian wet weight/body weight.

2.4. Vaginal Smear Analysis. Vaginal smear analysis was performed daily at 9 am from 10 days after DHEA treatment. Vaginal cells were collected via saline lavage and dropped onto a glass slide. After air-drying, they were stained with 0.1% methylene blue (Solarbio, China). Estrous stages were determined based on the predominant cell type as described previously [21].

2.5. Implantation Sites' Number Examination. The whole uteri dissection was performed rapidly on ice and collected for implantation sites' number. After removing fat and the connective tissue, the uteri were separated and the conceptuses were removed. The number of implantation sites was recorded.

2.6. Enzyme-Linked Immunosorbent Assay (ELISA). Rat serum sex hormones (T, E2, LH, FSH) and eNOS and NO in the endometrium of the rat were measured with an ELISA kit (MEIMIAN, Yancheng, China) according to the manufacturer's instructions. Briefly, the assay was applied to rat serum in a 96-well low-adherent white-luminescent plate. First, diluent sample of 100 μl was infused into the corresponding pore of an enzyme scale plate, gently mixed for 30 s, and incubated for 90 min at 37°C. Second, we discarded all of the liquid in the enzyme scale plate, washed the enzyme scale plate with eluent, and removed the water with drying paper. The washing and drying were repeated three times. Subsequently, 100 μl of enzyme-labeling reagent was added to each pore, except for the blank pore, and incubated for 30 min at 37°C away from light; the washing and drying were repeated five times. Thereafter, the enzyme-labeling reagent was replaced with 50 μl each of chromogenic agents A and B, and the plate was gently mixed for 10s and incubated for 15 min at 37°C away from light. Stop buffer was added, and the solution was gently mixed for 30 s. Each pore's OD value was then measured at 450 nm with a spectrophotometer within 15 min of the addition of stop solution.

2.7. Hematoxylin-Eosin (H&E) Staining. After fixation in 4% paraformaldehyde, the ovaries and uterus were embedded in paraffin, and 4-μm serial sections were produced. The sections were mounted on slides and immersed in xylene (10 min, twice) and rehydrated in a decreasing ethanol series diluted in distilled water (100%, 100%, 95%, 90%, 80%, and 70%, 1 min each). The sections were then rinsed in deionized water, stained in hematoxylin for 80 s, rinsed in deionized water, and finally stained in eosin for 3 s. After the color reaction, the sections were dehydrated through an ethanol series in xylene. The sections were taken using an IX73 microscope (Olympus Corporation, Japan). ImageJ (Image in Java, USA) software was used to observe the morphology of ovary, determine the thickness of the endometrium (the vertical distance from the endometrial junction to the myometrium to the uterine cavity), and calculate the number of blood vessels in three different fields of view, with their averages recorded.

2.8. Scanning Electron Microscope (SEM). Ultrastructural changes of pinopodes in the endometrium were observed using the BA600Mot scanning electron microscope (SEM) (MOTIC, Canada). Briefly, the endometrium tissues were prefixed with 2.5% glutaraldehyde. Following this, they were dehydrated with acetone and soaked in isoamyl acetate for 2 h. Then, vacuum drying and metal coating were used for the tissue treatment. The treated tissues were observed by SEM.

2.9. Immunohistochemistry (IHC). Samples were fixed with fresh 4% paraformaldehyde and underwent conventional histological procedures for embedding in paraffin. These samples were cut into 4.5-μm-thick sections, processed for IHC staining with anti-VEGF polyclonal antibody (GB11034B, Servicebio, China) and rabbit anti-Ang-1 polyclonal antibody (ab95230, Abcam, UK), and then incubated with secondary antibodies. The images were digitized by fluorescence microscopy using an IX73 microscope (Olympus Corporation, Japan). The positive cells' density of each slice was measured by image analysis software Image-Pro Plus 6.0.

2.10. Western Blotting. Total protein was extracted from endometrium tissues using RIPA buffer (Servicebio, China) supplemented with 1% PMSF (Servicebio, China). Protein quantification was performed using an Enhanced BCA Protein Assay Kit (Servicebio, China). Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out for protein separation. Then, the protein samples were transferred onto the PVDF membrane (Servicebio, China) and blocked in 5% BSA (Servicebio, China). Subsequently, the membranes were incubated with the primary antibodies against HOXA10 (ab191470, Abcam, UK), LIF (ab138002), VEGF (GB11034B, Servicebio, China), VEGFR2 (GB11190, Servicebio, China), P-PI3K (GB11190, Servicebio, China), AKT (GB111114, Servicebio, China), P-AKT (AF3242, Affinity, USA), and ACTIN (GB15001, Servicebio, China) at 4°C overnight. After washing with TBS-T three times, the membranes were then incubated with the secondary antibody at RT for 1h and detected using an ECL plus kit (G2019, Servicebio, China). PhotoShop software (alphaEaseFC, Alpha Innotech, USA) was used to remove the color, and Alpha software (Adobe PhotoShop, Adobe, USA) was used to analyze the optical density of the target band.

2.11. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted from the harvested uteri using the TRIzol reagent (Gibco), and 1 μg of total RNA was subjected to the reverse transcription of mRNA using oligo dT as a primer and a reverse transcription

kit (Servicebio, Wuhan, China) to generate total cDNA. A quantitative PCR was then carried out using the primers shown in Table 1 and a fluorescence ration PCR instrument (Bio-Rad, California, USA). R-Actin was used for normalization. The quantitative expression level was analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.12. Statistical Analysis. Data analysis was performed with SPSS 26.0 software. The data were expressed as means ± standard deviation (SD). The means and SD were calculated from three independent experiments. Student's *t*-test was used to compare the differences between two groups. One-way analysis of variance (ANOVA) was used for comparisons between multiple groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Inhibition of PI3K/AKT Pathway Reversed the Beneficial Effect of Acupuncture on Rats with PCOS. As shown in Figures 1(a) and 1(b), the body weight gain and the ovarian index in the model group were much higher than those in the control group ($P < 0.05$). The body weight gain and the ovarian index of the acupuncture group were lower than those in the model group ($P < 0.05$). However, the PI3K inhibitor reversed the effect of acupuncture on weight gain and the ovarian index ($P < 0.05$)

In addition, SD rats have an estrous cycle of about 4-5 days. The vaginal smear results of the rats are shown in Figure 1(c). The vaginal smear in the proestrus stage is mainly composed of nuclear epithelial cells (NE, the red arrows). The estrous stage is mainly composed of epithelial keratinocytes (EK, the green arrows). Leukocytes (L, the black arrows) and nuclear epithelial cells are the main components at the metestrus stage with keratinocytes occasionally. The diestrus stage is almost entirely leukocytes. Figures 1 1-1, 1-2, 1-3, and 1-4 are the vaginal smears of rats in the control group on days 1 to 4. 1-1: The proestrus stage is mainly composed of nuclear epithelial cells; 1-2: the estrous stage is mainly composed of epithelial keratinocytes; 1-3: leukocytes and nuclear epithelial cells are the main components at the metestrus stage with keratinocytes occasionally; 1-4: the diestrus stage is almost entirely leukocytes. In the Acu group, there was a complete estrous cycle as the control group (3-1, 3-2, 3-3, and 3-4 same as 1-1, 1-2, 1-3, and 1-4). But in the model and PI + Acu groups, 2-1, 4-1 are at the vaginal smear during the metestrus stage (same as 1-3); 2-2, 4-2 are at the vaginal smear during the metestrus stage (same as 1-3); 2-3, 4-3 are at the vaginal smear during the proestrus stage (same as 1-1); and 2-4, 4-4 are at the vaginal smear during the proestrus stage (same as 1-1). In our research, we discovered that the control group experienced a full estrous cycle. The model group experienced the estrous cycle abnormality almost at the proestrus and metestrus stages. Anovulation is a sign that there is no estrus. The acupuncture group's estrous cycle steadily improved. The inhibition of PI3K/AKT caused by PI3K inhibitors undid the effects of acupuncture on the typical estrous cycle.

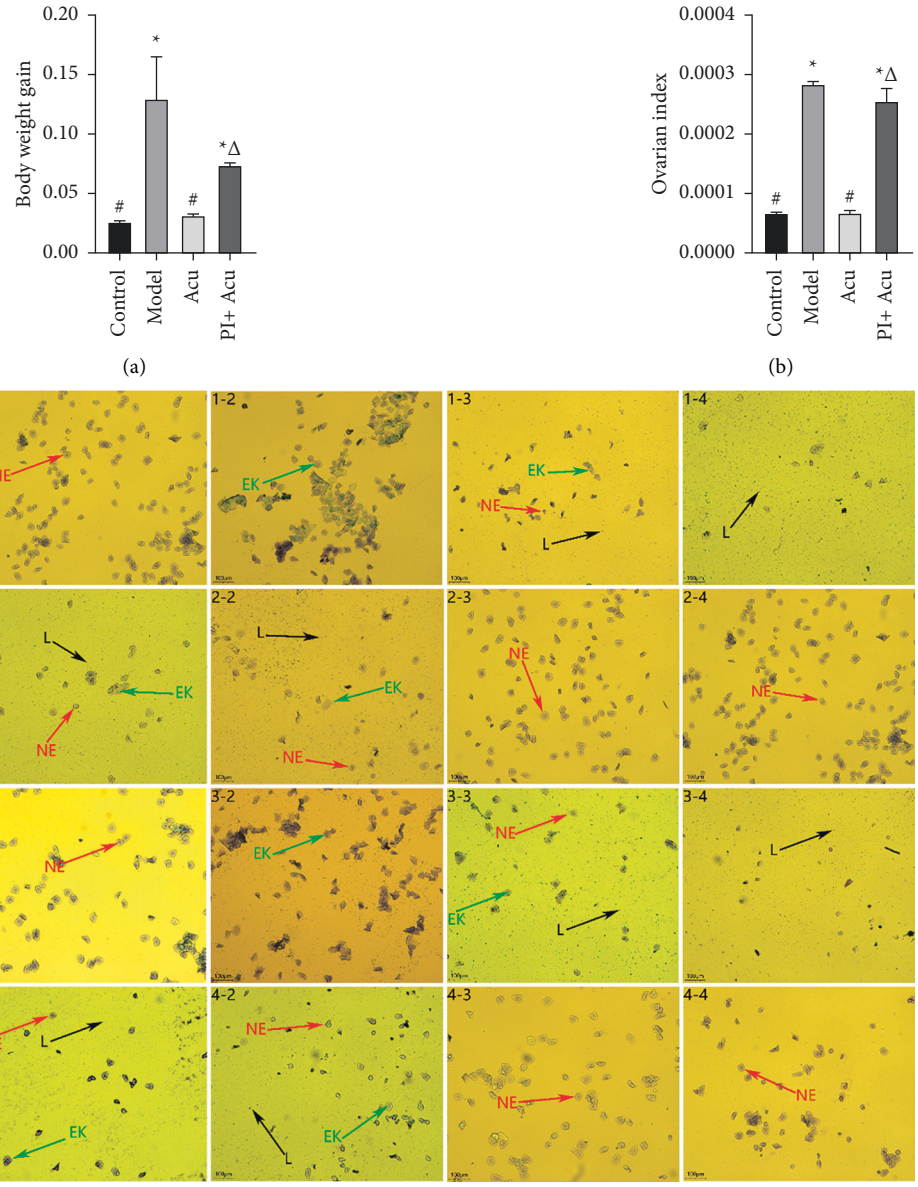
Table 1: Primers for qRT-PCR.

Gene name	Primer sequence
R-*Gapdh*-S	CTGGAGAAACCTGCCAAGTATG
R-*Gapdh*-A	GGTGGAAGAATGGGAGTTGCT
R-*Vegf* (2)-S	TGTGAGCCTTGTTCAGAGCG
R-*Vegf* (2)-A	GGTCTAGTTCCCGAAACCCTGA
R-*Vegfr*2 (4)-S	CAAGTCCGAATCCCTGTGAAGT
R-*Vegfr*2 (4)-A	GGTGAGGATGACCGTGTAGTTTC
R-*Pi3k* (5)-S	CCTGGTGATTGAGAAGTGTAAAGTG
R-*Pi3k* (5)-A	CGTAAGGCAGAAGGCACAGGT
R-*Akt* (4)-S	CTGGAGGACAACGACTATGGC
R-*Akt* (4)-A	AGCCTCTGTGTAGGGTCCTTCTT

Moreover, as shown in Figure 1(d), the serum levels of E2, T, LH, and LH/FSH ratio in model rats detected by ELISA were remarkably elevated compared to control rats, while the serum P and FSH levels were reduced by almost half, whereas these changes were attenuated by acupuncture intervention. Effects of acupuncture were reversed by the PI3K inhibitor.

Besides, the results of HE staining of ovary tissue were presented in Figure 1(e). In the control group, the ovarian tissue from the control group had a normal appearance. Different stages of ovarian follicles, multilayer follicle granulosa cells (8-9 layers), oocytes, and corona in follicles were observed. While the number of atretic follicles and cystic dilating follicles increased significantly with fewer layers of granular cells, there is a lack of oocytes and corona radiating within the follicles in the model group. The corpus luteum was significantly reduced. The ovarian tissue from the acupuncture group showed increased granular cell layers and some ovulation phenomena. After a combination intervention of acupuncture and the PI3K inhibitor, the number of atretic follicles and cystic dilating follicles increased significantly with fewer layers of granular cells, and there is a lack of oocytes and corona radiating within the follicles. The above results suggested that compared with the control and acupuncture groups, the number of atretic follicles and cystic dilating follicles increased significantly with fewer layers of granular cells, and there is a lack of oocytes and corona radiating within the follicles. The corpus luteum was significantly reduced. DHEA-induced increased atretic follicles and cystic dilating follicles were relieved by acupuncture intervention; however, the PI3K inhibitor counteracted the therapeutic effect of acupuncture.

3.2. Inhibition of PI3K/AKT Pathway Reversed the Acupuncture-Mediated Changes in Implantation Sites Number and Endometrial Receptivity on Rats with PCOS. We further investigated the effectiveness of acupuncture in implantation sites' number and endometrial receptivity, as well as the involvement of the PI3K/AKT pathway in acupuncture-mediated changes, since a decreased implantation site number and endometrial receptivity are two of the key pathological features of PCOS. As can be seen from Figure 2(a), there were fewer implantation sites in the model group as compared to the control group ($P < 0.05$). The number of implantation sites was shown to be increased in



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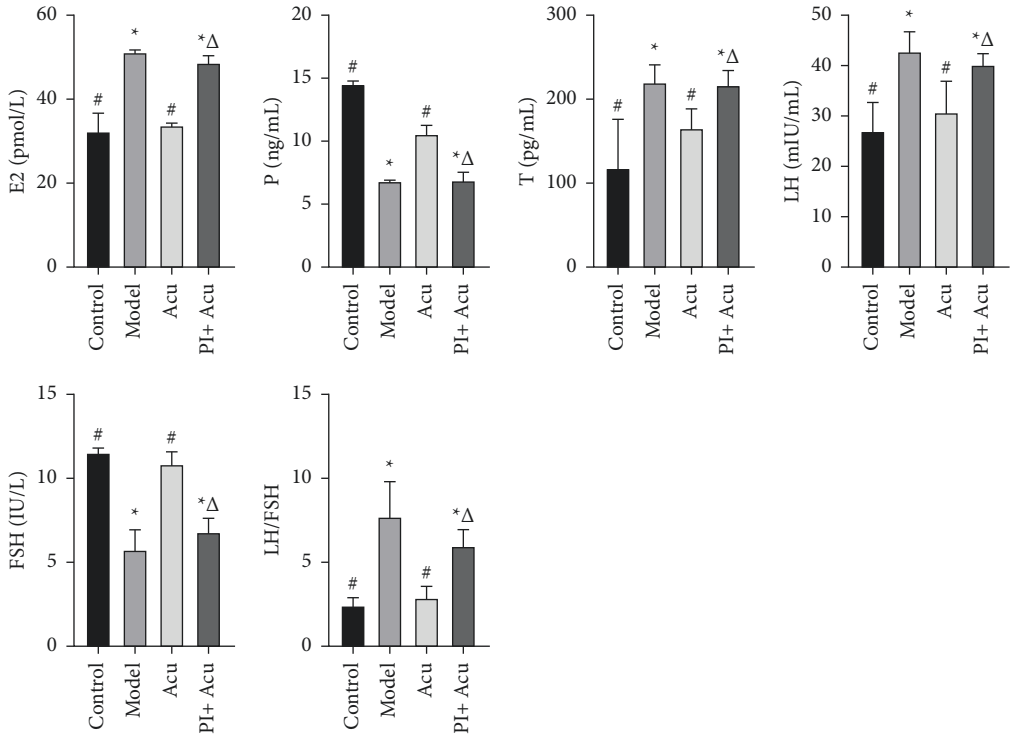
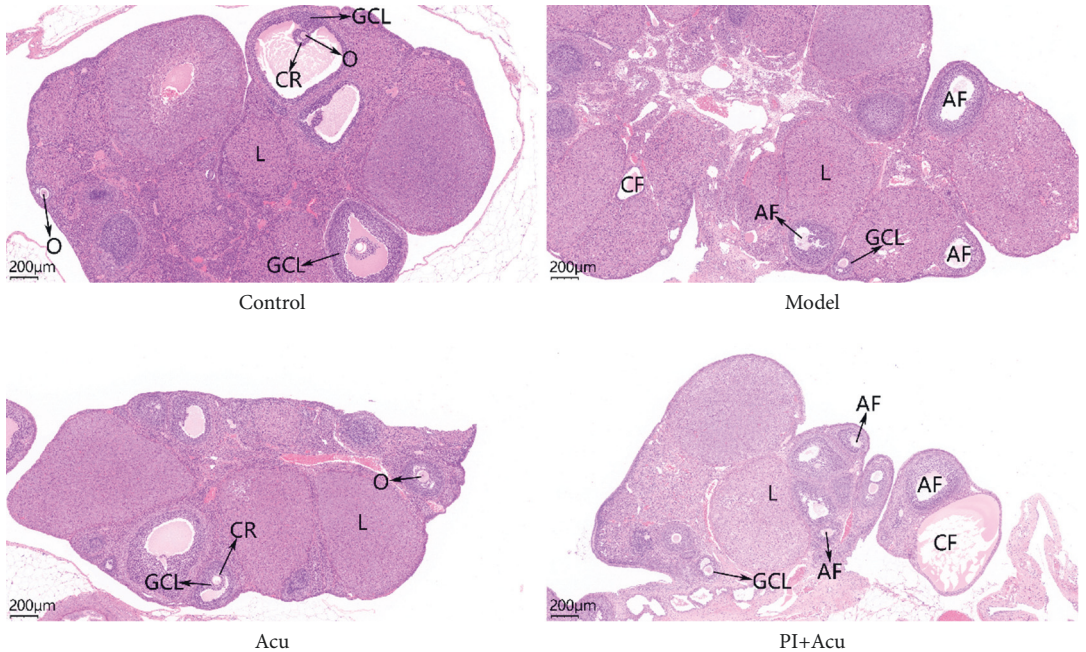
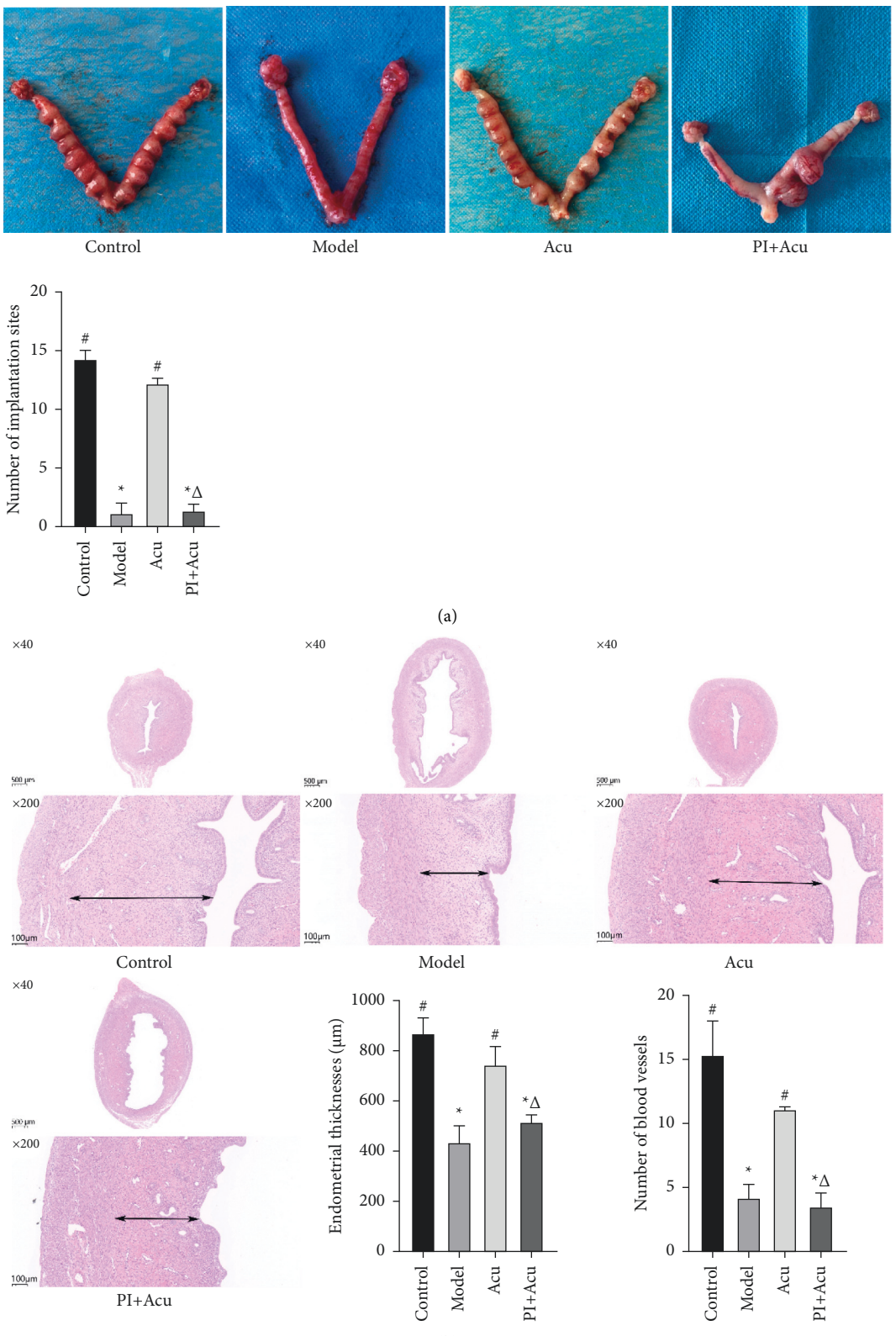


(d)



(e)

Figure 1: Inhibition of the PI3K/AKT pathway reversed the beneficial effect of acupuncture on rats with PCOS. (a) Body weight gain. (b) Ovarian index. (c) Cycle detection by vaginal smear analysis (×200). Red arrow: nuclear epithelial cell (NE). Green arrow: epithelial keratinocyte (EK). Black arrows: leukocyte L. (d) Sex hormones by ELISA. (e) H&E staining of the rat ovary tissue (×100). AF: atretic follicle, CF: cystic follicle, CR: corona radiate, GCL: granular cell layer, L luteal, O oocyte, TCL: theca cell layer. Control: the control group; model: the model group; Acu: the acupuncture treatment group; and PI + Acu: the PI3K inhibitor combined with the acupuncture treatment group. $^{*}P < 0.05$ as compared to the control group; $^{\#}P < 0.05$ as compared to the model group; $^{\triangle}P < 0.05$ as compared to the acupuncture group.



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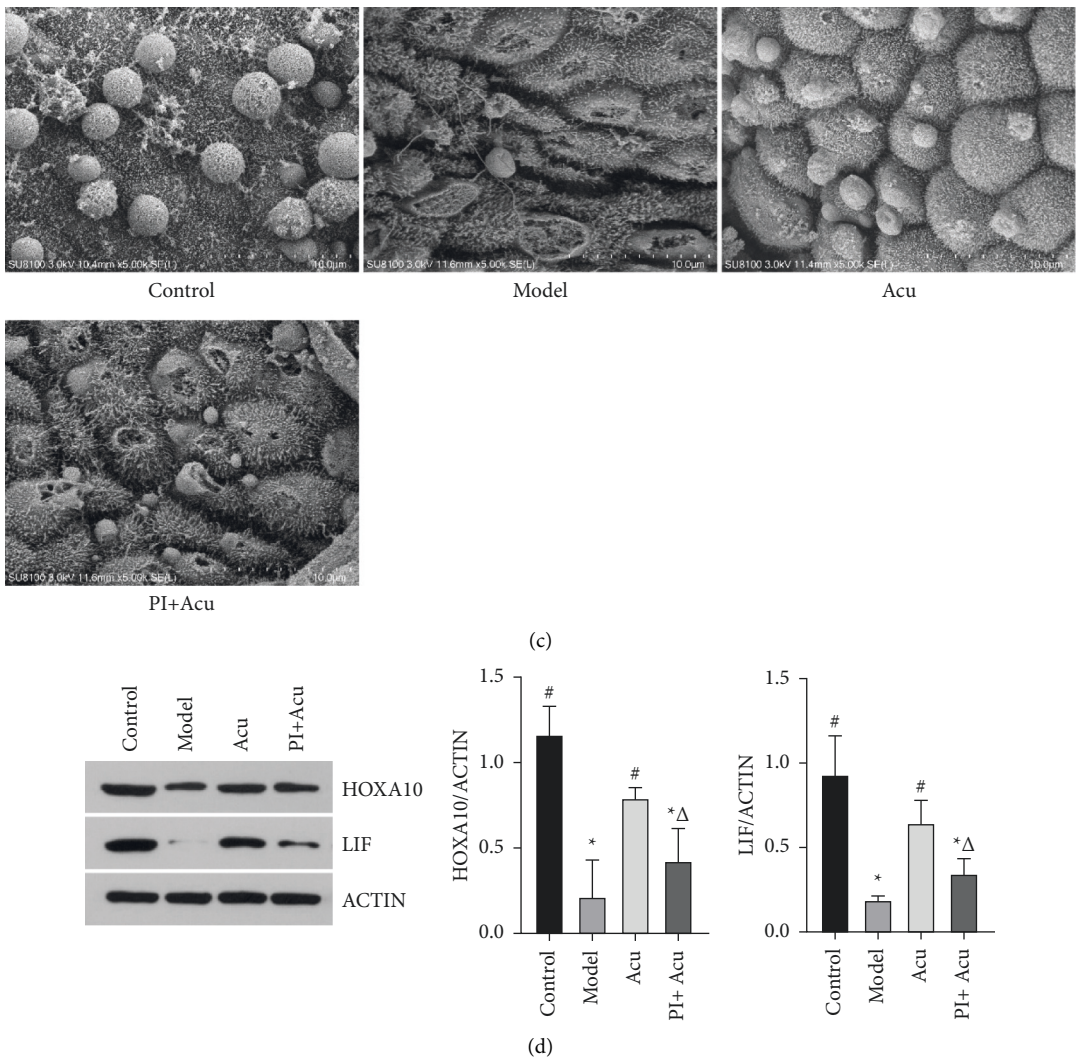

Figure 2: Inhibition of the PI3K/AKT pathway reversed the acupuncture-mediated changes in blastocysts number and endometrial receptivity on rats with PCOS. (a) The number of blastocysts. (b) H&E staining of rat uterine tissue (×40, ×100). Arrows: endometrial thickness. Endometrial thicknesses and blood vessels in each group. (c) Ultrastructural changes of endometrial pinopodes by SEM (x5000). (d) WB results and the histograms of western blot analysis for HOXA10 and LIF. Control: the control group; Model: the model group; Acu: the acupuncture treatment group; and PI + Acu: the PI3K inhibitor combined with the acupuncture treatment group. $^{*}P < 0.05$ as compared to the control group; $^{\#}P < 0.05$ as compared to the model group; $^{\triangle}P < 0.05$ as compared to the acupuncture group.

the acupuncture group; however, $P < 0.05$. The reduction in the number of implantation sites caused by acupuncture was reversed by the inhibition of PI3K/AKT by the PI3K inhibitor.

Furthermore, the endometrial structure in the control group was intact, the epithelial cells were neatly aligned, and the blood vessels and glands were clearly apparent from the HE scans, as shown in Figure 2(b). The endometrial glands and blood arteries were in scarce in the model group, and there was obvious significant endometrial thinning. The endometrial thicknesses in the acupuncture group ($P < 0.05$) considerably increased as compared to the model group. The acupuncture group also experienced an increase in endometrial blood vessels. However, acupuncture's healing effects were negated by PI3K inhibitors. In addition, the endometrial pinopodes detected by SEM shown in Figure 2(c) showed that there were a major number of pinopodes on the surface of endometrium in the control and acupuncture groups, while few pinopodes can be found in the model group. An acupuncture-mediated increase in pinopodes was significantly repressed by combination with the PI3K inhibitor.

HOXA10 and LIF, indicators of endometrial receptivity, were also shown to be expressed by western blotting, as shown in Figure 2(d). According to the western blotting results, both the control group and the acupuncture group had significantly higher levels of HoxA10 and LIF expression as compared to the model group. HoxA10 and LIF expression changes brought on by acupuncture, however, were reversed when PI3K/AKT was inhibited by the PI3K inhibitor.

3.3. Inhibition of PI3K/AKT Pathway Reversed the Acupuncture-Mediated Changes in Endometrial Angiogenesis on Rats with PCOS. Since the PI3K/AKT pathway is an important representative of angiogenesis, next, we investigated the improvement in endometrial angiogenesis underlying the beneficial effects of acupuncture on PCOS and the involvement of the PI3K/AKT pathway in acupuncture-mediated changes. As shown in Figure 3(a) which was detected by IHC, the localization of VEGF was observed in an IHC analysis. The cytoplasm of vascular endothelial cells, mesenchymal cells, and endometrial glandular epithelial cells all expressed VEGF protein and displayed brownish yellow granules. The findings demonstrated that the model group's VEGF-positive cell density was significantly lower than that of the control group ($P < 0.05$). Following acupuncture, the endometrial tissue displayed a brown-yellow particle accumulation with positive staining. The acupuncture group had a higher density of positive cells than the model group did. In contrast, the combination of a PI3K inhibitor and acupuncture greatly reduced the increase in VEGF caused by acupuncture.

According to Figure 3(b), immunohistochemistry examination revealed that the majority of the perivascular matrix contained Ang-1, which presented brownish yellow granules, suggesting that Ang-1 promoted angiogenesis by acting on the blood vessels. The findings demonstrated that the model group's positive Ang-1 cell density was significantly lower than that of the control group ($P < 0.05$). When compared to the model group, the acupuncture group experienced a substantial increase in brown-yellow particle deposition with positive staining ($P < 0.05$). In contrast, the combination of a PI3K inhibitor and acupuncture greatly suppressed the increase in Ang-1 caused by acupuncture.

3.4. Effect of Acupuncture on PI3K/AKT Pathway Expression in Rats with PCOS. Since PI3K/AKT pathway promotion participated in the protection of acupuncture against PCOS in PCOS-like symptoms, implantation sites' number, endometrial receptivity, and endometrial angiogenesis, the regulation of acupuncture in PI3K/AKT pathway expression was further investigated in PCOS rats. As presented in Figure 4(a), the western blotting results showed that as compared to the model group, the expression of VEGF, VEGFR2, P-PI3K, AKT, and P-AKT in the endometrium tissue were upregulated in the acupuncture group with a significant increase, whereas an acupuncture-mediated increase in VEGF, VEGFR2, AKT, P-PI3K, and P-AKT was repressed by combination with the PI3K inhibitor. Compared with the acupuncture group, the levels of VEGF, VEGFR2, P-PI3K, AKT, and P-AKT in the PI3K inhibitor + acupuncture group were significantly decreased.

As shown by the results of the qRT-PCR of *Vegf*, *Vegfr*, *Pi3k*, and *Akt* expression in the endometrium at the mRNA level in Figure 4(b), *Vegf*, *Vegfr*, *Pi3k,* and *Akt* mRNA levels were reduced in the model group as compared to the control group ($P < 0.05$). In contrast, the upregulated expression of *Vegf*, *Vegfr*, *Pi3k*, and *Akt* mRNA was detected in the acupuncture group ($P < 0.05$). The suppression of PI3K/AKT by the PI3K inhibitor reversed acupuncture-mediated changes in *Vegf*, *Vegfr*, *Pi3k*, and *Akt* mRNA levels.

Since eNOS and NO are important molecules of the PI3K/AKT signaling pathway, we detected the expression of eNOS and NO in uterine tissues by ELISA. As shown in Figure 4(c), the endometrial tissue eNOS and NO expression in model rats was remarkably reduced compared to control rats ($P < 0.05$). However, these changes were attenuated by acupuncture intervention. Effects of acupuncture were reversed by the PI3K inhibitor.

4. Discussion

PCOS, a complex disease of reproductive endocrine system, is the main cause of infertility. Reduced endometrial receptivity caused by angiogenesis abnormalities may lead to a high abortion rate and a low implantation rate in patients with PCOS. A majority of studies suggested that acupuncture can improve ER and angiogenesis effectively in women with PCOS [22, 23]. However, the potential mechanisms of acupuncture underlying the efficacy of PCOS have not been fully illuminated.

Previous studies have demonstrated that the DHEA-induced rat model in PCOS displays the clinical characteristics of PCOS in humans, including hyperandrogenism, obesity, and pathological morphology of polycystic ovary [24]. Yu et al. indicated that decreased endometrial receptivity and abnormal ovarian morphology existed in mice exposed by DHEA [25]. Therefore, a rat model of PCOS induced by DHEA was established in the study, which showed typical PCOS-like symptoms like considerable increases in body weight and ovarian index, disruption of the estrous cycle, abnormal sex hormone levels, and abnormal ovarian morphology, in line with the previous study [26]. According to previous studies, acupuncture can relieve PCOS-like symptoms by balancing the levels of sex hormones and autophagy in ovarian tissues via regulating PI3K/AKT pathway [27]. In our study, acupuncture-induced alleviation in PCOS-like symptoms can be reversed by the PI3K inhibitor, indicating the PI3K/AKT pathway may be involved in the mechanism of acupuncture in PCOS, which is consistent with previous studies. Acupuncture can also control weight gain in rats with PCOS effectively due to the regulation of lipid metabolism and insulin resistance. Our earlier study suggested that acupuncture decreased leptin resistance by boosting the number of leptin receptors in the hypothalamus, altering the architecture of fat, and controlling blood lipid levels, which led to weight loss [28].

SP4, PC6, EX-CA1, and CV4 were selected due to their effectiveness in acupuncture-mediated treatment and for further investigation underlying the mechanism of acupuncture on patients with PCOS. Acupuncture points, SP4, PC6, EX-CA1, and CV4, were curative in ER-improving in patients with PCOS, as well as gonadal hormone concentrations and clinical pregnancy rate according to our previous study [17–19]. In the theory of traditional Chinese medicine, SP4 is luo-connecting point of spleen meridian, which can tonify qi and replenish blood, as well as promote local angiogenesis in the endometrium [29]. PC6 is luo-

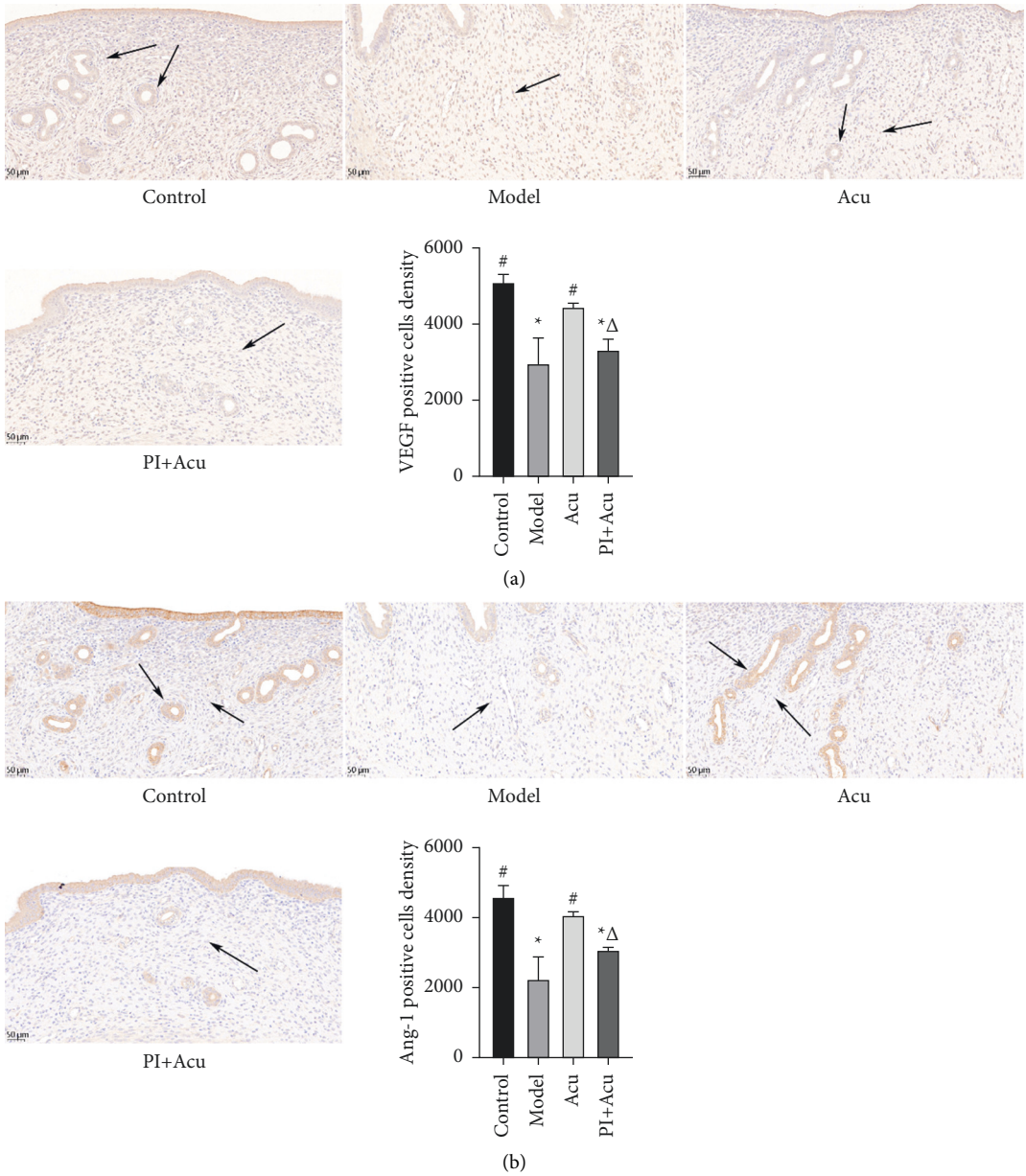

Figure 3: Inhibition of the PI3K/AKT pathway reversed the acupuncture-mediated changes in endometrial angiogenesis on rats with PCOS. (a) VEGF protein expression by IHC (×400). (b) Ang-1 protein expression by IHC (×400). Arrows: the site where brown-yellow particles were deposited. Control: the control group; model: the model group; Acu: the acupuncture treatment group; and PI + Acu: the PI3K inhibitor combined with the acupuncture treatment group. $^{*}P < 0.05$ as compared to control group; $^{\#}P < 0.05$ as compared to the model group; $^{\triangle}P < 0.05$ as compared to the acupuncture group.

connecting point of the pericardium meridian, which can promote blood circulation to remove blood stasis [30]. EX-CA1 is a local point selection, which can increase endometrial vascular permeability and CV4 is point of conception vessel, which has effect on pregnancy and PCOS [31]. Some studies suggested that forementioned acupoints can regulate the hypothalamic-pituitary-ovarian axis bidirectionally and thus restore the secretion of FSH, LH, and E2 in patients with PCOS [32]. Also the clinical pregnancy rate was elevated by acupuncture on the above acupoints of patients with PCOS as well, whose molecular mechanism may be related to the expression of HOXA10, LIF, and some other endometrial receptivity factors [33].

Defected endometrial receptivity will lead to a reduced pregnancy rate due to endometrial epithelial cell attachment failure or poor embryo reception, which indicates that endometrial receptivity is crucial for embryo implantation [34]. The endometrial epithelium permits implantation during a brief period known as the implantation window (WOI) due to the endometrial receptivity [6]. And the characteristic pathology of PCOS, such as hyperandrogenemia, inflammation, insulin resistance, and

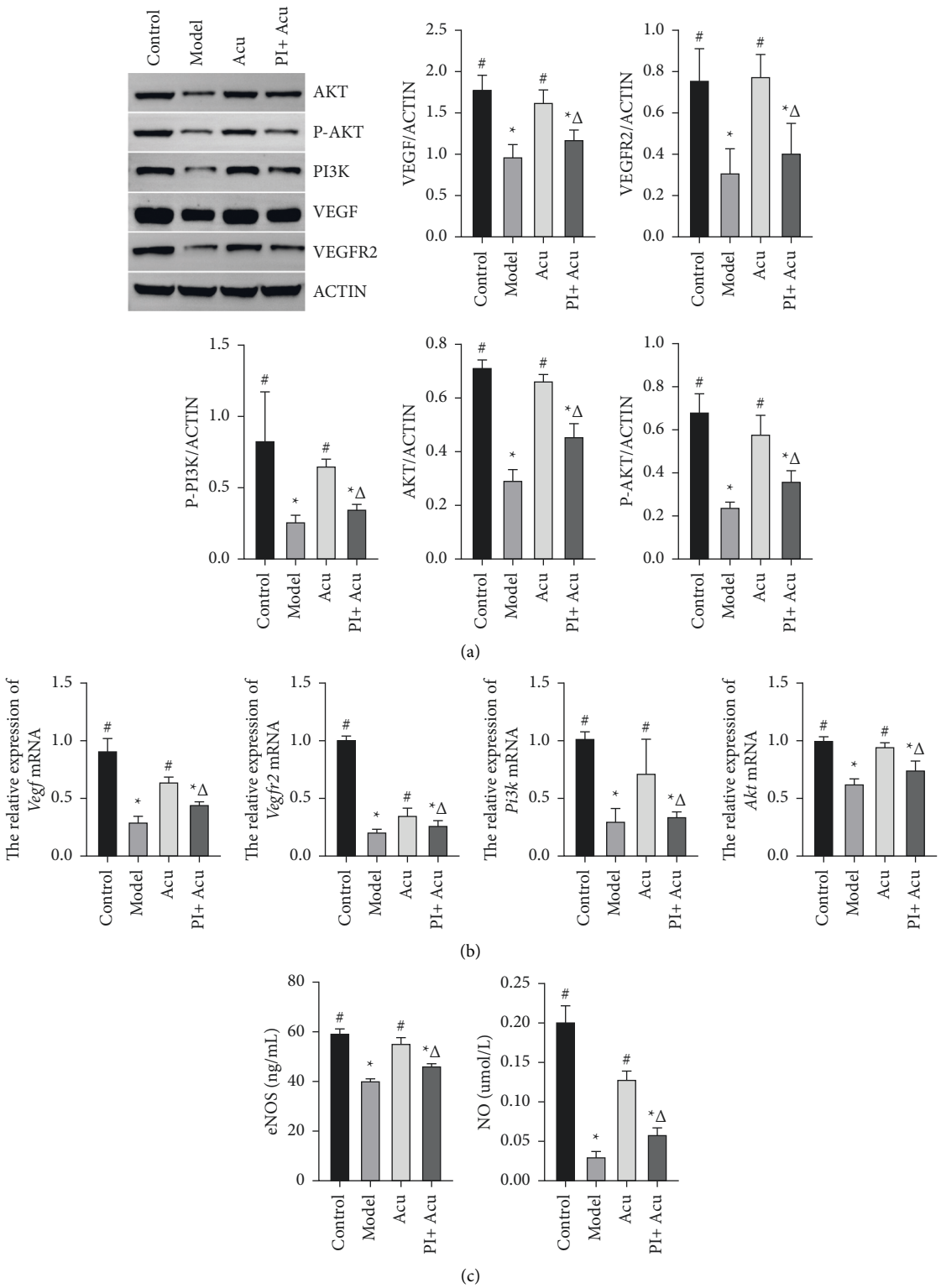

Figure 4: Effect of acupuncture on PI3K/AKT pathway expression in rats with PCOS. (a) WB results and the histograms of western blot analysis for VEGF, VEGFR2, P-PI3K, AKT, and P-AKT. (b) qRT-PCR result of VEGF, VEGFR2, PI3K, and AKT mRNA expression. Control: the control group; model: the model group; Acu: the acupuncture treatment group; and PI + Acu: the PI3K inhibitor combined with the acupuncture treatment group. $^{*}P < 0.05$ as compared to the control group; $^{\#}P < 0.05$ as compared to the model group; $^{\triangle}P < 0.05$ as compared to the acupuncture group.

obesity, can all result in the forementioned disorder [35]. Dechaud et al. reported that when the endometrial thickness ≤7 mm, the endometrial receptivity and pregnancy rate decreased, which implied endometrial thickness as an indicator to predict endometrial receptivity [36]. Liu considered blood vessel numbers as representative marker of blood supplement in endometrium [37]. Pinopodes, a smooth projection on the endometrial epithelium's membrane tip, are spatiotemporally synchronized with endometrial receptivity and as a morphological indicator of excellent endometrial receptivity [38]. HOXA10 is a crucial regulator of embryo implantation, controlling the expression of downstream genes to alter endometrial decidualization and embryo adhesion [39]. LIF is a pleiotropic cytokine that affects blastocyst implantation by encouraging the development of endometrial receptors and is directly targeted at endometrial epithelial cells [40]. In our study, the rat model with PCOS showed considerably fewer implantation sites, thinner endometrium thickness, fewer blood vessels, and pinopodes in the endometrium with lower levels of HOXA10 and LIF protein, all of which indicated defected endometrial receptivity in PCOS, while acupuncture can enhance the endometrial receptivity-related indicators mentioned above. As previous study mentioned before, acupuncture can prominently improve endometrial receptivity by different ways. You et al. reported that electroacupuncture could improve the endometrial receptivity and promote the blastocyst implantation in COH rats by reducing cell adhesion molecules and enhancing the LIF/STAT3 signaling pathway [41]. Also, significantly increased embryo implantation, endometrial thickness, numbers of glands, and blood vessels were observed in the acupuncture group, as well as significantly higher expression levels of pinopode-related markers, including integrin v3, homeobox A10 (HOXA10), heparin-binding EGF-like growth factor (HBEGF), estrogen receptor alpha (ER), and progesterone receptor (PR), which indicates that EA had a positive effect on the endometrial receptivity of thin endometrium model rats by improving pinopode formation through multiple molecular targets according to Xi's study [42]. Endometrial receptivity factors including endometrial morphology, pinopodes, HOX-10, and LIF protein expression were elevated after acupuncture in rats with PCOS detected by our study, which indicates that there is a notably curative effect of acupuncture on promoting endometrial receptivity in rats with PCOS.

Angiogenesis is the fundamental connection strongly tied embryo implantation and endometrial receptivity together [43]. Angiogenesis of the endothelial function layer and capillary epithelium in the early stages of endometrial proliferation and secretion, along with increased vascular endothelial growth factor and angiogenesis-related factor expression, leads to the thickening of the intima layer and subsequent growth of curly spiral artery, and makes the lining after ovulation and the implantation window receptively, which are prepared for embryo implantation [44]. Endometrial Ang-2 blocks Tie-2 signaling pathways with increased angiogenesis-related factor expression and exposes vascular epithelial cells, stromal cells, cell solution, and initial angiogenic factor recognition sites to endothelial surface; thus, angiogenesis is promoted with increased endometrial implant site blood perfusion during the budding stage, and endometrium decidualizes quickly when prepared for pregnancy [45]. VEGF and Ang have an impact on angiogenesis, which are key indicators for ER evaluation [46]. The process of the blastocyst on apposition, adhesion attachment, invasion penetration, and decidualization is ensured by VEGF, one of the earliest contacting-endometrium genes, which was activated at the point of implantation [44]. Ang-1 is strongly expressed in the endometrium during the embryo implantation period, which modulates the angiogenesis and is crucial for the successful implantation of blastocysts [47]. The immunohistochemical results in our study revealed that decreased levels of VEGF and ANG-1 existed in the endometrium of rats with PCOS rats, which was significantly elevated after acupuncture treatment, indicating that acupuncture can treat endometrial angiogenesis disorders brought on by PCOS in WOI.

The PI3K/AKT pathway is the center of regulation for angiogenesis, which controls endothelial cell survival, proliferation, and vascular permeability [48]. VEGF binding to VEGFR-2 will activate the release of NO by endothelial cells and ultimately trigger eNOS. eNOS and NO are indicators of vascular permeability and angiogenesis, which indicates that the PI3K/AKT pathway is tightly linked to angiogenesis and permeability. P-PI3K and P-AKT represent the activation of the PI3K/AKT pathway. When combining with the PI3K inhibitor, acupuncture-mediated improvements in PCOS-like symptoms, endometrial receptivity, the number of implantation sites, and endometrial angiogenesis were altered, indicating that the PI3K/AKT signaling pathway may be involved in the mechanism of acupuncture on rats with PCOS. As a result, we detected acupuncture's impacts on PI3K/AKT pathway-related proteins and mRNA expression. The results demonstrated that acupuncture dramatically enhanced the expression of VEGF, VEGFR2, P-PI3K, AKT, P-Akt protein and VEGF, VEGFR, PI3K, and AKT mRNA in the endometrium of PCOS rats, along with the rising of vascular permeability factors eNOS and NO. Acupuncture-induced alterations in the expression of the PI3K/AKT signaling pathway were, however, restored by PI3K inhibitors. Notably, P-PI3K and P-AKT protein expression by WB and PI3K, and AKT mRNA expression by qRT-PCR were considerably lower in the PI + Acu group compared to the Acu group. It has been proposed that the phosphorylation, or activation, of the PI3K/AKT pathway may be connected to the mechanism of acupuncture. According to previous studies, P-PI3K and P-Akt total protein expression were significantly higher in the endometrium of women who were successfully pregnant during the WOI stage compared to the other stages, indicating that the PI3K/AKT signaling pathway is activated and may play a role in the development of endometrial receptivity and pregnancy [49].

As a result, our study demonstrated that acupuncture notably improved the implantation sites' number, endometrial receptivity factors including endometrial morphology, pinopodes, HOX-10, and LIF protein expression, as well as angiogenesis and PI3K/AKT pathway factors such as

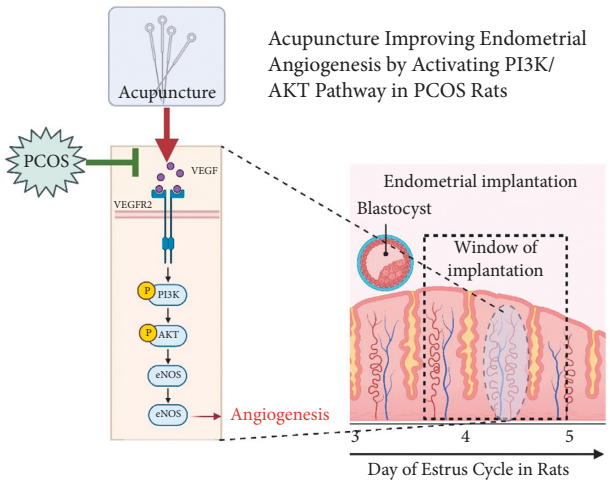

FIGURE 5: Mechanism of acupuncture improving endometrial angiogenesis by activating the PI3K/AKT pathway in PCOS rats.

VEGF, VEGFR2, Ang-1, PI3K, AKT, P-AKT gene/protein expression, and the level of eNOS and NO in the endometrium of rats with PCOS; PCOS-like symptoms were alleviated as well. The efficacy of acupuncture on the rat model with PCOS was counteracted by the combination with the PI3K inhibitor.

The above results indicate that acupuncture improves endometrial angiogenesis by activating the PI3K/AKT pathway, thus promoting endometrial receptivity and the number of implantation sites in PCOS rats, as illustrated in Figure 5.

5. Conclusion

Taken together, our findings indicate that acupuncture improves endometrial angiogenesis by activating the PI3K/AKT pathway, thus promoting endometrial receptivity and the number of implantation sites in PCOS rats. Our study provides novel insight into the mechanisms underlying the treatment of acupuncture in PCOS, which offers more theoretic foundation for its clinical application.

Data Availability

The data used to support the findings of this study can be obtained from the corresponding author upon request.

Ethical Approval

All of the procedures were strictly performed in accordance with the Provision and General Recommendation of the Chinese Experimental Animals Administration Legislation and were approved by the Yunnan University of Chinese Medicine (Approval no. R-062021038).

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Liwei Xing and Yang Chen contributed equally to this work and drafted the manuscript. Xingliwei, Yang Chen, Zhe He, Yuhuan Sun, Ming He, Jinlon Xu, Jian Wang, and Haina Zhuang conducted the experimental intervention; Zeqin Ren, Ying Chen, Jun Yang, and Shuluo Cheng conducted statistical analysis; Rong Zhao guided the experiment implementation and manuscript drafting.

Acknowledgments

This study was supported by the Yunnan University of Traditional Chinese Medicine Joint Special Project of Applied Basic Research (2019FF002-004), the National Natural Science Foundation of China (81860881), Hainan Province Clinical Medical Center (QWYH202175), and the General Project of Yunnan Applied Basic Research Program (Grant nos. 2019FB118, 202101AT070580, and 202001AZ07 0001-020).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 2977636, 7 pages
https://doi.org/10.1155/2022/2977636

Research Article

The Effect of Age on IVF-ET Outcome in Infertile Patients with PCOS Based on Tiangui Theory in Chinese Medicine

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Received 22 January 2022; Accepted 20 April 2022; Published 22 August 2022

Academic Editor: Yong Wang

Objective. Our aim was to investigate the effect of age on the outcome of IVF-ET and ICSI in infertile PCOS patients under the guidance of Tiangui theory in traditional Chinese medicine. *Method*. This was a retrospective analysis of 532 infertile women with PCOS and 1,392 women with infertility due to tubal factors as the controls. All of the participants were divided into different age groups-aged 20–28 years, 29–35 years, and ≥36 years-according to the stages of female reproductive development in Tiangui theory as described in the Canon of Internal Medicine-Treatise of Ancient Natural Truth. We explored the effect of age on controlled ovarian hyperstimulation (including the initial dosage and duration of Gn and the estradiol level on the day of human chorionic gonadotropin administration); the numbers of retrieved oocytes, 2PN zygotes, and embryos; and the rates of fertilization, clinical pregnancy, abortion, live birth, and OHSS incidence. *Results*. Compared to controls, the maximum follicular diameter and the numbers of follicles with d ≥ 20 mm, retrieved oocytes, and 2PN zygotes were greater in the PCOS group with age >28 years ($p < 0.05$). The abortion rate of PCOS patients with age ≤28 years was higher than that of the controls. All PCOS groups and the control group showed reduced numbers of retrieved oocytes and live births with age. The difference in age was not significant in the PCOS groups but was significant in the control group ($p < 0.05$), and the trend in the PCOS groups was more gradual. *Conclusion*. The fertility of all subjects decreased with age, but PCOS patients decreased more slowly than in controls at the same age, which verified the applicability of the guiding principles of Tiangui theory in the clinic.

1. Introduction

Polycystic ovary syndrome (PCOS) is a fairly common endocrine disorder in young reproductive-age women. PCOS is a multifactorial disorder and is characterized by a combination of clinical (anovulation and hyperandrogenism), biochemical (excessive androgen and luteinizing hormone concentrations), and ovarian (polycystic ovaries) features [1]. Infertility is the main reason for PCOS patients of reproductive age to seek medical treatment, and current treatment measures include drug ovulation induction, laparoscopic ovarian drilling, or acupuncture to achieve the goal of ovulation induction and natural conception. However, 20% of patients still need assisted reproductive technology in order to conceive. Recently, it has been shown that the incidence of adverse pregnancy outcomes after in vitro fertilization-embryo transfer (IVF-ET) and intracytoplasmic sperm injection-embryo transfer (ICSI-ET) increases with age. Both the number and quality of follicles decrease in an age-dependent manner, especially in women over 35 years. After 38 years of age, follicular atresia accelerates sharply along with a lower pregnancy rate, higher abortion rate, and lower delivery rate.

It has also been reported that although the clinical pregnancy rate in PCOS patients over 38 years is lower than in healthy women, the numbers of retrieved oocytes do not show significant differences [2]. In addition, Tannus and Colleagues found that PCOS patients over 40 years of age had higher oocyte numbers and a higher cumulative live birth rate than in healthy women of the same age [3].

In traditional Chinese medicine, Tiangui theory seeks to describe the development and maintenance of reproduction and expounds upon, how reproductive function varies at different stages in humans. For females, the length of one stage is 7 years. The filling and exhaustion of Tiangui, which is derived from the kidney essence, is closely related to female fertility. It is said in the Canon of Internal Medicine-Treatise of Ancient Natural Truth (hereafter referred to as the Internal Classic) that "Girls of seven years have abundant kidney qi, they get their adult teeth, and they grow long hair; at the second seven Tiangui arrives, the conception vessel circulates smoothly, the thoroughfare vessel is abundant, menstruation occurs regularly, and pregnancy is possible. . .." With the age changing from the third seven to the seventh seven, female fertility changes along with Tiangui's growth and cessation [4]. Premature ovarian failure is characterized as "Tiangui disorder."

Based on Tiangui theory, we performed a retrospective analysis to investigate the effect of age on ovarian function and IVF-ET outcomes in infertile PCOS patients.

In this paper, we retrospectively analyzed the differences in ovarian function and IVF-ET reproductive outcomes among infertile PCOS patients at different ages under the guidance of age-dependent female fertility according to Tiangui theory.

2. Research Data

2.1. Research Subjects. This was a retrospective analysis of data collected from October 2009 to October 2016 in Dalian Municipal Women and Children's Medical Center. A total of 532 infertile PCOS patients who had undergone their first IVF/ICSI-ET cycle were selected as the research subjects along with 1,392 patients, who underwent IVF/ICSI-ET due to tubal factors as the controls.

2.2. Diagnostic Criteria. The diagnostic criteria for infertility were those in the 8th edition of Obstetrics and Gynecology [5], and the diagnostic criteria for PCOS were the 2012 revision of the Rotterdam criteria [4].

2.3. Exclusion Criteria. IVF/ICSI patients were excluded if they reported endometriosis, hypothyroidism, hyperprolactinemia, congenital adrenocortical hyperplasia, chromosomal abnormalities, ovarian surgery history, or other diseases affecting fertility.

2.4. Grouping. According to Tiangui theory, patients were divided into three groups based on age and female reproductive stage: group A (aged 20–28 years, which is equivalent to the fourth seven in the Internal Classic), group B (aged 29–35 years, which is equivalent to between the fourth and fifth seven in the Internal Classic), and group C (aged ≥36 years, which is equivalent to the fifth seven in the Internal Classic).

All IVF/ICSI-ET patients involved in the study provided informed consent, and the study protocol was approved by the ethics committee of Dalian Municipal Women and Children's Medical Center and the ethics committee of the First Affiliated Hospital, Heilongjiang University of Chinese Medicine.

3. Materials and Methods

3.1. Pretreatment. All PCOS patients started taking oral Diane-35 at 1 tablet/day on the third day of menstruation before the treatment cycle and continued for 21 days.

3.2. Controlled Ovarian Hyperstimulation (COH). All patients underwent the long standard protocol of COH. Pituitary down-regulation was achieved with gonadotropin-releasing hormone agonist (GnRH-a) administered on Day 5 to Day 7 after ovulation or on the 21st day of the menstrual cycle if using oral contraceptives. The dose of GnRH-a was 0.05 mg or 0.1 mg daily (according to the ovarian response) by subcutaneous injection. Serologic examination and transvaginal ultrasonography inspection were performed after 14 to 21 days of application of GnRH-a. Treatment with recombinant follicle-stimulating hormone (rFSH) (Gonal-F) to promote ovulation was initiated until the day of human chorionic gonadotropin (hCG) injection when the serum estradiol (E2) level, luteinizing hormone (LH), and vaginal ultrasonography evaluation met the criteria of down-regulation (E2 < 5 pg/ml, LH < 5 IU/L, endometrial thickness <4-5 mm, and nonfunctional cysts). The starting dose of gonadotropin (Gn) was 112.5–225 IU based on the patient's age, antral follicle count, basal E2 level, and body surface area.

3.3. Oocyte Retrieval, Semen Treatment, and Insemination. The follicular development was monitored by transvaginal ultrasonography and serologic examination on the 6th day after oocyte retrieval. Once 2-3 follicles reached the diameter of 18 mm by transvaginal ultrasonography, 5000–10000 IU hCG was added at night to induce follicular maturation, and transvaginal oocyte retrieval was performed 34–36 h after injection. The partner of the patient needed to ejaculate once 2–7 days before oocyte retrieval and sperm were obtained by masturbation on the day of oocyte retrieval. The semen was placed in an aseptic sperm retrieval cup and liquefied after 30 minutes of standing, and the sperm were capacitated after separation, washing, and culturing. At 2–4 h after oocyte retrieval, IVF or ICSI was performed and the fertilization status was observed after 16–18 h. Normal fertilization results in the presence of two pronuclei or two polar bodies in the cytoplasm. Embryo evaluation was based on the criteria of Peter [6].

3.4. Embryo Transplantation and Clinical Pregnancy Detection. On the third day after oocyte retrieval, high-quality embryos were selected for transplantation when the patient's endometrial thickness was up to 8 mm and were selected according to the patient's age, endometrial receptivity, and risk of ovarian overstimulation. The remaining embryos were cryopreserved by vitrification. Luteal phase support was provided by oral, intramuscular injection, or intravaginal progesterone. At 14 days after embryo transfer, biochemical pregnancy was determined by serum hCG concentration, and 30 days later the position and number of gestational sacs were determined by transvaginal ultrasonography. Biochemical pregnancy was defined by serum β-hCG concentration >10 IU/L and the absence of a gestational sac detected by transvaginal ultrasonography. Clinical pregnancy was defined as an ultrasound finding of the pregnancy sac (intrauterine or extrauterine) with a heartbeat.

3.5. Primary Measurements

3.5.1. Serum Hormone Levels Measurement. The fasting blood sample of patients was instructed to be taken on the second or third day of a regular menstrual cycle or after progesterone withdrawal bleeding. The serum levels of E2, LH, FSH, testosterone, prolactone, and thyroid stimulating hormone were monitored by microparticle enzyme immunoassay. A fasting blood sample was taken to detect serum E2 on the day of oocyte retrieval.

3.5.2. Clinical Indexes Calculation. The implantation rate was calculated as the number of patients with fetal heartbeat observed under B-ultrasound divided by the number of transplanted embryos.

The rate of clinical pregnancy was calculated as the sum of the number of patients with fetal heartbeat observed under B-ultrasound and the number of patients with β-HCG-positive serum divided by the total number of subjects.

The fertilization rate was calculated as the number of patients with fertilization (including the presence of clinical pregnancy or abortion) divided by the total number of subjects.

The clinical pregnancy rate was calculated as the number of patients with more than 28 weeks of pregnancy divided by the total number of subjects.

The abortion rate was calculated as the number of patients with abortions that occurred 28 weeks ago divided by the total number of subjects.

The live birth rate was calculated as the number of patients with ≥28 weeks of pregnancy divided by the total number of subjects.

The incidence rate of OHSS was calculated as the number of patients with OHSS divided by the total number of subjects.

4. Statistical Analyses

All statistical analyses were performed using SPSS version 19.0. All data are shown as the mean and standard deviation. The comparisons between groups used a one-way analysis of variance. The numerical data were compared using the chi-squared test, and rates were compared using Fisher's exact test. $p < 0.05$ was considered statistically significant. The clinical pregnancy rate, live birth rate, and OHSS data with positive primary outcomes were analyzed by logistic regression.

5. Results

5.1. Baseline Characteristics of PCOS Patients. As shown in Table 1, the PCOS group had a higher BMI, longer duration of infertility, a lower initial dose of Gn, shorter duration of Gn use, higher LH and LH/FSH, and lower FSH compared with the control group ($p < 0.05$). The PCOS group above the age of 35 years had higher initial doses of Gn and lower E2 levels compared with the other two PCOS groups ($p < 0.05$).

5.2. Ovulation Induction Outcomes in PCOS Patients. As shown in Table 2, the maximum follicular diameter, number of follicles with d ≥ 20 mm, number of retrieved oocytes, and number of 2PN zygotes in the PCOS group B and group C were significantly higher compared with the group b and group c ($p < 0.05$).

5.3. Pregnancy Outcomes and Neonatal Outcomes in PCOS Patients. The implantation rate, clinical pregnancy rate, presence of clinical pregnancy rate, and live birth rate in the PCOS group aged ≥36 years were significantly lower than in the other two PCOS groups. The rate of abortion in the PCOS group aged 20–28 years was higher than the controls, while the OHSS rate in the PCOS group aged ≥36 years was significantly higher than the controls, and the difference remained significant after adjusting for BMI, the initial dose of Gn, and the duration of Gn. The days of pregnancy in the PCOS group aged ≥36 years was less than the controls (Table 3).

5.4. Comparison of the Number of Retrieved Oocytes and Live Birth Rate in PCOS Patients of Different Ages. With increasing age, the number of retrieved oocytes and the live birth rate in the PCOS groups did not decrease significantly ($p > 0.05$), while these all showed significant declines in the control group ($p < 0.05$) (The slope of the number of retrieved oocytes: PCOS group vs. control group = –0.028 vs. –0.25; the slope of the live birth rate: PCOS group vs. control group = –0.014 vs. –0.023) (Figures 1 and 2).

6. Discussion

Tiangui is first seen in the Internal Classic, which states, "Girls of seven years have abundant kidney qi, they get their adult teeth and grow long hair; at the second seven Tiangui arrives, the conception vessel circulates smoothly, the thoroughfare vessel is abundant, menstruation occurs regularly, and pregnancy is possible." Tiangui is thus, closely related to female fertility. As the woman ages through the third seven, fourth seven, fifth seven, sixth seven, and

Table 1: The baseline characteristics of PCOS patients.

	PCOS			Tubal factor		
	Group A age, 20–28	Group B age, 29–35	Group C age, ≥36	Group a age, 20–28	Group b age, 29–35	Group c age, ≥36
Subjects (n)	81	350	101	114	753	525
Age (years)	26.7 ± 1.4	32.0 ± 1.9	38.3 ± 2.1$^{\#}$	26.7 ± 1.5	32.4 ± 1.9	38.6 ± 2.3$^{\#}$
BMI (kg/m^2)	23.9 ± 3.6*	23.9 ± 3.4*	24.6 ± 4.0*	21.5 ± 3.0	22.6 ± 3.3	22.8 ± 2.9$^{\#}$
Duration of infertility (years)	3.3 ± 1.6*	4.3 ± 2.6*	6.3 ± 4.1$^{*\#}$	2.7 ± 1.8	4.0 ± 2.8	5.6 ± 4.4$^{\#}$
Initial dose of Gn (IU)	159.5 ± 59.5*	182.7 ± 64.9*	212.4 ± 76.4$^{*\#}$	196.4 ± 75.3	243.2 ± 68.5	276.9 ± 64.3$^{\#}$
Duration of Gn use (days)	9.3 ± 2.0*	9.4 ± 2.7*	10.7 ± 2.1*	9.9 ± 1.7	10.2 ± 1.9	11.2 ± 2.3
LH (U/L)	6.6 ± 3.6*	6.3 ± 4.1*	6.3 ± 5.0*	4.6 ± 2.4	4.3 ± 2.0	4.2 ± 1.8
FSH (U/L)	6.6 ± 2.3*	6.7 ± 2.0*	7.0 ± 2.0*	7.3 ± 2.1	7.5 ± 2.2	9.5 ± 33.8$^{\#}$
LH/FSH	1.2 ± 1.6*	0.95 ± 0.5*	0.9 ± 0.7*	0.7 ± 0.4	0.6 ± 0.3	0.6 ± 0.6$^{\#}$
E2 (pg/ml)	40.9 ± 26.2	36.9 ± 16.8	36.5 ± 15.2$^{*\#}$	38.6 ± 21.7	40.6 ± 22.5	42.1 ± 22.2

$^{*}p < 0.05$ compared with control at the same age. $^{\#}p < 0.05$ compared within the PCOS group and control group.

Table 2: The comparison of ovulation induction outcomes in PCOS patients of different ages.

	PCOS			Tubal factor		
	Group A age, 20–28	Group B age, 29–35	Group C age, ≥36	Group a age, 20–28	Group b age, 29–35	Group c age, ≥36
Subjects (n)	81	350	101	114	753	525
E2 level on the day of hCG injection (pg/ml)	3487.9 ± 2038.1	3103.9 ± 1879.1	2472.5 ± 1686.6*	3156.8 ± 2123.0	2945.3 ± 1851.7	2441.4 ± 1829.1$^{\#}$
Maximum follicular diameter (mm)	22.6 ± 1.8	22.5 ± 2.1*	22.9 ± 1.5*	22.5 ± 1.6	22.3 ± 1.7	21.8 ± 1.5$^{\#}$
Number of follicles with d ≥ 20 mm	3.6 ± 1.9	3.4 ± 1.7*	3.7 ± 2.0*	3.2 ± 1.6	2.9 ± 1.5	2.3 ± 1.4$^{\#}$
Number of retrieved oocytes	7.9 ± 3.7	8.3 ± 3.8*	7.5 ± 3.3*	8.4 ± 3.2	7.6 ± 3.4	4.8 ± 2.9$^{\#}$
Number of 2PN zygotes	6.8 ± 3.5	6.8 ± 3.2*	6.2 ± 3.1*	6.8 ± 2.8	6.1 ± 3.0	4.8 ± 2.9$^{\#}$
Number of embryos	5.5 ± 2.8	5.5 ± 2.8	5.1 ± 2.8*	6.2 ± 2.9	5.3 ± 2.9	4.1 ± 2.5$^{\#}$

$^{*}p < 0.05$ compared with control at the same age. $^{\#}p < 0.05$ compared within the PCOS group and control group.

Table 3: The pregnancy outcomes and neonatal outcomes in PCOS patients.

	PCOS			Tubal factor		
	Group A age, 20–28	Group B age, 29–35	Group C age, ≥36	Group a age, 20–28	Group b age, 29–35	Group c age ≥36
Subjects (n)	81	350	101	114	753	525
Number of embryos transferred	166	779	269	248	2494	1369
Implantation rate (%)	74/166*	252/779*	58/269$^{*\#}$	79/248	479/2494	227/1369$^{\#}$
Fertilization rate (%)	53/81	198/350	45/101$^{\#}$	62/114	390/753	198/525$^{\#}$
Clinical pregnancy rate (%)	45/81	175/350	38/101$^{\#}$	58/114	352/753	167/525$^{\#}$
Presence of clinical pregnancy rate (%)	41/81	153/173	29/101$^{\#}$	53/114	305/753	128/525$^{\#}$
Abortion rate (%)	16/53^{*a}	49/198	16/45	9/114	87/390	71/198$^{\#}$
Live birth rate (%)	37/81	149/350	29/101$^{\#}$	53/114	303/753	127/525$^{\#}$
Ectopic pregnancy rate (%)	2/81	7/350	2/101	3/114	10/753	7/525
OHSS incidence rate (%)	1/81	6/350	4/101^{*a}	1/114	11/753	3/525
Neonatal weight (g)	2909 ± 664.8	2958 ± 696.7	2881 ± 844.8	2948 ± 562.8	2990 ± 623.0	2931 ± 625.3
Duration of pregnancy (d)	262.7 ± 16.8	265.8 ± 16.0	261.7 ± 16.9*	264.1 ± 13.0	265.5 ± 14.5	268.2 ± 16.3

$^{*}p < 0.05$ compared with control at the same age. $^{\#}p < 0.05$ compared within the PCOS group and control group. $^{a}p < 0.05$ compared with control at the same age after adjusting for BMI, the initial dose of Gn, and the duration of Gn.

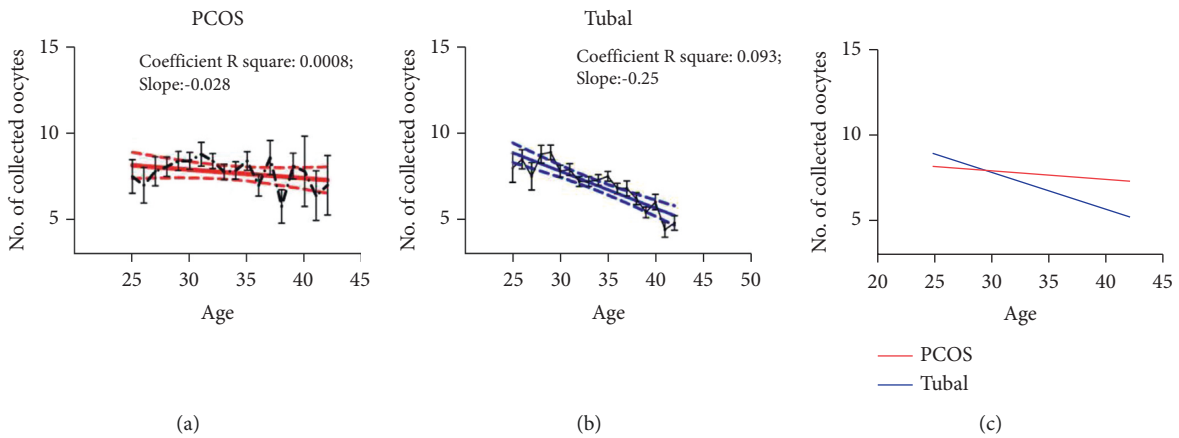

Figure 1: Age-related changes in the number of retrieved oocytes. The number of retrieved oocytes declined with age both in the two groups, while the number of retrieved oocytes in the PCOS group was significantly better than that in the control group.

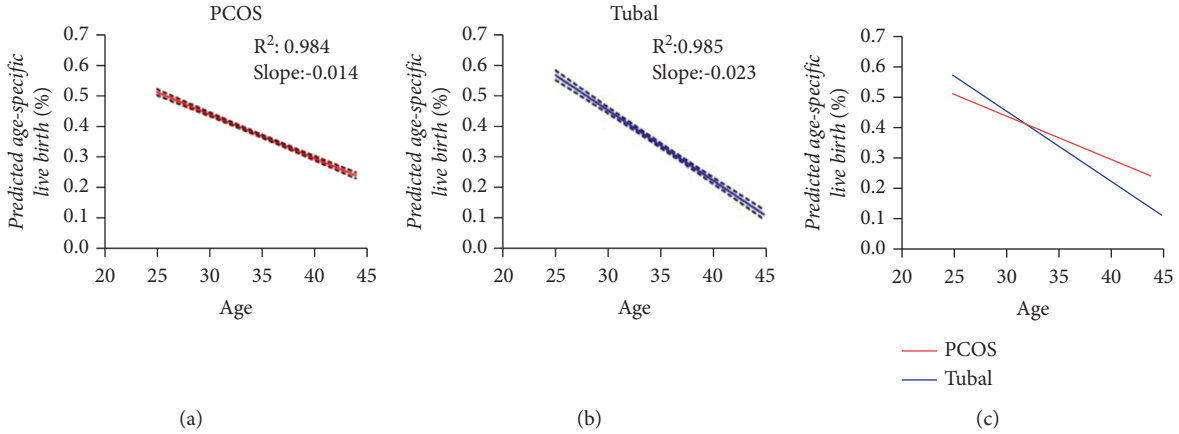

Figure 2: Age-related changes in the live birth rate. The live birth rate declined with age both in the two groups, while the live birth rate in the PCOS group was significantly better than that in the control group, as the change in the number of retrieved oocytes.

seventh seven, Tiangui grows to a peak and then decreases leading to the eventual failure of female fertility [4]. The theory of the kidney qi-Tiangui thoroughfare-conception vessels-uterus reproductive axis is well established. Kidney qi gradually grows until it becomes exuberant, which promotes the emergence of Tiangui, and together they play a central role in the physiology of the uterus, and thus female fertility declines along with deficiency of kidney qi and the exhaustion of Tiangui [7]. Because the qi-blood and yin-yang relationships in the reproductive axis are disturbed in Tiangui disorders because kidney deficiency is a root aspect of PCOS and turbid phlegm is a branch aspect, PCOS patients usually have oligomenorrhea, anovulation, blocked thoroughfare vessel and conception vessel, and phlegm congestion in the uterus, and they present the main manifestations of obesity, hirsutism, acne, and other metabolic disorders. Invigorating the kidney and resolving phlegm are the starting points of treatment [8, 9].

It is currently proposed that PCOS patients might have a wider reproductive window than healthy women. Minooee et al. observed that PCOS patients have significantly higher serum anti-Müllerian hormone (AMH) than normal ovulatory women and that they might have a 2-year longer reproductive lifespan when compared with their normal ovulatory counterparts [10]. In terms of reproductive outcomes, a retrospective cohort study explored the effect of age on the cumulative live birth rate after the first ovarian stimulation in IVF in PCOS patients and showed that declines in treatment outcomes were slower in PCOS patients compared to non-PCOS patients [11]. However, another study found that clinical pregnancy and live birth rates between PCOS patients and women with tubal factor infertility were similar, and the hypothesis that the reproductive window is extended in PCOS patients was not supported [12].

Age is one of the predictors of ovarian reserve function and is an independent factor affecting female fertility. As women age, the number of follicles decreases along with a decline in ovarian reserve function, and the incidence of infertility in women aged 40 is twice as high as in women aged 30–35 [5]. AMH is generally considered the best currently available measure of ovarian reserve in various clinical conditions. Low AMH levels represent low ovarian reserve, and it is common for AMH levels to gradually decline as a woman ages [13]. Researchers have compared AMH mRNA expression in cumulus and mural granulosa

cells and measured the AMH level in follicular fluid of women aged 21–35 years and 40–45 years. The expression of small noncoding microRNA also differs in the follicular fluid between PCOS patients and normal healthy women, and age is one of the factors, that is, correlated with these changes [14]. The results showed that AMH was highly expressed and secreted from cumulus granulosa cells in women of advanced age [15]. Another study of AMH levels at different ages among women receiving IVF-ET or ICSI treatment found a statistically significant difference in AMH between groups aged 24–30 and 41–45 years. When it comes to pregnancy rates, significant differences were seen between groups aged under 30 and other groups, as well as between the groups aged 31–35 and 41–45 years [16]. It can thus be speculated from the studies above that the decline of ovarian function is more obvious in women over 40 years old.

An analysis of the follicular fluid of IVF patients at different ages found that with the increase in age the quality and quantity of oocytes tended to decline, while the level of reactive oxygen species gradually increased in follicles, and the follicular fluid microenvironment, which is closely related to the development of the oocyte, also changed [17]. Shi Li-hong et al. [18] found that the live birth rate and abortion rate of IVF patients over the age of 43 who underwent the long protocol of COH were reduced and increased, respectively, and they proposed that age was an independent factor affecting the pregnancy outcome of IVF-ET attempts. For women over 37 years old receiving IVF/ICSI-ET, the clinical pregnancy decreased and the spontaneous abortion rate increased with increasing age, especially for women over 44 years old. This is consistent with the traditional Chinese medicine notion that "At the fifth seven, the Yangming meridian starts to decline, her face begins to wither, and her hair begins to thin" and supports the theory of changes in female fertility at different ages. The fertility window of PCOS patients may extend to 40 years of age, but at ages, over 40 years old come declining ovarian reserve, the poor effect of ovulation-stimulating treatments, and a low pregnancy rate [19]. Despite serum AMH decreases over time in all of the women, the decrease in the PCOS patients was less pronounced than in controls, and this may suggest better preservation of the ovarian reserve and thus a prolonged reproductive life span [20]. A study showed that the live birth rate and cumulative live birth rate in PCOS patients aged 35–40 years were significantly higher than in the control group [11]. In another study, AMH levels in PCOS patients aged 20–30 and those aged 30–40 were not significantly different, but in non-PCOS patients AMH levels in the group aged 20–30 were significantly higher than those in the group aged 30–40 [21]. It has been reported that the threshold of AMH might be more accurate in predicting PCOS based on age stratification, which may account for the difference in age-related AMH levels between PCOS patients and healthy women [6]. Moreover, it has been shown that AMH levels gradually decrease with age, although the rate of AMH decline may not be the same for all women of reproductive age. There is also evidence that the ovarian pool is depleted in a more gradual manner in the ovaries of PCOS patients compared to healthy women [22].

In this study, the number of retrieved oocytes, 2PN zygotes, and embryos formed in the controls showed a significantly decreasing trend with increasing age, whereas there were no differences in the PCOS group with age but they were all higher than the control group ($p < 0.05$). The number of retrieved oocytes is an important indicator of ovarian function, suggesting that PCOS patients have a better ovarian reserve function according to the results of this study. Meanwhile, we found that the implantation success rate, conception rate, clinical pregnancy rate, ongoing pregnancy rate, and live birth rate both in the PCOS group and control group declined with age and were greater in the PCOS group compared to the control group but the differences were not statistically significant. A study indicated that the number of retrieved oocytes, the pregnancy rate, and the live birth rate in PCOS patients remained relatively stable but showed a significant decreasing trend in the control group [23]. This was consistent with our results (Figure 2), which indicated that the ovarian function in PCOS patients was better than that in healthy women of the same age.

In this study, the rate of abortion in the PCOS group aged 20–28 years was higher than that in the control group, while the OHSS incidence rate in the PCOS group aged ≥36 was higher than in the control group, and both remained significantly different after adjusting for BMI, the initial dose of Gn, and the duration of Gn. This indicated that the high abortion rate in PCOS patients before the age of 28 may be related to changes in the uterine and endocrine environment needed to maintain pregnancy, while the ovaries have a relatively good reserve and response over 35 years, resulting in the higher risk of OHSS occurrence. Hwang [2] found that the number of retrieved oocytes remained stable and that the conception rate, clinical pregnancy rate, and live birth rate declined in the PCOS group with age, but were superior to controls. The women with PCOS had a lower miscarriage rate than the controls, although it increased with age. This finding also supports our hypothesis that the decrease in fertility with age is slower in PCOS than in controls and suggests that ovarian reserve function was superior in PCOS compared with the control group at the same age.

Our study has several strengths. It is the first to evaluate IVF outcomes with Tiangui theory of TCM, which provides clinical evidence in support of Tiangui theory of TCM. We found that fertility in the women declined with age but the fertility in PCOS might be superior to that of the control group. Furthermore, it had a large sample size with a total of 532 infertile women with PCOS and 1924 women with tubal factor infertility who did not have PCOS, which has important significance for clinical guidance. A limitation of this study is that this was a retrospective analysis and some important indicators could not be collected, such as AMH, which is one of the most important factors for predicting ovarian reserve function, and thus these values were not fully tested in this paper.

Data Availability

The data are available from the corresponding author upon request.

Conflicts of Interest

All authors declare no conflicts of interest.

Authors' Contributions

Concept and design were proposed by Hui Chang, Jian Li, and Xiaoguang Shao. Acquisition, analysis, and interpretation of data were performed by Hui Chang and Xiaoguang Shao. Drafting of the manuscript was done by Hui Chang, Mengyi Zhu, and Hang Ge. Statistics were collected by Jian Li, Qi Wu, and Baichao Shi. Critical revision of the manuscript for important intellectual content was carried out by Yanhua Han and Xin Fu.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Youth Project) (Grant No. 82004404), the grant of China Postdoctoral Science Foundation (Grant Nos. 2021T140188 and 2018M641886), and the Natural Science Foundation of Heilongjiang Province (Grant No. YQ2020H031).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 2624434, 14 pages
https://doi.org/10.1155/2022/2624434

Research Article

Mechanism Study of Cinnamomi Ramulus and *Paris polyphylla* Sm. Drug Pair in the Treatment of Adenomyosis by Network Pharmacology and Experimental Validation

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Received 22 April 2022; Revised 28 June 2022; Accepted 11 July 2022; Published 16 August 2022

Academic Editor: Yue Hui Zhang

Objective. To explore the molecular mechanism of the Cinnamomi ramulus and *Paris polyphylla* Sm. (C-P) drug pair in the treatment of adenomyosis (AM) based on network pharmacology and animal experiments. *Methods*. Via a network pharmacology strategy, a drug-component-target-disease network (D-C-T-D) and protein–protein interaction (PPI) network were constructed to explore the core components and key targets of C-P drug pair therapy for AM, and the core components and key targets were verified by molecular docking. Based on the results of network pharmacology, animal experiments were performed for further verification. The therapeutic effect of the C-P drug pair on uterine ectopic lesions was evaluated in a constructed AM rat model. *Results*. A total of 30 components and 45 corresponding targets of C-P in the treatment of AM were obtained through network pharmacology. In the D-C-T-D network and PPI network, 5 core components and 10 key targets were identified. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the PI3K signaling pathway was the most significantly enriched nontumor pathway. Molecular docking showed that most of the core components and key targets docked completely. Animal experiments showed that the C-P drug pair significantly ameliorated the pathological changes of endometriotic lesions in AM model rats and inhibited PI3K and Akt gene expression, and PI3K and Akt protein phosphorylation. In addition, treatment with the C-P drug pair promoted AM cell apoptosis; upregulated the protein expression of Bax, Caspase-3, and cleaved Caspase-9; and restrained Bcl-2 expression. *Conclusions*. We propose that the pharmacological mechanism of the C-P drug pair in the treatment of AM is related to inhibition of the PI3K/Akt pathway and promotion of apoptosis in AM ectopic lesions.

1. Introduction

Adenomyosis (AM) is a gynecological disorder characterized by the abnormal appearance of endometrial glands and stroma in the myometrium. Increased dysmenorrhea, abnormal uterine bleeding (AUB), and infertility are the most typical symptoms of AM and severely affect the quality of life of patients [1]. Hormone therapy, which is often used as an effective palliative strategy, has side effects such as impaired ovarian reserve function, irregular vaginal bleeding, and infertility [2, 3]. Surgical hysterectomy, currently the only definitive treatment, is typically physiologically and psychologically traumatic for patients of reproductive age, but uterine-sparing surgery also has disadvantages: namely, removal of lesions is difficult and recurrence is common [4].

Among alternative medicines, traditional Chinese medicine (TCM) has accumulated considerable clinical experience in AM treatment [5–7]. Cinnamomi ramulus (Guizhi), or branch of cinnamon, is believed in TCM to be able to relax muscles, warm the body and dredge meridians. Cinnamomi ramulus was first used for gynecological treatment in the formula Guizhi-Fuling Wan, as recorded in

the Synopsis of the Golden Cabinet (Jin Gui Yao Lue), written by Zhang Zhongjing, and Cinnamomi ramulus is still used in clinics with remarkable effects [8, 9]. *Paris polyphylla* Sm. (Chonglou) is recorded in Shennong's Herbal Classic (Shen Nong Ben Cao Jing) and is believed to be able to relieve heat, remove toxic material, detumescence, and analgesic effects. Gongxuening capsules (Manufacturing Approval Number: Z20053256), which are commonly used in the clinical treatment of AM, are developed from *Paris polyphylla* Sm. and have been used to treat uterine diseases and blood stasis syndrome for decades [10–12].

In TCM treatment theory, prescriptions based on the concept of "drug pairs" can often achieve better results than individual drug prescriptions, as two drugs can often increase the effectiveness of each other. We extracted the monarch drugs (the drugs that play major roles in a formula), namely, Cinnamomi ramulus and *Paris polyphylla* Sm. (C-P), from Guizhi-Fuling Wan and Gongxuening capsules as a drug pair and explored their synergistic regulatory effects on an AM rat model in this study [13]. A previous study by our team demonstrated that when used in combination, Cinnamomi ramulus, *Paris polyphylla* Sm. and *Panax notoginseng* (Sanqi) reduced the levels of matrix metalloproteinases (MMP-2 and MMP-9), increased the expression of Bax and Caspase-3, and significantly reduced the proliferation and invasion ability of endometrial cells in AM model rats [14]. Using C-P drug pair is a promising therapeutic strategy for AM. However, the pharmacological mechanism of the C-P drug pair in the treatment of AM remains unclear.

TCMs are characterized by multiple components and multiple targets, which makes it difficult to further study the mechanism of the C-P drug pair in the treatment of AM. In recent years, the emergence of network pharmacology has provided an effective means to study the pharmacological mechanisms of TCMs. Construction and analysis of drug–component–target–disease (D-C-T-D) networks can enable the core components of TCMs and potential targets for disease treatment to be effectively discovered [15]. In this study, we used network pharmacology to construct D-C-T-D and protein–protein interaction (PPI) networks and searched for core components and key targets for C-P drug pairs to treat AM. Subsequently, we constructed a rat AM model to verify the network pharmacology results. Our findings provide a valuable reference for further study of the pharmacological mechanism of C-P in the treatment of AM.

2. Materials and Methods

2.1. Acquisition of Active Components and Targets for the C-P Drug Pair. The ingredients and targets of Cinnamomi ramulus were obtained from the TCMSP database (https://old.tcmsp-e.com/index.php). The active ingredients obtained from the TCMSP database were screened under the conditions of oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18, and the corresponding targets of the active ingredients were further obtained. The ingredients and corresponding targets of *Paris polyphylla* Sm. were obtained from the HERB database (https://herb.ac.cn/) and a literature search [16–22]. Standard name correction was performed for the obtained active ingredients using PubChem, and duplicate values were deleted. The SwissTargetPrediction database (https://www.swisstargetprediction.ch/) was used to predict the targets for the components obtained from the literature search [23, 24].

2.2. Search for AM-Related Targets. AM-related targets were searched in the GeneCards (https://www.genecards.org/), Coremine (https://coremine.com/medical/), and PharmGKB (https://www.pharmgkb.org/) databases using "Adenomyosis" as the retrieval keyword. The correlation score was used as a reference for each included target. The common targets between C-P and AM were identified using a Venn diagram.

2.3. Construction of the D-C-T-D Network. Microsoft Excel was used to determine the relationships among the drugs, active ingredients, common targets, and disease. The data were then imported into Cytoscape software, and a D-C-T-D network model was built. In the model, the nodes represent the herbs, components, targets, and disease, and edges represent relationships between two nodes. The number of associations of each node is used to calculate the "degree."

2.4. Construction of the PPI Network and Screening of Key Targets. The overlapping targets between the C-P active ingredient targets and the AM-related targets were imported into the STRING database (https://www.string-db.org/cgi/input.pl), and "*Homo sapiens*" was used as the screening criterion to obtain the PPI relationship [25]. The results were then imported into Cytoscape 3.8.0 software to construct a PPI network of intersecting targets of the C-P drug pair and AM. The core targets were obtained by screening related targets according to the degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC).

2.5. Gene Ontology (GO) Biological Process and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analyses. DAVID 6.8 (https://david.ncifcrf.gov/) was utilized to perform GO biological process enrichment and KEGG pathway analysis on the overlapping targets between C-P and AM [26]. The top 20 KEGG pathways and the top 20 biological processes from GO analysis with the most significant enrichment were plotted.

2.6. Molecular Docking of Core Components and Key Targets. The five components with the highest degrees were identified as core components in the D-C-T-D network, and the 2D structures of the core components were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. The 3D structures of key target proteins were downloaded from the RCSB database (https://www.rcsb.org/search). Chem3D software was used to preprocess the components, and AutoDock Vina software was used for molecular

docking analysis of the core components and key targets. The binding energy between the component and the protein was used to assess the degree of binding. PyMOL software was used to visually display part of the structure.

2.7. Experimental Drugs. The herbal medicines were supplied by the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (Jinan, China) and verified by Prof. Feng Li. Cinnamomi ramulus and *Paris polyphylla* Sm. were mixed at a standard ratio of 5 : 4 and were subjected to reflux extraction twice with 10 times the volume of distilled water for 1 h each. The extracts were then mixed thoroughly and concentrated to a relative density of 1.20–1.25 (70–80°C). The dosage of the C-P drug pair liquid used in subsequent animal studies was 2.7 g/kg/day. Mifepristone was purchased from Beijing Zizhu Pharmaceutical Co., Ltd. (National Medicine Standard H20010633). The final dosage was 1.25 mg/kg/day [27].

2.8. Reagents. Rabbit anti-Caspase-3 (bs-0081R) and Caspase-9 (bs-0049R) polyclonal antibodies were obtained from Beijing Bioss Biotechnology Co., Ltd. (Beijing, China). A Bax monoclonal antibody (60267-1-Ig) and a Bcl-2 polyclonal antibody (12789-1-AP) were obtained from Wuhan Proteintech Biotechnology Co., Ltd. (Wuhan, China). PI3 kinase p85 alpha (phospho-Y607, p-PI3K) (ab182651), GAPDH (EPR16891, loading control) (ab181602) and Akt (phospho-T308, p-Akt) (ab38449) antibodies were obtained from Abcam, Inc. (Cambridge, MA, USA).

2.9. Experimental Grouping, Modeling, and Intervention. Forty female Wistar rats and 30 male Wistar rats purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., were housed in the SPF Animal Experimental Center of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine. All rats had free access to a commercial diet and tap water. All the experimental procedures conformed to the regulations described in the Guide to the Care and Use of Laboratory Animals of the USA National Institutes of Health. The ethical review was approved by the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (AWE-2019-009). According to the random number table method, 40 female Wistar rats were divided as follows: 10 mice served as the blank group, and the remaining 30 were prepared for modeling. Animal models were established according to previous studies [28]. The pituitary gland of male rats was implanted into the uterine cavity of female rats, and 800,000 U of penicillin was injected for a week to prevent infection. Three months later, female rats were randomly divided into the model group, C-P group, and positive control group. According to the "Equivalent Dose Table for Conversion of Human and Animal Body Surface Areas," the rats were administered normal saline, C-P drug pair liquid, and mifepristone by gavage. After 30 days of treatment, the rats were euthanized by cervical dislocation, and their uterine tissues were collected.

2.10. Pathological Observation. The uterine tissues were fixed in 4% paraformaldehyde solution and embedded in paraffin, and 5 μm-thick sections were prepared for hematoxylin and eosin (H&E) staining. All images were acquired using a NanoZoomer S60 Digital slide scanner (Hamamatsu, Japan).

2.11. Immunohistochemistry. Bax, Bcl-2, Caspase-3, and Caspase-9 protein expressions in uterine tissues were detected using the immunohistochemistry streptavidin-peroxidase (SP) method. The regions were randomly selected under the microscope, and the cumulative optical density, area, and average density of the regions were measured using ImageJ (version 1.8.0).

2.12. Real-Time PCR. The expression of genes was quantified using the real-time PCR ΔΔCT method with a LightCycler 480II platform (Roche, Switzerland). Total RNA was purified using a Simply P Total RNA Extraction Kit according to the manufacturer's instructions. cDNA was synthesized with a ReverTra Ace qPCR RT Kit. Quantitative PCR was performed using a LightCycler 480II Real-Time PCR Detection System. All primers were synthesized by Integrated DNA Technology (BioSune, China). The primer sequences for mRNA analysis were as follows: phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA) forward 5′-CTGCAGTTCAACAGCCACAC-3′ and reverse 5′-CCAGCTGCCATCTCAGTTCA-3′; Akt1 forward 5′-AAGGTTTGCTGGGTGAGTGA-3′ and reverse 5′-CTCCTCAGGCGTTTCCACAT-3′. GAPDH expression was used to normalize gene expression.

2.13. Western Blotting. Uterine tissues (50 mg) collected from each group were homogenized and lysed in RIPA lysis buffer and then analyzed to detect the protein concentration using a BCA kit. Tissue total proteins were loaded onto 10% SDS–PAGE gels and transferred onto a PVDF membrane. The membrane was blocked using 5% skim milk and then incubated overnight with primary antibodies (p-PI3K, p-Akt, Bax, Bcl-2, and Caspase-9) at 4°C. The membrane was then incubated with a secondary antibody. Western blot analysis was performed using a ChemiDoc Imaging System (BIO-RAD, USA). The density of the signal was quantified by ImageJ.

2.14. Statistical Analyses. All data were analyzed using GraphPad Prism 8 (version 8.0.2) and are expressed as the mean ± standard deviation (SD). Comparisons between two groups were performed using Student's *t*-tests, whereas the significance of differences among three or more experimental groups was determined by one-way analysis of variance. A value of $P < 0.05$ was considered to indicate statistical significance. Diagrams were drawn using GraphPad Prism 8.

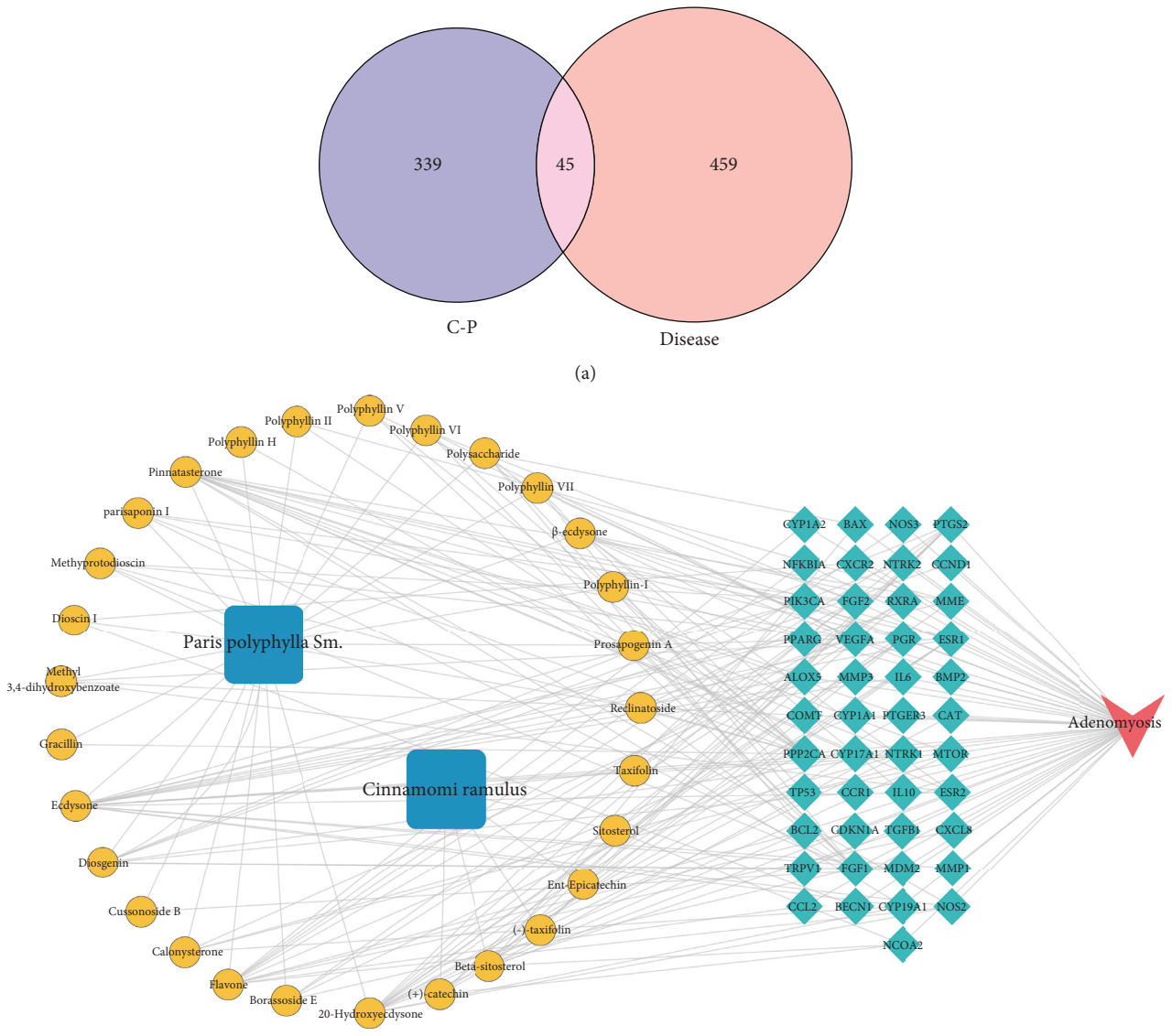


Figure 1: Venn diagram and drug-active component-target-disease network. (a) The Venn diagram shows 45 overlapping targets between AM-related targets and active compound-related targets. (b) Drug-component-target-disease (D-C-T-D) network of C-P in the treatment of AM. The blue nodes represent drugs, the yellow nodes represent active components, the dark green nodes represent targets, and the red nodes represent disease.

3. Results

3.1. Acquisition of Active Components and Targets for the C-P Drug Pair. A total of six active components and 50 corresponding targets of Cinnamomi ramulus were obtained from the TCMSP database. A total of 24 active components of *Paris polyphylla* Sm. were obtained from the HERB database and a literature search, and 360 targets corresponding to the active components of *Paris polyphylla* Sm. were obtained. A total of 384 potential targets of the C-P drug pair were identified after removing the repetition. A total of 504 targets related to AM were obtained through database retrieval. A Venn diagram revealed 45 intersecting targets between the C-P drug pair and AM (Figure 1(a)).

3.2. Construction of the D-C-T-D Network. Two herbs, 30 components, 45 targets, and 1 disease were classified and imported into Cytoscape 3.8.0 software to draw the D-C-T-D network (Figure 1(b)), which was composed of 77 nodes and 208 edges. "Degree" refers to the number of connections between each node and other nodes. Components in the network were screened according to a degree. The top five nodes based on the number of degrees were as follows: ecdysone (degree = 17), pinnatasterone (degree = 14), 20-hydroxyecdysone (degree = 14), flavone (degree = 12), and diosgenin (degree = 9). These molecules are thought to be the core components of the C-P drug pair in the treatment of AM (Table 1).

Table 1: Core components.

Component name	PubChem CID	Molecular formula	Molecular weight	Degree
Ecdysone	19212	C27H44O6	464.6	17
Pinnatasterone	15214617	C27H44O7	480.6	14
20-Hydroxyecdysone	146158258	C33H52O7	560.8	14
Flavone	10680	C15H10O2	222.24	12
Diosgenin	99474	C27H42O3	414.6	9

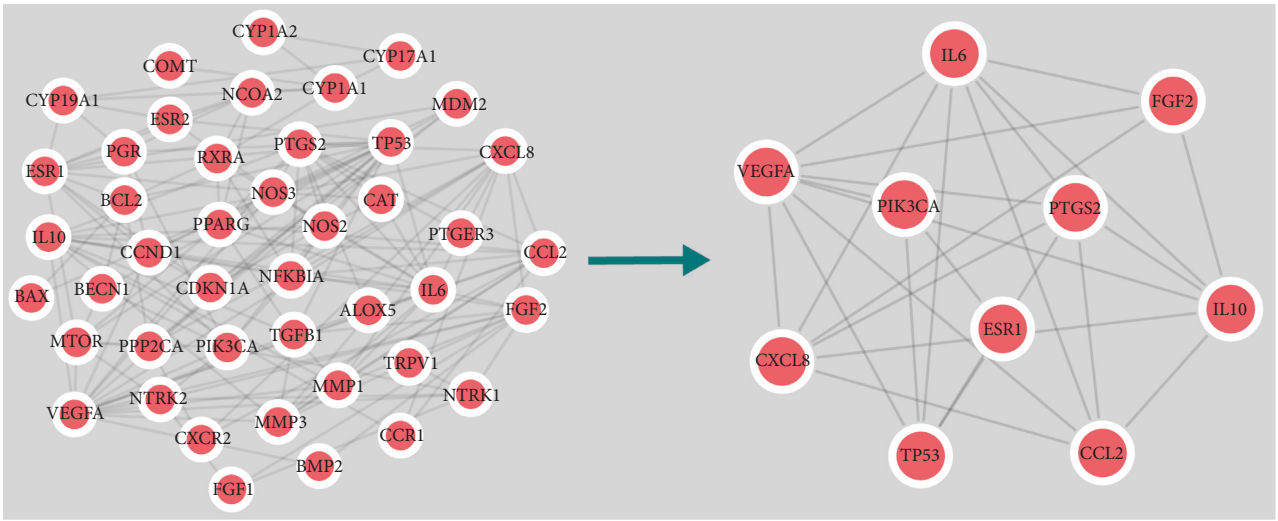


Figure 2: PPI network and core targets. (a) PPI network of potential C-P therapeutic targets for AM. (b) 10 core targets in the PPI network.

3.3. Construction of the PPI Network and Screening of Key Targets. Forty-five common targets were imported into the STRING database to obtain the PPI relationship data, which were imported into Cytoscape 3.8.0 software to construct the PPI network (Figure 2(a)). Finally, the first ten targets were selected as the key targets for C-P in the treatment of AM according to the BC, CC, and DC (Figure 2(b)). The key targets included PIK3CA, interleukin-8 (CXCL8), interleukin-6 (IL6), estrogen receptor (ESR1), cellular tumor antigen p53 (TP53), c-c motif chemokine 2 (CCL2), vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), prostaglandin G/H synthase 2 (PTGS2), and interleukin-10 (IL10) (Table 2).

Table 2: Information of the 10 key targets.

Gene	Degree centrality	Betweenness centrality	Closeness centrality
TP53	18	0.189524	0.558442
VEGFA	17	0.15469	0.52439
PTGS2	13	0.117131	0.518072
ESR1	12	0.086981	0.477778
PIK3CA	9	0.068537	0.488636
IL6	15	0.063904	0.5375
CXCL8	12	0.048844	0.472527
FGF2	11	0.046362	0.43
CCL2	11	0.036549	0.457447
IL10	12	0.031854	0.467391

3.4. GO Biological Process and KEGG Pathway Enrichment Analyses. We used the DAVID database to conduct an enrichment analysis of GO and KEGG pathways for the intersected targets of the C-P drug pair and AM and obtained a total of 327 biological processes (BPs) and 118 signaling pathways with a P value <0.05 as screening conditions. R 4.0.2 software and its expansion package were used to draw bubble charts to show the top 20 significantly enriched BP and KEGG terms (Figure 3(a), 3(b)). The results of KEGG pathway enrichment showed that the PI3K-Akt signaling pathway was the most significant non-tumor-related pathway. In the GO analysis results, phosphorylation regulation of the PI3K signal and positive regulation of protein phosphorylation were also significantly enriched. In addition, PIK3CA, the core protein of the PI3K-Akt signaling pathway, was a key target in the PPI network screening. Therefore, we concluded that the PI3K-Akt signaling pathway plays a key role in the C-P treatment of AM. This conclusion was verified in the animal experiments performed for this study.

3.5. Verification of Molecular Docking. The 2D structures of the five core components in the D-C-T-D network were obtained from the PubChem database. The 3D structures of 10 key targets in the PPI network were obtained from the RCSB database. Five core components and 10 targets were

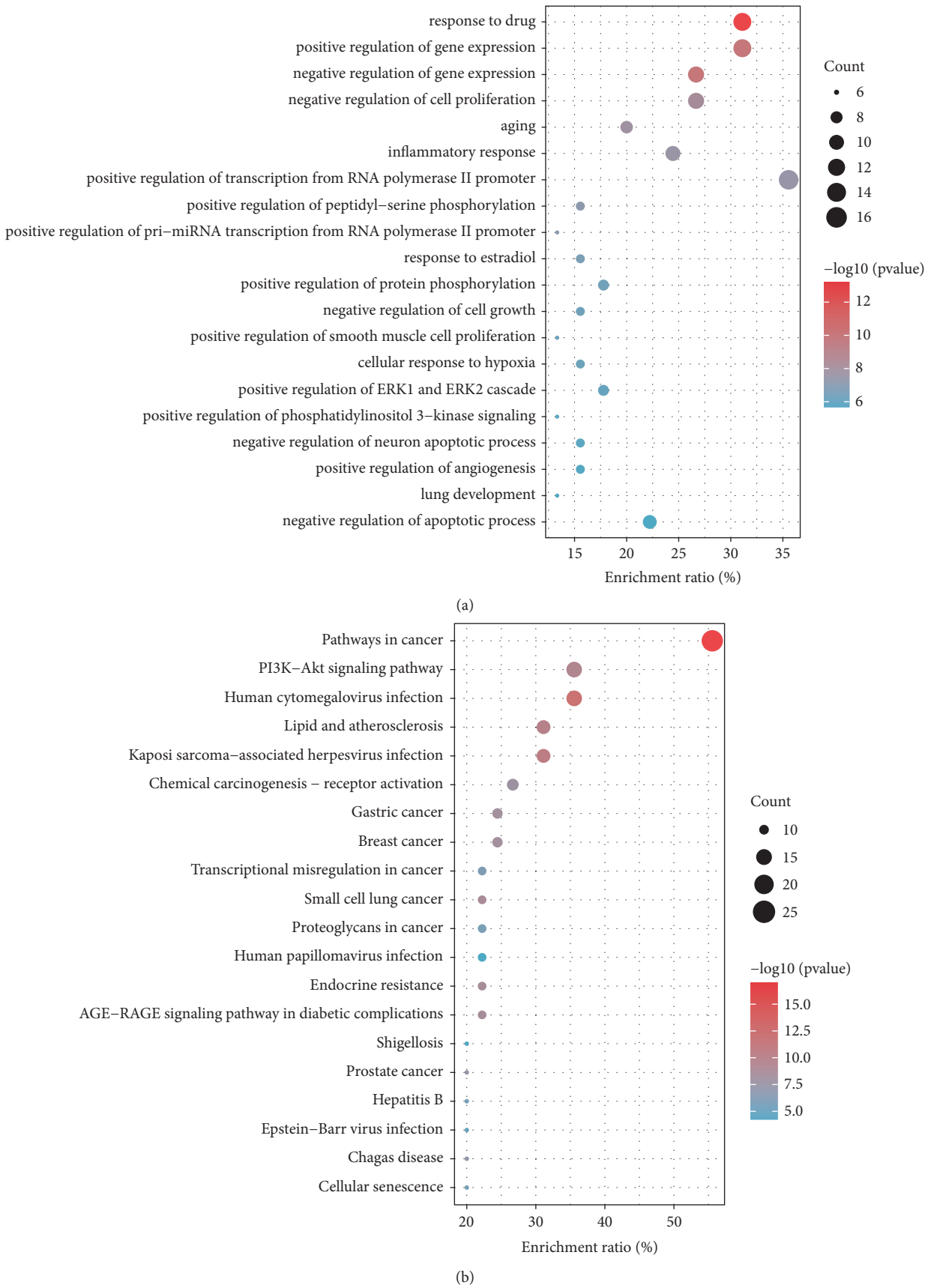
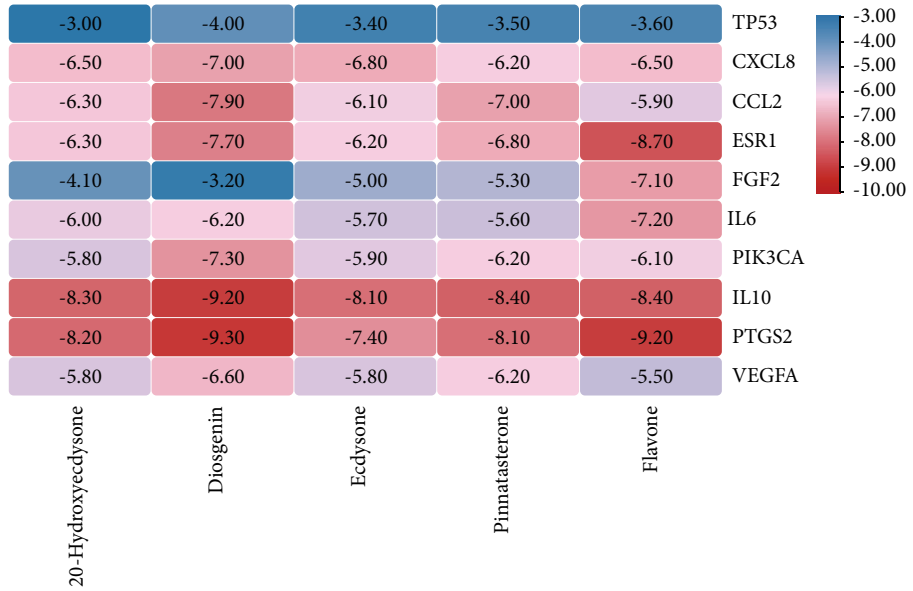
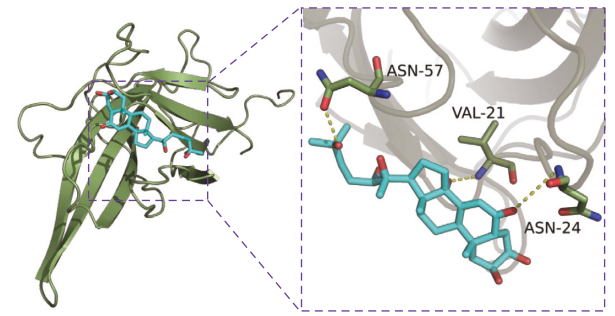


Figure 3: Bubble charts. (a) Bubble chart for GO enrichment analysis. (b) Bubble chart for KEGG enrichment analysis. The closer the bubble color is to red, the smaller the P value is, and the bubble size reflects the number of enriched genes.



(a)



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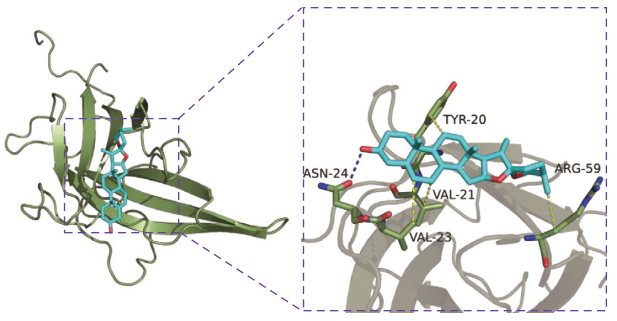

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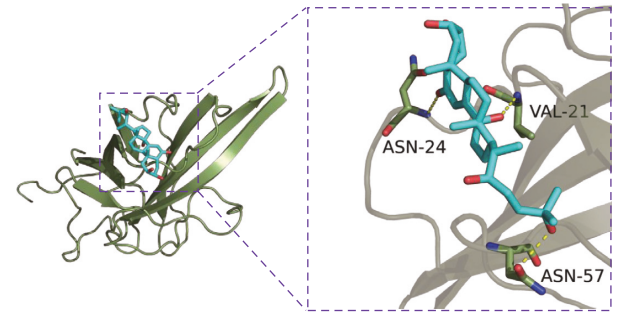

(d)

Figure 4: Continued.

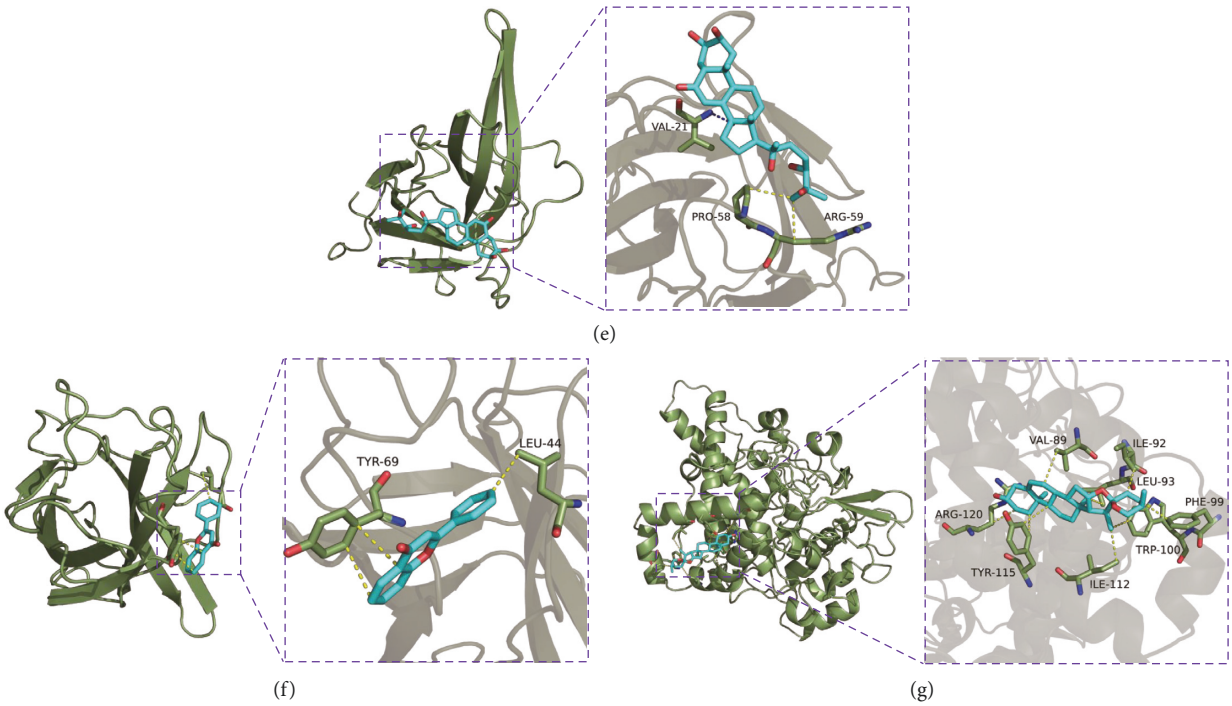

Figure 4: Results of molecular docking. (a) Heatmap of binding energy. Unit: kcal/mol. (b) Molecular docking of PIK3CA with 20-hydroxyecdysone. (c) Molecular docking of PIK3CA with diosgenin. (d) Molecular docking of PIK3CA with ecdysone. (e) Molecular docking of PIK3CA with pinnatasterone. (f) Molecular docking of PIK3CA with flavone. (g) Molecular docking of PTGS2 with diosgenin.

docked. Binding energy was used to evaluate the degree of binding of ingredients to proteins, and a binding energy <−5 kcal/mol was considered to indicate that component and target could bind. The results of our study showed that the core components had good overall docking with the key targets (Figure 4(a)), indicating that these components may play a role in the treatment of AM by acting on the key targets. We used PyMOL to demonstrate some of the well-matched results (Figure 4(b)–4(g)). The blue lines between molecules represent hydrogen bonds and the yellow lines represent hydrophobic interactions.

3.6. H&E Staining of Uterine Tissue. In the control group, the uterine morphology was intact and the muscular layer was continuous. The boundary between the muscular layer and the endometrium was clear, and no gland or endometrium tissue invasion was observed. However, the muscle layer in the model group was thickened with gland invasion and the boundary was blurred. Compared with that in the model group, the muscle layers in the C-P and mifepristone groups were almost intact. The boundary between the muscle layer and the intima layer was fairly clear, and fewer glands invaded the muscle layer (Figure 5)(a).

3.7. Effect of the C-P Drug Pair on the PI3K-Akt Signaling Pathway. The results of real-time PCR analysis indicated that PIK3CA and Akt1 mRNA expression was significantly higher in the AM model group than in the control group. The C-P drug pair and mifepristone significantly suppressed PIK3CA and Akt1 mRNA expression in AM rats compared with that in model rats (Figure 5(b), 5(c)). The expression trend of the P-PI3K and P-Akt proteins in Western blot analysis was the same as that observed with real-time PCR (Figure 6(a)).

3.8. The C-P Drug Pair Promoted Apoptosis in Ectopic Uterine Lesions. We observed changes in the apoptosis-related indicators Bax, Bcl-2, Caspase-3, and Caspase-9 in AM rats after C-P treatment based on tissue- and protein-level detection. The immunohistochemistry results showed that Bax (Figure 7(a)), Caspase-3 (Figure 7(c)) and Caspase-9 (Figure 7(d)) expression in the AM model group was significantly weaker than that in the control group, while Bcl-2 (Figure 7(b)) expression was stronger than that in the control group. Bax, Caspase-3, and Caspase-9 expressions were significantly enhanced in the C-P and mifepristone-treated AM groups than in the AM model group, while Bcl-2 expression was attenuated. The Western blot analysis results were consistent with the immunohistochemistry results. Compared with that in the control group, Bax and C-Caspase-9 protein expression in the model group was significantly reduced, and Bcl-2 protein expression was significantly induced. Compared with that in the model group, Bax and C-Caspase-9 protein expression in the C-P group and mifepristone group was significantly increased, whereas Bcl-2 protein expression was significantly suppressed. However, no significant difference in Caspase-9

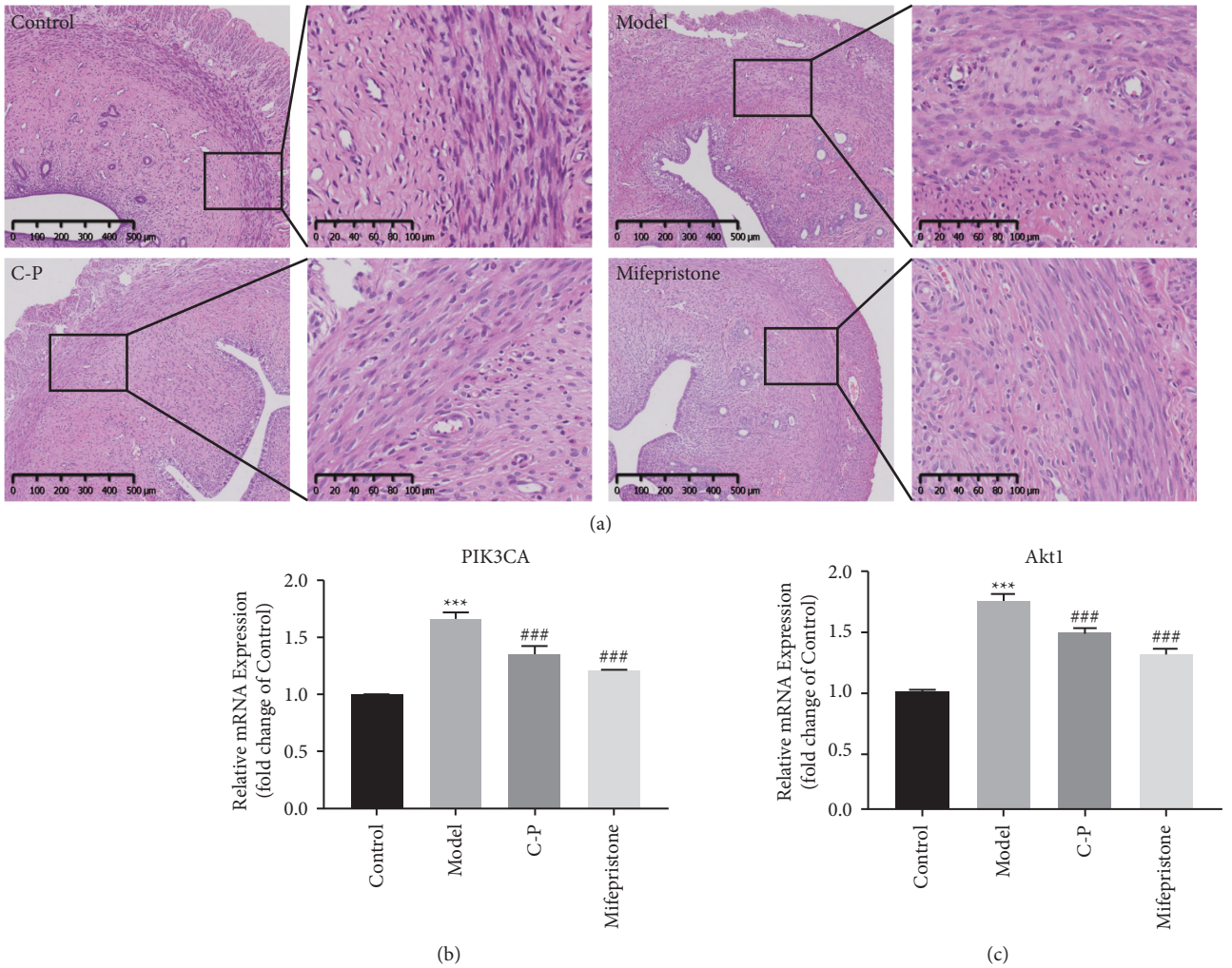

Figure 5: H&E staining and results of real-time PCR. (a) H&E staining. (b)The mRNA expression of PIK3CA and Akt1 (compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Compared with model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$).

expression was noted between groups, suggesting that C-P might promote Caspase-9 activation (Figure 6(b)).

4. Discussion

Although AM is a condition of benign gynecological lesions, the lesions show abnormal tumor characteristics including endometrial cell proliferation, apoptosis, invasion, and migration. Compared with radical hysterectomy, TCM treatment has positive effects on women's fertility. Cinnamomi ramulus and *Paris polyphylla* Sm. are commonly used in TCM [29–31]. The pathological basis of AM in the theory of TCM is that the blood of the meridian in the uterus is cold and stagnant. In TCM, it is believed that Cinnamomi ramulus can warm the meridian and promote blood circulation and that *Paris polyphylla* Sm. can remove blood stasis. Cinnamomi ramulus and *Paris polyphylla* Sm. are commonly used to warm meridians, remove blood stasis and relieve pain.

Modern pharmacological studies have shown that the active components of Cinnamomi ramulus can inhibit the activity, migration, and glycolysis of endometrial stromal cells by inhibiting pyruvate kinase PKM (PKM2) transcription induced by the NF-κB signaling pathway [32]. *Paris polyphylla* Sm. and its derivatives have been widely used to treat abnormal endometrial bleeding [11]. *Paris polyphylla* Sm. is also rich in a variety of saponins that have been widely used to combat abnormal cell proliferation and migration and to promote apoptosis of abnormal cells [33, 34].

TCM is characterized by the use of multiple drugs with multiple components and targets for the treatment of diseases. This complexity can hinder the exploration of the molecular mechanisms of TCM drugs in the treatment of AM from the perspective of precision medicine. Network pharmacology is useful for constructing D-C-T-D networks and PPI networks of drug–disease intersection targets based on the abovementioned characteristics of TCM. Through network analysis, the core components and key targets of TCM in the treatment of diseases can be obtained. This research method is beneficial for precision research on TCM and provides an effective means for identifying new targets for disease treatment.

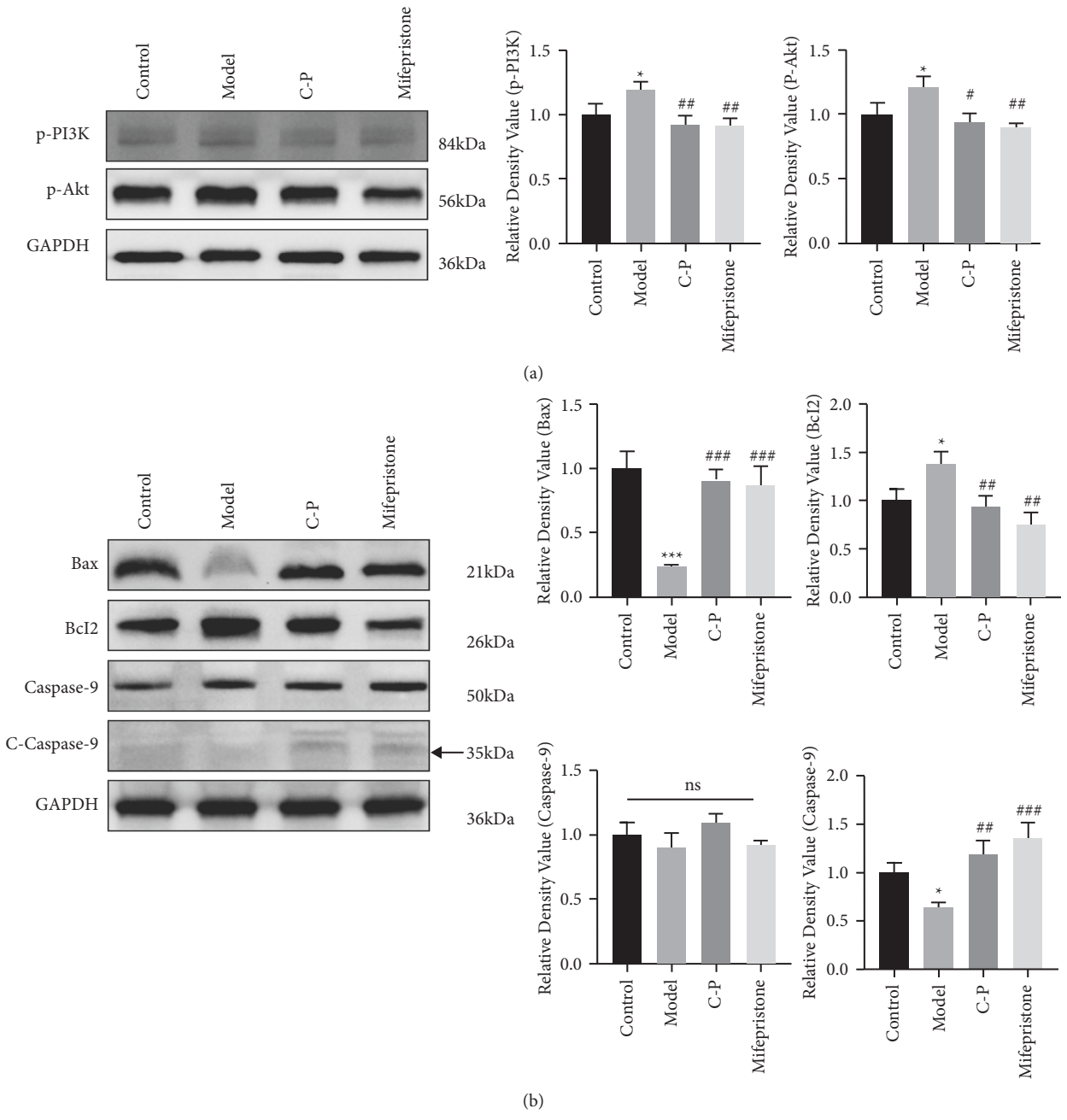

Figure 6: Results of western blotting assays. (a) The expression of p-PI3K and p-Akt. (b) The expression of Bax, Bcl-2, Caspase-9 and C-Caspase-9 (compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Compared with model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$).

In our study, a total of 30 components and 45 corresponding targets of the C-P drug pair related to AM were obtained through network pharmacology. In the D-C-T-C network, ecdysone, pinnatasterone, 20-hydroxyecdysone, flavone and diosgenin exhibited high degree numbers. These results suggest that these compounds may play a major role in the pharmacological mechanism of C-P in the treatment of AM. 20-Hydroxyecdysone has been reported to be a natural active component with the potential to promote apoptosis and autophagy in cancer cells [35]. In addition, as a natural steroid saponin, diosgenin can promote apoptosis of abnormally proliferating endometrial cells by regulating the apoptosis-related proteins Bcl-2, Caspase3, and Caspase9 [36]. Secretory dysfunction of the ovaries is considered to be an important factor affecting AM [37]. The synthesis of ecdysone in humans is regulated by the estrogen receptor (ER) [38]. Studies have shown that exogenous supplementation of ecdysone in ovariectomized female mice can inhibit a variety of metabolic diseases caused by the ovarian loss [39, 40].

Through analysis of the PPI network, we obtained 10 potential core targets of C-P therapy for AM: PIK3CA, IL10,

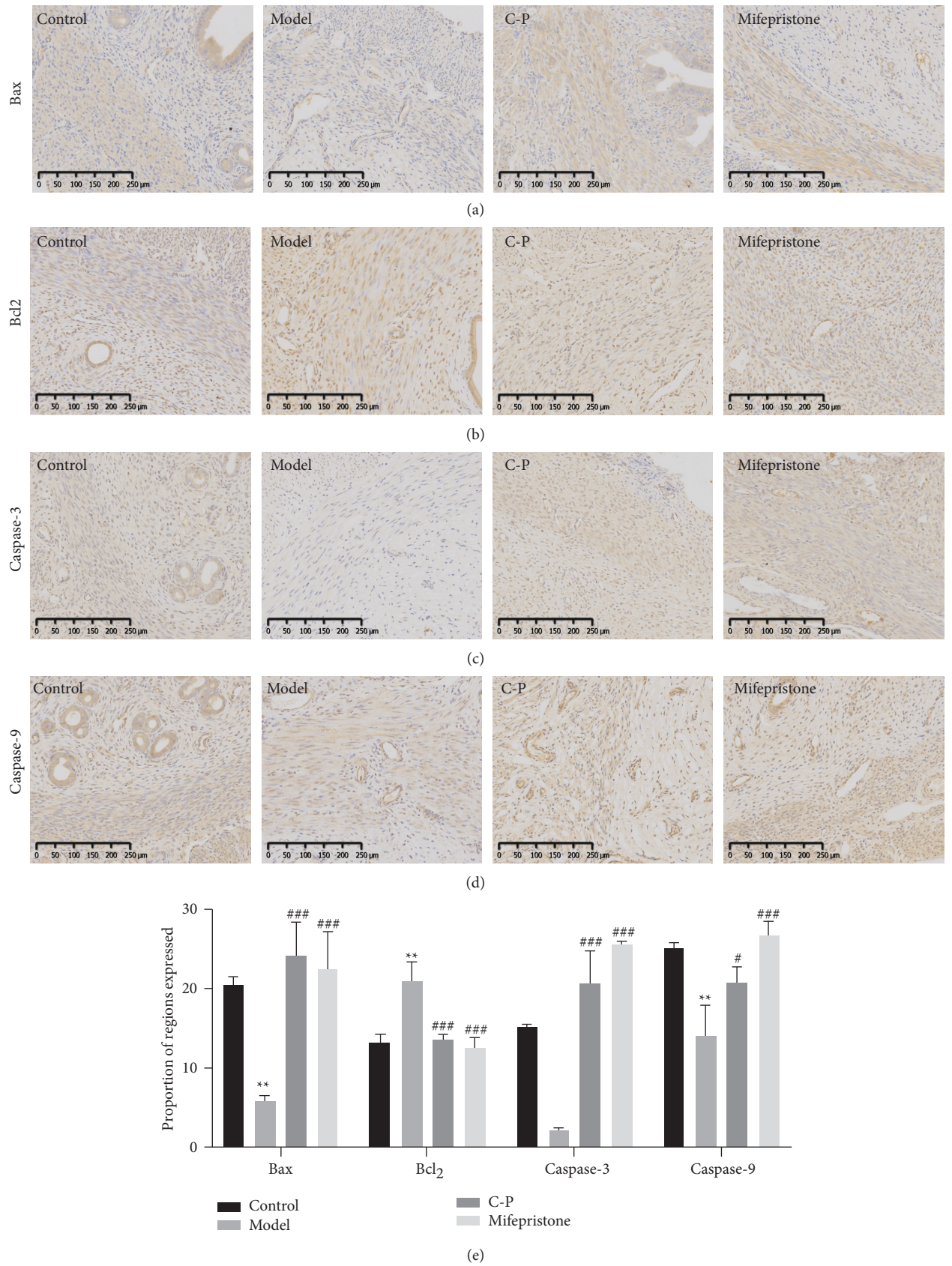

Figure 7: The results of immunohistochemistry. (a) Bax. (b) Bcl-2. (c) Caspase-3. (d) Caspase-9. (e) Quantitative analysis of immunohistochemistry (compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Compared with model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$).

IL6, ESR1, CCL2, TP53, VEGFA, FGF2, PTGS2, and CXCL8. PIK3CA is the catalytic subunit of the dimeric protein PI3K. As the core mediator of the PI3K signaling pathway, PI3K is a critical mediator of cell survival, and its downstream MTOR protein is an important protein involved in autophagy to avoid apoptosis [41]. IL6 and VEGFA are involved in inflammation and abnormal angiogenesis in AM, respectively [42, 43]. ESR1, a receptor protein of estrogen, is involved in the balance of estrogen in women, and studies have shown that disruption of this balance is an important factor in the occurrence and progression of AM [44]. IL10 is one of the major anti-inflammatory cytokines, and abnormal expression of IL10 in AM may impair endometrial receptivity and thus affect embryo implantation [45]. In summary, multiple core targets obtained through PPI network analysis play important roles in the pathological process of AM.

Through KEGG enrichment analysis of overlapping C-P and AM targets, we found that the PI3K signaling pathway was the most significant nontumor pathway, and GO enrichment analysis showed that phosphorylation (activation) of the PI3K signaling pathway was a significantly enriched biological process. In the molecular docking results, PIK3CA (PI3K) was able to dock with multiple core components. Therefore, PIK3CA may represent an important target of C-P in the treatment of AM. Previous studies have demonstrated that PI3K-Akt activation in AMs promotes endometrial cell proliferation [46]. In addition, PI3K and Akt gene and protein expression are upregulated in AM patients compared with controls without endometrial lesions [47]. The most widely studied PI3K is a heterodimer composed of a regulatory subunit (P85) and a catalytic subunit (PIK3CA, PIK3CB, PIK3CD, and PIK3CG). To generate the active form of PI3K, the phosphorylation sites in the SH2 and SH3 domains of the P85 subunit bind to the corresponding binding proteins, and phosphorylation occurs. Phosphorylated PI3K also activates the downstream protein Akt through phosphorylation and ultimately regulates a variety of biological pathways. In this study, we assessed the status of the PI3K/Akt signaling pathway by detecting P-PI3K and P-Akt protein levels. The PI3K/Akt pathway was activated in the AM model group compared with the control group, whereas PI3K and Akt phosphorylation was inhibited after C-P intervention.

The PI3K/Akt signaling pathway is closely related to Bcl-2 family proteins. When stimulated, active Akt leads to the phosphorylation of the proapoptotic protein Bad and inhibits the translocation of Bad from the cytoplasm to the mitochondria, thereby inhibiting mitochondrial dysfunction. Mitochondrial dysfunction stimulates downstream Caspase-3 and Caspase-9. The cleaved forms of Caspase-3 and Caspase-9 regulate other protein substrates, ultimately triggering apoptosis [48]. Moreover, the PI3K/Akt pathway mediates apoptosis in many gynecological diseases. PI3K/Akt is involved in uterine leiomyoma apoptosis and proliferation [49, 50]. Naringenin increases apoptosis of human endometriosis cells through the inactivation of the PI3K pathway [51]. CD47 overexpression activates the PI3K/Akt/mTOR signaling pathway in endometrial carcinoma cell lines to reduce cancer cell apoptosis [52]. In this study, through detection of apoptosis-related indicators, we found that the proapoptotic proteins Bax, Caspase-3, and Caspase-9 were significantly downregulated in the AM model group, whereas the antiapoptotic protein Bcl-2 was significantly upregulated. After C-P intervention, the Bax, Caspase-3, and Caspase-9 proteins were significantly upregulated, whereas Bcl-2 was significantly downregulated. In conclusion, the pharmacological mechanism by which C-P improves AM is related to the inhibition of the PI3K signaling pathway and the promotion of AM lesion cell apoptosis.

5. Conclusion

Network pharmacology and animal experiments showed that the C-P drug pair can significantly delay disease progression in AM rats. We propose that the pharmacological mechanism of C-P in the treatment of AM is related to inhibition of the PI3K pathway and promotion of apoptosis in AM ectopic lesions. However, unfortunately, the pharmacological components of C-P are not well characterized, and whether C-P regulates apoptosis through the PI3K/Akt pathway remains unclear. We will further explore the effective components and pharmacological mechanism of C-P in the treatment of AM through subsequent experiments.

Data Availability

All datasets for this study are included in the manuscript, further inquiries can be directed to the corresponding authors.

Conflicts of Interest

All authors have no financial or scientific conflicts of interest with regard to the research described in this manuscript.

Authors' Contributions

Keke Zhang, Zhou Zhou, and Chuchu Wang have contributed equally to this work.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 81873330), Shandong Taishan Scholar Project (No. tsqn201909185), the National Natural Science Foundation of China (No. 82104916), and the Science and Technology Development Program of Jinan (201907009).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 4892215, 14 pages
https://doi.org/10.1155/2022/4892215

Review Article

The Efficacy of Chinese Herbal Medicine in Animal Models of Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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Received 18 April 2022; Revised 20 June 2022; Accepted 22 July 2022; Published 13 August 2022

Academic Editor: Min Hu

Objective. This study aimed to evaluate the efficacy of Chinese herbal medicine (CHM) on ovarian mass, weight, sex hormone disorders, and insulin resistance in animal models of polycystic ovary syndrome (PCOS). *Methods*. This systematic review and meta-analysis was conducted through a comprehensive search in three databases to find studies testing CHM in animal models of PCOS. Two researchers independently reviewed the retrieval, extraction, and quality assessment of the dataset. The pooled effects were calculated using random-effect models; heterogeneity was explored through subgroup analysis; and stability was assessed through sensitivity analysis. In addition, publication bias was assessed using the Egger's bias test. *Results*. Fifteen studies with twelve mice and 463 rats published from 2016 to 2021 met the inclusion criteria. The results of primary outcomes revealed that CHM therapy was significantly different with control animals in ovarian mass and testosterone (SMD, −1.01 (95% CI, −1.58, −1.45); SMD, −1.62 (95% CI, −2.07, −1.16), respectively). The secondary outcomes as well showed an overall positive effect of CHM compared with control animals in weight (SMD, −1.02 (95% CI, −1.39, −0.65)), follicle-stimulating hormone (FSH) (SMD, 0.58 (95% CI, 0.19, 0.97)), luteinizing hormone (LH) (SMD, −0.94 [95% CI, −1.25, −0.64)), homeostasis model assessment-insulin resistance (HOMA-IR) (SMD, −1.24 (95% CI, −1.57, −0.92)). Subgroup analyses indicated that PCOS induction drug, formula composition, random allocation, and assessment of model establishment were relevant factors that influenced the effects of interventions. The stability of the meta-analysis was showed robust through sensitivity analysis. The publication bias was substantial. *Conclusions*. Administration with CHM revealed a statistically positive effect on ovarian mass, weight, sex hormone disorders, and insulin resistance. Moreover, these data call for further high-quality studies investigating the underlying mechanism in more depth.

1. Introduction

Polycystic ovary syndrome (PCOS), characterized mainly by hyperandrogenism (androgen excess, acne and/or hirsutism) and ovarian dysfunction (failure or absence of ovulation and/or polycystic ovarian morphology), is one of the most common endocrine and metabolic disorders. It affects approximately 8% to 18% of women of reproductive age [1]. However, most of the drugs treating the symptoms of PCOS were used in an off-label fashion because no drug was approved specifically for PCOS neither by the FDA nor by the European Medicines Agency [2]. The first-line therapy for PCOS is lifestyle modification via diet and physical activity [3]. Patients with mild symptoms might not require any drug intervention. Besides, letrozole and clomiphene are considered the most common drugs recommended for patients with PCOS who are seeking fertility [4]. According to a consensus statement, the oral contraceptive pill and antiandrogens are suggested in PCOS patients who are not attempting to conceive [5]. Notwithstanding their efficacy, clinical resistance, multiple pregnancy along with ovarian hyperstimulation syndrome are prone to occur with chronic treatment [6]. Considering that PCOS is a

heterogeneous and lifelong disorder, treatment for PCOS should be symptom-oriented and adapted to the expectations of the individual patient.

Traditional Chinese medicine (TCM), attaching great importance to individualized treatment, follows an independent theoretical pathway to make diagnosis and treatment plans by systematically evaluating patients' signs and symptoms. Gynaecological and infertility problems of PCOS have been widely treated by TCM in China [7, 8]. Some women in southern Australia, the UK, and the USA have also begun to use TCM in subfertility clinics [9]. Chinese herbal medicine (CHM), a kind of TCM therapy consisting of a variety of herbs, remains controversial because it is hard to be standardized. CHM has different compatibility for each patient which coincides with the principle that we should pay attention to individualized treatment in PCOS. Notwithstanding the growing interest in CHM, rigorous clinical trials addressing their specific effects are lacking. These trials showed quite a few biases related to age, genetic background, or other interfering variables [10]. In this regard, animal models may represent a useful tool to study the efficacy and mechanisms of various formulas used in PCOS models that were induced by letrozole, dehydroepiandrosterone, prasterone sodium sulfate, or testosterone propionate. In addition, rats and mice are ideal animal models for PCOS because they are sensitive to sexual hormone stimulation and have a stable estrous cycle that is easy to observe [11, 12].

The components of different formulas act synergistically in various ways [13]. Our previous study demonstrated that Bushen Huatan Granules (BHG) and Kunling Wan (KW) respectively ameliorated DHEA-induced PCOS symptoms such as irregular estrous cycle, high levels of testosterone and insulin in serum. Both BHG and KW may attenuate the apoptosis in granulosa cells (GCs), while BHG targets the mitochondria-dependent apoptotic pathway and KW targets the endoplasmic reticulum stress-dependent apoptotic pathway [14]. In addition, it is reported that Guizhi Fuling Wan (GFW) inhibited GCs' autophagy by activating the phosphatidylinositol-3-Kinase (PI3K)/Protein Kinase B (AKT)/mammalian Target of Rapamycin (mTOR) pathway and alleviated ovulation disorder in PCOS-IR rats [15]. Considering the optimal pattern of CHM therapy in various symptoms of PCOS remains unanswered, analysis that evaluates the specific efficacy of different formulas in rodent models of PCOS will be prospective.

Herein, we report a systematic review and meta-analysis of data from studies testing the efficacy of CHM in animal models of PCOS. The purpose of this study was to provide evidence relating to the efficacy of CHM on ovarian mass, weight, sex hormone disorders, and insulin resistance in animal models of PCOS.

2. Methods

We conducted the systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [16, 17]. No animal work was performed in this study. Moreover, the protocol was based on SYRCLE's systematic review protocol format for animal intervention [18] was registered in PROSPERO (registration number: CRD42022310345).

2.1. Search Strategy. We conducted a comprehensive search of three electronic databases which included PubMed, Web of Science, and Scopus from inception to February 2022. Searches were limited to English-language publications. Details of the search strategies are shown in Supplementary File 1.

2.2. Inclusion and Exclusion Criteria. The experts formulated the eligibility criteria for the current study. Publications were considered eligible based on the following inclusion criteria: (1) Experimental subjects are animal models of PCOS. (2) Intervention is Chinese herbal medicine. (3) Comparison with the PCOS group in the animal experimental studies, with no treatment. (4) Outcomes include effects of Chinese herbal medicine on the development or treatment of PCOS, morphological and hormonal alterations in the animal experimental studies. The original trials should include at least one of the following outcomes: weight, ovarian mass, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and homeostasis model assessment-insulin resistance (HOMA-IR). (5) Types of study are experimental animal studies.

Furthermore, two authors examined the titles and abstracts of retrieved studies. Exclusion criteria were: (1) nonoriginal studies and no full-text articles (e.g. reviews, editorials); (2) in vitro and in silico studies; (3) interventions different from Chinese herbal medicines (e.g. single traditional Chinese medicine, acupuncture or monomer composition); (4) all species different from mouse and rat; (5) presence of concomitant interventions in the control group. In addition, full-texts of retrieved studies were assessed for eligibility independently by two authors. Studies for which was no full-text available or had no relevant outcomes reported were excluded.

2.3. Data Extraction. Two reviewers independently assessed the extraction of data. Any disagreement between them was solved through discussion. Detailed data extraction was collected by using the following characteristics: (1) publication details (author and year); (2) intervention used (prescription composition, route, dose and timing); (3) PCOS induction method; (4) animal used (species, strain, age and weight); (5) underlying mechanism of the intervention; (6) information of outcomes. Moreover, for each comparison, we extracted data reporting the sample size per group, mean value, and variance (SD or SEM) for both the treatment and control group.

The sample size of the control group was divided by the number of treatment groups to adjust the impact of the control group [19]. When treatment was administered in multiple doses, each dose's data was extracted and performed in separate studies. For studies only presented

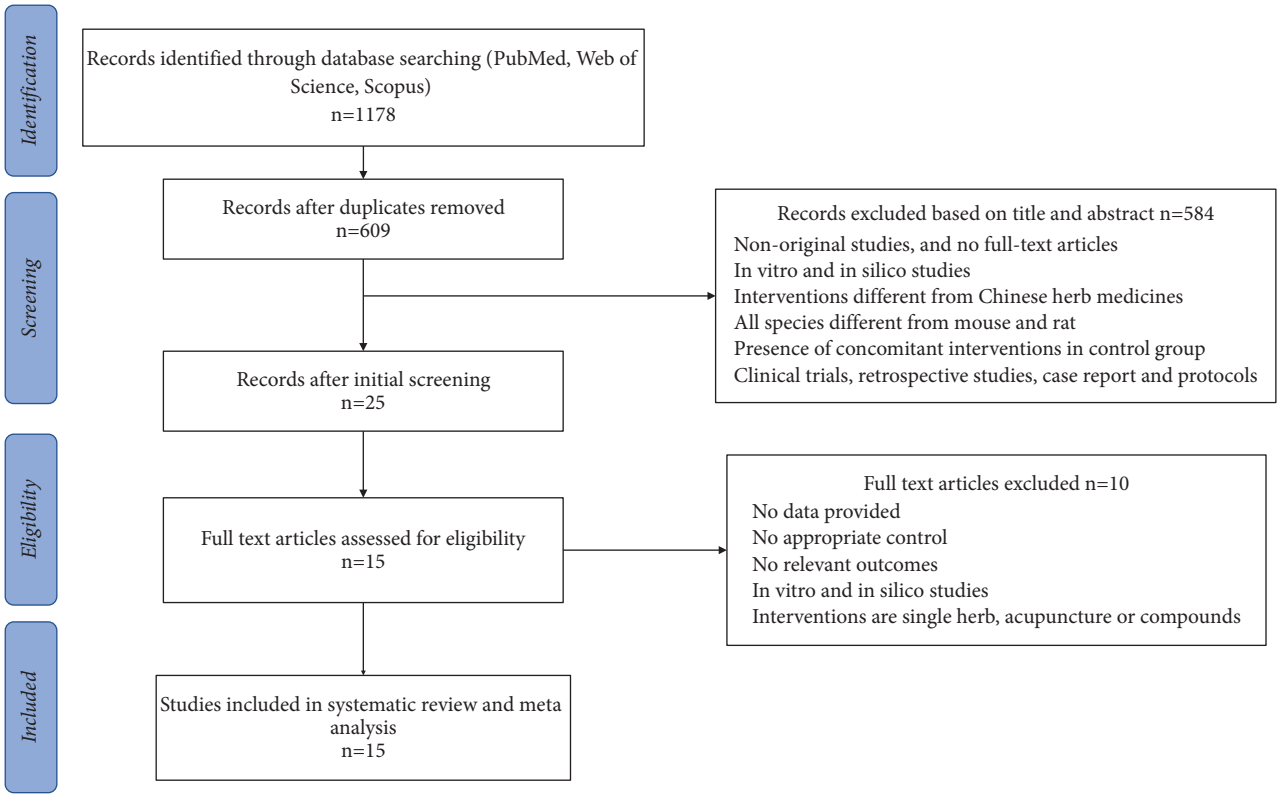


Figure 1: Flow diagram of publication inclusion.

graphically, we contacted the corresponding authors by e-mail to request data. If no response was received, we used the ImageJ software to quantify the results. We will exclude studies when essential data could not be obtained.

2.4. Risk of Bias Assessment. The internal validity of the included studies was assessed by two reviewers independently, referencing the SYRCLE's risk of bias tool for animal studies [20]. The 10-item checklist of evaluation included: (1) publication in a peer-reviewed journal; (2) control of temperature; (3) random allocation to groups; (4) assessment of PCOS model establishment; (5) blinded assessment of outcome; (6) accurate drug production institutions; (7) detection of estrous cycles; (8) the use of comorbidity animals; (9) compliance with animal welfare regulations; 10) statement of potential conflicts of interest.

2.5. Statistical Analysis. When the measure of variance reported was Standard Error of Mean (SEM), we inverted SEM to Standard Deviation (SD) first. Considering that different species and measurements in animals vary greatly, we used a standardized mean difference (SMD) to standardize the results to a uniform scale [21].

We performed a random-effect meta-analysis to calculate the SMD values with 95% confidence intervals (CI) as the overall effects for the combined pooled outcomes which were continuous. Heterogeneity was assessed by the Q statistic and quantified using the I^2 statistic [22]. Further, subgroup analyses, related to the impacts of interventions with different mechanisms and characteristics, were conducted to explore the sources of heterogeneity. Sensitivity analyses were performed by repeating the primary meta-analysis to confirm the robustness of the results. Moreover, publication bias was assessed statistically with Egger's bias test with $p < 0.05$ indicating asymmetry. RevMan 5.3 and STATA 17 were used to perform the statistical analyses.

3. Results

3.1. Study Selection. Initially, 1178 studies were retrieved through a comprehensive search of three databases (66 for PubMed, 1069 for Web of Science, and 43 for Scopus), out of which 609 nonduplicate studies were filtered out. After reviewing the titles and abstracts, 584 studies were removed based on predetermined exclusion criteria. Furthermore, 10 studies were excluded in the second selection phase, and 15 studies were finally included in the systematic review. A flowchart depicting the research selection process is presented in Figure 1.

3.2. Study Characteristics. These 15 articles [14, 15, 23–35] investigated 13 formulas and 35 treatment arms according to dose and herb composition. The main characteristics are summarized in Table 1. All the included studies were conducted between 2016 and 2021. Rats were used in most of the studies, only one study used mice. The quantity of herbal medicine used in compounds varied greatly, which ranged from 2 to 31. PCOS models were induced by letrozole,

Table 1: Characteristics of included studies.

Author, year	Intervention	Mechanism	Quantity of herb types	Dose(s)	Route	Treatment duration	PCOS induction methods	Species	Strain	Outcomes
Pan, 2021	BJTF	PERK-ATF4-CHOP↓	8	BJTF-L: 9.32 g/kg BJTF-M: 18.63 g/kg BJTF-H: 37.26 g/kg	Gavage	21 days	Daily gavage of letrozole, 1 mg/kg, for 28 days	Rat	SD	①②③④⑤
Jiang, 2021	BSZY	PI3K/AKT/mTOR↑	6	BSZY-L: 20 g/kg BSZY-H: 40 g/kg	Oral	6 estrous cycles	Daily s.c injection of DHEA, 60 mg/kg, for 28 days	Rat	SD	①③
Chang, 2021	SGD	TLR4/NF-κB↓	2	25 g/kg	NM	2 weeks	Daily oral administration of letrozole, 1 mg/kg, for 21 days	Rat	SD	②③③⑤
Xu, 2021	BHG; KW	BHG: regulation of mitochondria KW: protection of endoplasmic reticulum stress.	BHG: 10 KW: 31	BHG-L: 0.75 g/kg BHG-M: 1.49 g/kg BHG-H: 2.99 g/kg KW-L: 0.46 g/kg KW-M: 0.91 g/kg KW-H: 1.82 g/kg	Gavage	28 days	Daily s.c injection of DHEA, 60 mg/kg, for 20 days	Rat	SD	②③⑥
Liu, 2021	GFW	PI3K/AKT/mTOR↑	5	GFW-L: 0.31 g/kg GFW-M: 0.62 g/kg GFW-H: 1.24 g/kg	Gavage	30 days	Daily oral administration of letrozole combined with intragastric high-fat emulsion, for 21 days	Rat	SD	②④⑤⑥
Wang, 2020	CFD	IGF-1-PI3K/Akt-Bax/Bcl-2↓	16	CFD-L: 15 g/kg CFD-H: 30 g/kg	Gavage	4 weeks	Daily intraperitoneal injection of letrozole, 1 mg/kg, combined with HFD, for 21 days	Rat	SD	①②③④⑤⑥
Xu, 2021	FTZ	Release of adiponectin↑	8	2.892 g/kg	Gavage	5 weeks	Daily intraperitoneal injection of letrozole, 50 μg/d, combined with HFD, for 35 days	Mouse	C57 BL/6J	②⑤
Yi, 2021	CFD	OATP2B1 and OATP3A1↑	9	CFD-L: 1.42 g/kg CFD-H: 5.68 g/kg	Gavage	2 weeks	Daily s.c injection of prasterone sodium sulfate, 90 mg/kg, combined with Kcal fat diet, for 40 days	Rat	SD	④⑤

Table 1: Continued.

Author, year	Intervention	Mechanism	Quantity of herb types	Dose(s)	Route	Treatment duration	PCOS induction methods	Species	Strain	Outcomes
Zhu, 2020	GFW	Reshape the intestinal flora	5	GFW-L: 0.31 g/kg GFW-M: 0.62 g/kg GFW-H: 1.24 g/kg	Gavage	35 days	Daily gavage of letrozole, 1 mg/kg, combined with intragastric high-fat emulsion, for 35 days	Rat	SD	②⑥
Lian, 2020	BGC	P450c17α↓, P450arom↑, GLUT4↑	12	BGC-L: 0.28 g/kg BGC-H: 0.57 g/kg	Gavage	3 weeks	Daily gavage of letrozole, 1 mg/kg, for 21 days	Rat	SD	②③
Qiu, 2020	LWDH	PI3K/AKT/FOXO1A↑	6	LWDH-L: 1.2 g/kg LWDH-H: 3.6 g/kg	NM	21 days	Daily gavage of letrozole, 1 mg/kg, combined with HFD, for 21 days	Rat	SD	①②④⑤⑥
Azeemuddin, 2019	DXB-2030	GLUT4↑	5	100 mg/kg	NM	60 days	S.c injection of TP, 1.25 mg/pup, for 70 days.	Rat	Wistar	①②
Shao, 2019	SGD	NF-κB↓	2	SGD-L: 12.5 g/kg SGD-M: 25 g/kg SGD-H: 50 g/kg	Oral	2 weeks	Daily oral administration of letrozole, 1 mg/kg	Rat	NM	①②③④⑤
Zhao, 2017	HEQI	PI3K/AKT↑	12	8.1 g/kg	Oral	30 days	Daily s.c injection of DHEA, 60 mg/kg, for 20 days	Rat	SD	②④⑤⑥
Wang, 2016	SJD	IRS-1↑, PI3K p85α↑	6	SJD-L: 9.2 g/kg SJD-M: 18.4 g/kg SJD-H: 36.8 g/kg	Gavage	18 days	Daily s.c injection of prasterone sodium sulfate, 90 mg/kg, combined with HFD, for 42 days	Rat	SD	②④⑤⑥

Interventions. BGC, bao gui capsule; BHG, bushen huatan granules; BJTF, bushen jieyu tiaochong formula; BSZY, bu-shen-zhu-yun decoction; CFD, cangfudaotan decoction; FTZ: fu fang zhenzhu tiaozi; GFW, guizhi fuling wan; HEQI, heqi san; KW, kunling wan; LWDH, liuwei dihuang pills; SGD, shaoyao-gancao decoction; SJD, shouwu jiangqi decoction. Mechanism, ATF4: activation transcription factor 4; AKT, protein kinase B; Bax, BCL2-associated X; Bcl-2, B-cell lymphoma-2; CHOP, C/EBP homologous protein; FOXO1A, forkhead box protein O1A; IGF-1, insulin-like growth factor-1; IRS-1, insulin receptor substrate-1; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-B; OATP2B1, organic anion transporting polypeptide 2b1; OATP3A1, organic anion transporting polypeptide 3a1; PERK, PKR-like reticulum kinase; PI3K: phosphatidylinositol-3-kinase; P450arom, P450 aromatase; P450c17α, cytochrome P450, family 17, subfamily A, polypeptide 1; TLR4: toll like receptor 4. Route: NM: not mentioned. PCOS induction methods: DHEA: dehydroepiandrosterone; HFD: high-fat diet; S.c, subcutaneous; TP, testosterone propionate. Strain: SD, Sprague–Dawley. Outcome measurements: ① ovarian mass; ② testosterone; ③ weight; ④ FSH; ⑤ LH; ⑥ HOMA-IR.

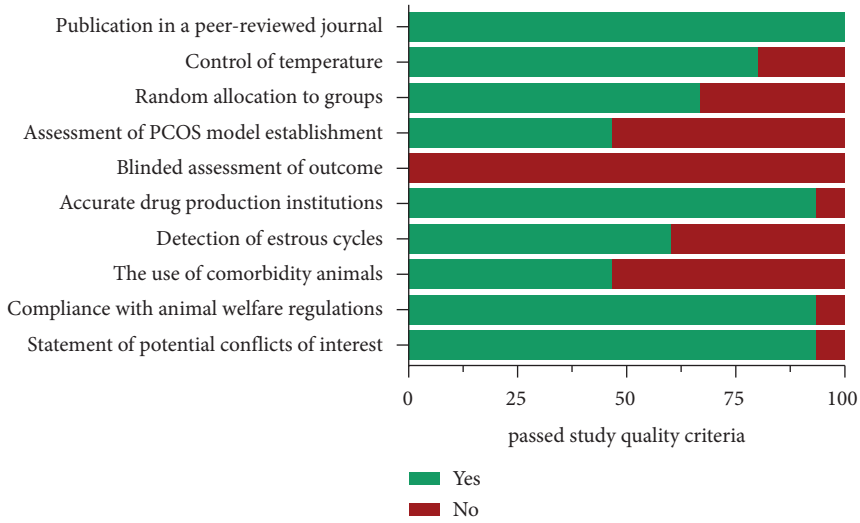


Figure 2: Study quality assessment of the included publications.

dehydroepiandrosterone (DHEA), prastenone sodium sulfate, or testosterone propionate (TP). Moreover, the characteristics of the administration route differed sketchily among the studies. Nine studies administered the CHM by gavage, three indicated the oral route without specifications, and three studies did not mention the administration route. With regard to the outcomes of interest to us, the 15 articles contained 13 comparisons for ovarian mass and 31 comparisons for testosterone as the primary outcomes. Weight (in 19 comparisons), FSH (in 20 comparisons), LH (in 21 comparisons), and HOMA-IR (in 18 comparisons) were assessed as the secondary outcomes.

3.3. Study Quality. The median study quality score was 7 of a possible 10 (interquartile range, 5 to 8). All studies included were published in peer-reviewed journals. Twelve articles (80%) reported control of temperature during study. Ten articles (67%) indicated random allocation. The criteria assessing PCOS model establishment were reported in seven articles (47%). No article reported a blinded assessment of the outcome, and only one publication did not report the accurate drug production institutions. Nine articles (60%) detected estrous cycles before the experiment. Relevant comorbidity was modeled in seven studies (47%). Besides, only one article did not report compliance with animal welfare and a conflicts of interest statement. Figure 2 and Supplementary Table 1 provide detailed information about each study quality.

3.4. Primary Outcomes

3.4.1. Ovarian Mass. Six studies, including 13 comparisons measured the efficacy of CHM in PCOS. Overall, the random-effect model showed that administration of CHM led to a significant decrease of ovarian mass in animal models of PCOS (SMD = −1.01, 95% CI: −1.58 to −0.45). Heterogeneity between studies was substantial ($I^2 = 52\%$) (Figure 3(a)).

3.4.2. Testosterone. Thirteen studies, including 31 comparisons reported testosterone. The random-effect model showed that CHM therapy was associated with a significant difference compared with the control group (SMD = −1.62, 95% CI: −2.07 to −1.16). Heterogeneity between studies was substantial ($I^2 = 59\%$) (Figure 3(b)).

3.5. Secondary Outcomes

3.5.1. Weight. Seven studies, including 19 comparisons reported weight. The random-effect model showed that CHM therapy was associated with a significant difference compared with the control group (SMD = −1.02, 95% CI: −1.39 to −0.65) (Figure 3(c)).

3.5.2. FSH. Nine studies, including 20 comparisons reported FSH. The random-effect model showed that CHM therapy was associated with a significant difference compared with the control group (SMD = 0.58, 95% CI: 0.19 to 0.97) (Figure 3(d)).

3.5.3. LH. Ten studies, including 21 comparisons reported LH. The random-effect model showed that CHM therapy was associated with a significant difference compared with the control group (SMD = −0.94, 95% CI: −1.25 to −0.64) (Figure 3(e)).

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
BJTF-H 2021	61.5	10.16	8	71.12	10.16	2	6.9%	−0.86 [−2.48, 0.77]
BJTF-L 2021	74.33	16.04	8	71.12	10.16	2	7.3%	0.19 [−1.37, 1.74]
BJTF-M 2021	57.75	10.16	8	71.12	10.16	2	6.6%	−1.19 [−2.88, 0.50]
BSZY-H 2021	77.91	20.15	10	110.15	30.9	5	9.4%	−1.27 [−2.46, −0.07]
BSZY-L 2021	107.46	28.21	10	110.15	30.9	5	10.2%	−0.09 [−1.16, 0.99]
CFD-H 2020	117.5	26.96	10	157.09	17.61	5	9.1%	−1.52 [−2.77, −0.28]
CFD-L 2020	139.55	11.99	10	157.09	17.61	5	9.5%	−1.18 [−2.36, −0.00]
DXB-2030 2019	57.95	13.41	10	67.49	19	10	11.5%	−0.56 [−1.45, 0.34]
LWDH-H 2020	133.68	29.02	10	145.08	21.76	5	10.1%	−0.40 [−1.48, 0.69]
LWDH-L 2020	139.9	21.76	10	145.08	21.76	5	10.2%	−0.22 [−1.30, 0.85]
SGD-H 2019	71.36	6.55	8	109.95	3.64	2	2.3%	−5.57 [−9.06, −2.07]
SGD-L 2019	91.75	5.83	8	109.95	3.64	2	4.5%	−2.93 [−5.20, −0.67]
SGD-M 2019	80.83	5.1	8	109.95	3.64	2	2.4%	−5.32 [−8.70, −1.95]
Total (95% CI)			*118*			*52*	*100.0%*	*−1.01 [−1.58, −0.45]*

Heterogeneity: $\tau^2 = 0.51$; $\chi^2 = 25.06$, df = 12 (P = 0.01); $I^2 = 52\%$
Test for overall effect: Z = 3.52 (P = 0.0004)

Std. Mean Difference IV, Random, 95% CI
−10 −5 0 5 10
Favours [experimental] Favours [control]

(a)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
BGC-H 2020	12.84	1.4	9	24.87	1.81	4	1.3%	−7.35 [−10.93, −3.77]
BGC-L 2020	14.04	1.2	9	24.87	1.81	4	1.3%	−7.23 [−10.76, −3.70]
BHG-H 2021	11.76	11.99	8	56.13	15.59	1	1.8%	−3.29 [−6.19, −0.39]
BHG-L 2021	9.86	10.79	8	56.13	15.59	1	1.6%	−3.81 [−6.95, −0.68]
BHG-M 2021	7.21	10.79	8	56.13	15.59	1	1.5%	−4.03 [−7.27, −0.79]
BJTF-H 2021	24.44	6.81	8	27.41	8.3	2	3.6%	−0.38 [−1.95, 1.18]
BJTF-L 2021	23.26	7.41	8	27.41	8.3	2	3.6%	−0.50 [−2.07, 1.08]
BJTF-M 2021	19.41	7.7	8	27.41	8.3	2	3.5%	−0.93 [−2.56, 0.71]
CFD-H 2020	60.73	6.31	10	68.61	11.04	5	4.6%	−0.92 [−2.06, 0.22]
CFD-L 2020	59.94	7.89	10	68.61	11.04	5	4.6%	−0.91 [−2.05, 0.23]
DXB-2030 2019	0.05	0.06	10	0.24	0.2	10	4.9%	−1.23 [−2.21, −0.26]
FTZ 2021	0.21	0.25	6	0.83	0.3	6	3.7%	−2.07 [−3.59, −0.55]
GFW-H 2020	0.76	0.31	12	1.17	0.28	4	4.3%	−1.28 [−2.52, −0.04]
GFW-H 2021	0.75	0.73	12	1.07	0.9	4	4.5%	−0.39 [−1.54, 0.75]
GFW-L 2020	0.79	0.28	12	1.17	0.28	4	4.3%	−1.28 [−2.53, −0.04]
GFW-L 2021	0.78	0.52	12	1.07	0.9	4	4.5%	−0.44 [−1.59, 0.70]
GFW-M 2020	0.76	0.31	12	1.17	0.28	4	4.3%	−1.28 [−2.52, −0.04]
GFW-M 2021	0.76	0.57	12	1.07	0.9	4	4.5%	−0.45 [−1.59, 0.70]
HEQI 2017	62.19	23.98	10	95.57	71.95	10	5.1%	−0.60 [−1.50, 0.30]
KW-H 2021	9.48	9.59	8	56.13	15.59	1	1.4%	−4.32 [−7.70, −0.94]
KW-L 2021	9.48	10.79	8	56.13	15.59	1	1.6%	−3.84 [−6.99, −0.69]
KW-M 2021	7.58	7.2	8	56.13	15.59	1	1.0%	−5.99 [−10.23, −1.76]
LWDH-H 2020	1.42	0.31	10	9.66	3.54	5	2.9%	−3.92 [−5.87, −1.96]
LWDH-L 2020	2.38	0.31	10	2.6	0.35	5	4.6%	−0.64 [−1.75, 0.46]
SGD 2021	26.95	8.04	10	34.24	8.84	10	5.1%	−0.83 [−1.75, 0.10]
SGD-H 2019	0.46	0.18	8	0.87	0.25	2	3.0%	−1.95 [−3.85, −0.05]
SGD-L 2019	0.48	0.22	8	0.87	0.25	2	3.2%	−1.57 [−3.36, 0.21]
SGD-M 2019	0.47	0.25	8	0.87	0.25	2	3.3%	−1.45 [−3.20, 0.30]
SJD-H 2016	1.85	0.23	8	4.98	0.46	2	0.5%	−10.48 [−16.58, −4.38]
SJD-L 2016	3.95	0.32	9	4.98	0.46	2	2.7%	−2.78 [−4.89, −0.67]
SJD-M 2016	4.11	0.42	9	4.98	0.46	2	3.2%	−1.87 [−3.69, −0.06]
Total (95% CI)			*288*			*112*	*100.0%*	*−1.62 [−2.07, −1.16]*

Heterogeneity: $\tau^2 = 0.84$; $\chi^2 = 73.11$, df = 30 (P < 0.0001); $I^2 = 59\%$
Test for overall effect: Z = 6.99 (P < 0.00001)

Std. Mean Difference IV, Random, 95% CI
−10 −5 0 5 10
Favours [experimental] Favours [control]

(b)

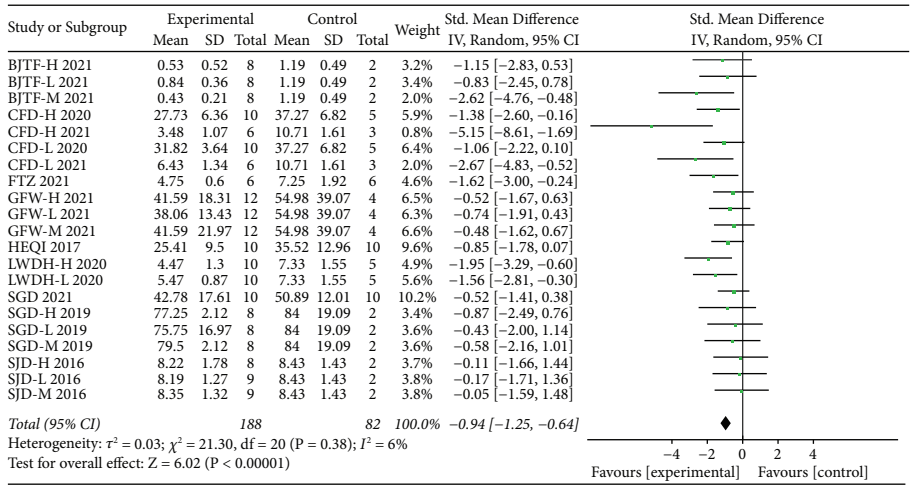
Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
BGC-H 2020	307.78	22.22	9	342.22	16.67	4	7.3%	−1.54 [−2.91, −0.16]
BGC-L 2020	314.44	7.78	9	342.22	16.67	4	5.4%	−2.36 [−3.96, −0.76]
BHG-H 2021	241.62	41.28	8	251.35	36.69	1	3.2%	−0.21 [−2.29, 1.87]
BHG-L 2021	238.38	32.11	8	251.35	36.69	1	3.2%	−0.36 [−2.45, 1.73]
BHG-M 2021	244.86	32.11	8	251.35	36.69	1	3.2%	−0.18 [−2.26, 1.90]
BJTF-H 2021	318.3	25.53	8	335.32	17.02	2	5.5%	−0.62 [−2.21, 0.96]
BJTF-L 2021	320	30.64	8	335.32	17.02	2	5.6%	−0.47 [−2.04, 1.10]
BJTF-M 2021	311.49	22.13	8	335.32	17.02	2	5.1%	−1.00 [−2.65, 0.65]
BSZY-H 2021	156.11	2.13	5	168.06	1.71	2	0.8%	−4.90 [−9.12, −0.69]
BSZY-L 2021	160.38	2.56	5	168.06	1.71	2	1.9%	−2.68 [−5.36, 0.00]
CFD-H 2020	366.73	12.06	10	393.74	18.1	5	8.1%	−1.79 [−3.10, −0.48]
CFD-L 2020	383.25	18.36	10	393.74	18.1	5	11.5%	−0.54 [−1.64, 0.56]
KW-H 2021	244.86	32.11	8	251.35	36.69	1	3.2%	−0.18 [−2.26, 1.90]
KW-L 2021	248.11	36.69	8	251.35	36.69	1	3.2%	−0.08 [−2.16, 2.00]
KW-M 2021	251.35	36.69	8	251.35	36.69	1	3.2%	0.00 [−2.08, 2.08]
SGD 2021	308.98	17.29	10	328.13	23.06	10	16.0%	−0.90 [−1.83, 0.03]
SGD-H 2019	295.56	18	8	329.49	22	2	4.2%	−1.65 [−3.46, 0.15]
SGD-L 2019	306.87	16	8	329.49	22	2	4.8%	−1.21 [−2.90, 0.48]
SGD-M 2019	305.45	14	8	329.49	22	2	4.5%	−1.43 [−3.17, 0.32]
Total (95% CI)			*154*			*50*	*100.0%*	*−1.02 [−1.39, −0.65]*

Heterogeneity: $\chi^2 = 15.45$, df = 18 (P = 0.63); $I^2 = 0\%$
Test for overall effect: Z = 5.36 (P < 0.00001)

Std. Mean Difference IV, Fixed, 95% CI
−10 −5 0 5 10
Favours [experimental] Favours [control]

(c)

Figure 3: Continued.



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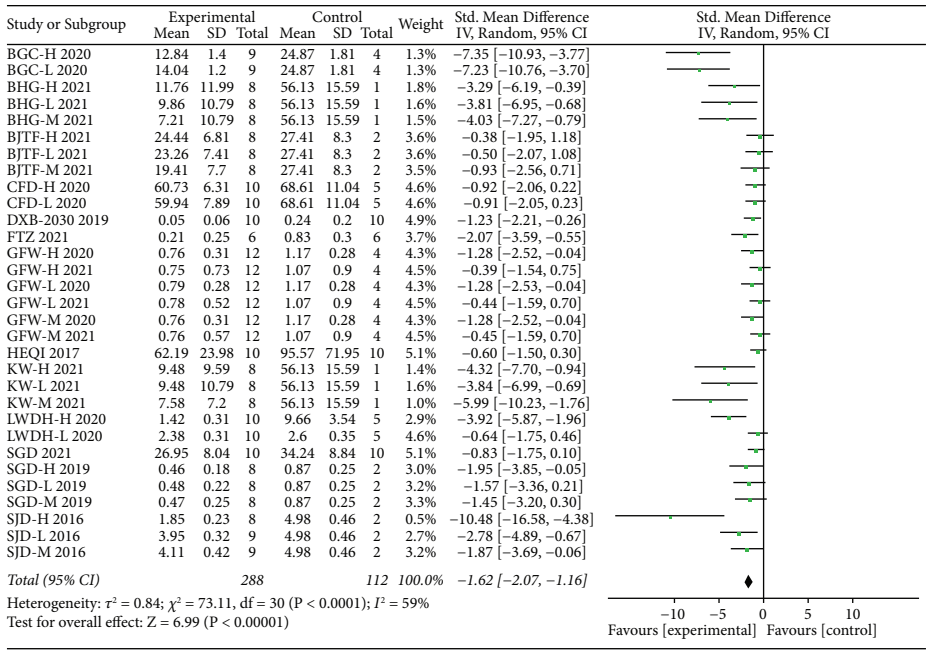

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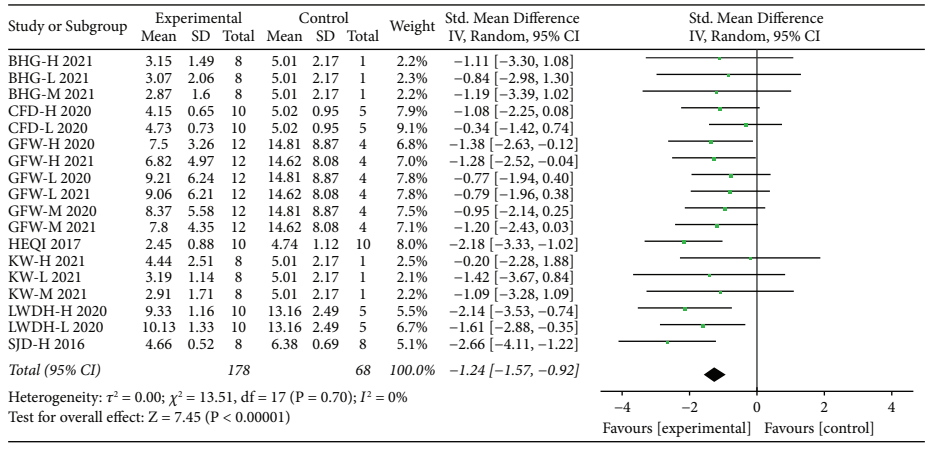

(f)

Figure 3: Forest plots of outcomes. (a) Ovarian mass; (b) testosterone; (c) weight; (d) FSH; (e) LH; (f) HOMA-IR.

3.5.4. HOMA-IR. Seven studies, including 18 comparisons reported HOMA-IR. The random-effect model showed that CHM therapy was associated with a significant difference compared with the control group (SMD = −1.24, 95% CI: −1.57 to −0.92) (Figure 3(f)).

3.6. Subgroup Analyses. The heterogeneity of the secondary outcomes which included weight, FSH, LH, and HOMA-IR is little ($I^2 = 0\%$; $I^2 = 40\%$; $I^2 = 6\%$; $I^2 = 0\%$, respectively). The pooled estimates for studies in meta-analyses of ovarian mass and testosterone exhibited substantial heterogeneity ($I^2 = 52\%$; $I^2 = 59\%$). Subgroup analyses were conducted to identify the source of heterogeneity with five covariates (PCOS induction drug, formula composition, control of temperature, random allocation, and assessment of model establishment).

For ovarian mass, administration of formulas with different compositions may be a possible source of heterogeneity ($P = 0.003$) (Supplementary Figure 1B). Besides, treatment effects were higher in studies that reported random allocation ($P = 0.02$) (Supplementary Figure 1D) and in studies that reported assessment of model establishment ($P = 0.05$) (Supplementary Figure 1E). Overall, analyses showed that PCOS induction drugs and control of temperature did not account for the heterogeneity and different estimates of efficacy for ovarian mass ($P = 0.44$; $P = 0.47$) (Supplementary Figures 1A/1C).

For testosterone, we found significant impact of different formulas used in the PCOS model ($P < 0.01$) (Supplementary Figure 2B). Analysis showed that PCOS induction drug may account for a proportion of the heterogeneity of the efficacy for testosterone ($P = 0.07$) (Supplementary Figure 2A). The other covariates including control of temperature, random allocation, and assessment of model establishment were shown to be irrelevant to the heterogeneity ($P = 0.7$; $P = 0.2$; $P = 0.28$, respectively) (Supplementary Figures 2C/2D/2E).

Furthermore, considering that various formulas have different emphasis on the improvement effects, subgroup analyses of formula composition for the other outcomes were conducted. Analyses showed that in concluded studies the effect of Shaoyao-Gancao Decoction (SGD) on reducing ovarian mass was the best (SMD = −4.16, 95% CI: −5.91 to −2.4), the effect of Bao Gui capsule (BGC) on reducing testosterone was the best (SMD = −7.29, 95% CI: −9.8 to −4.77), the effect of Bu-Shen-Zhu-Yun Decoction (BSZY) on reducing weight was the best (SMD = −3.32, 95% CI: −5.58 to −1.06), the effect of Liuwei Dihuang Pills (LWDH) on improving FSH was the best (SMD = 1.98, 95% CI: 0.18 to 3.79), the effect of Cangfudaotan Decoction (CFD) on reducing LH was the best (SMD = −1.89, 95% CI: −3.09 to −0.69), and the effect of Shouwu Jiangqi Decoction (SJD) on reducing HOMA-IR was the best (SMD = −2.66, 95% CI: −4.11 to −1.22) (Figure 4 and Supplementary Figure 3).

3.7. Sensitivity Analyses. The sensitivity analysis was performed by removing one study at a time to confirm and account for the stability of the results. The pooled sensitivity analyses showed that the results would not be affected by excluding any study, which suggested that the stability of the results remained robust (Supplementary Figure 4).

3.8. Publication Bias. The Egger's bias test was performed to identify the potential publication bias. For ovarian mass, FSH and testosterone, significant publication bias was detected ($P < 0.01$) (Figures 5(b), 5(c) and 5(e)).

4. Discussion

In this study, we performed a meta-analysis aimed at assessing the efficacy of CHM therapy in animal models of PCOS. Compared with other publications, the strengths of this study consisted of only animal experiments being included for the meta-analysis which differed from the analyses for comparing randomized controlled trials (RCTs) [10, 36], different formulas and doses being compared to verify the most appropriate treatment, and multivariate subgroup analyses of square law and experimental design. Overall, results of the study indicated that some specific features of PCOS (ovarian mass, weight, sex hormone disorders, and insulin resistance) were ameliorated by CHM. Specifically, the ovarian mass, testosterone, weight, LH, and HOMA-IR were decreased, and the FSH was increased. The outcomes of ovarian mass and testosterone represented substantial heterogeneity, and their sources mainly were formula composition, random allocation, assessment of model establishment, and PCOS induction drug. According to the subgroup analyses, different formulas showed different emphasis on the improvement effects on various symptoms of PCOS. Besides, the Egger's bias test showed substantial publication bias in the outcomes of ovarian mass, FSH, and testosterone.

Overall, the study quality of the studies included was moderate, and there was little difference in the quality of articles. We tried to perform a correlation analysis between the quality and the year of the study, but no statistical correlation was found (data not shown). Among the 15 studies, 10 publications reported the random allocation, and no publication mentioned the blinded assessment of outcomes, which implied that in the animal models blinding methods are usually seen as technical difficulties. Given that failure to perform blind assessment might lead to overestimation of effect sizes, we recommend following more standardized experimental standards in preclinical studies. Besides, contrary to our expectations, randomization to group was associated with a higher improvement in outcomes of testosterone. We speculate that expected results may be anticipated if they follow strict experimental criteria.

Seven publications reported the assessment of the PCOS model establishment. In our study, the best effect was seen in the publication that evaluated the PCOS model establishment before intervention. This was probably because the improvement of phenotype by CHM was more remarkable in the exact model. 7 publications employed comorbidity animals with obesity, hyperglycemia, or chronic stress states

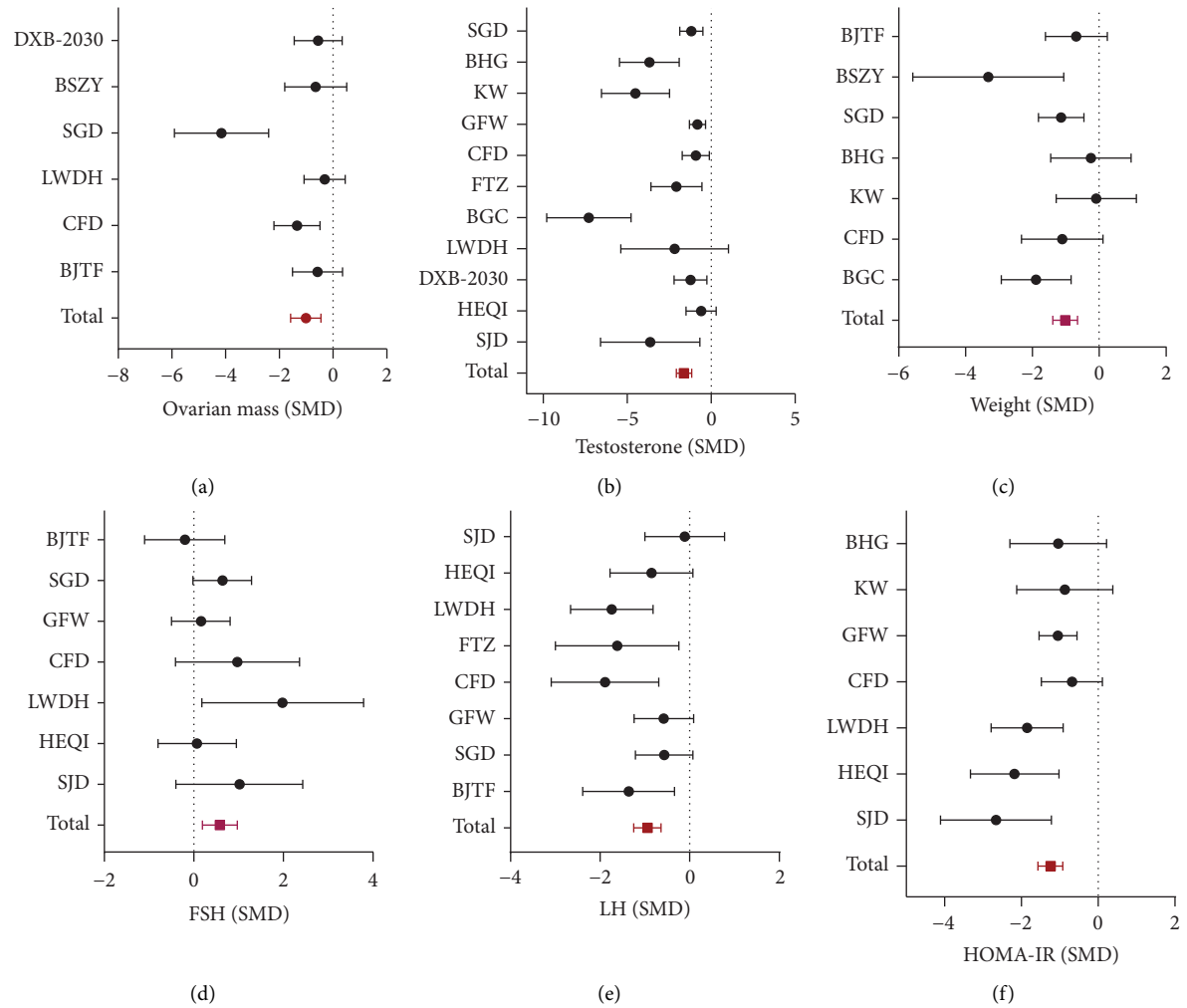

Figure 4: Forest plots of efficacy of different formulas on PCOS in animal models. (a) Ovarian mass; (b) testosterone; (c) weight; (d) FSH; (e) LH; (f) HOMA-IR.

which may be more in line with the pathophysiology of PCOS patients [37, 38].

It has been estimated that as many as 38% to 88% of women with PCOS are overweight or obese, but that does not mean obesity is a defining feature of PCOS as the syndrome is also seen in women of normal weight [39]. Hyperandrogen, metabolic dysfunction, and insulin resistance caused by PCOS are the significant predisposition of weight gain [40]. The subgroup analyses exploring the source of heterogeneity of ovarian mass and weight revealed that better effectiveness was shown in letrozole-induced models (Supplementary Figure 1A/Figure 5). Among the studies included, 9 publications used letrozole, 3 publications used prasterone sulfate sodium, and only one publication used testosterone propionate. As a nonsteroidal aromatase inhibitor, letrozole restrains the conversion of androgen to estrogen, leading to androgen accumulation and ultimately recapitulating both reproductive and metabolic PCOS phenotypes in rats and mice [41, 42]. The PCOS models induced by testosterone propionate were characterized by high blood free testosterone level, low LH and FSH values, which can last for a long time [43, 44]. As another androgen induction method, DHEA and prasterone sulfate sodium require shorter modeling time than testosterone propionate and are closer to the pathogenesis of PCOS [45, 46]. In addition to the modeling methods mentioned in this study, estradiol valerate, insulin combined with human chorionic gonadotrophin (HCG), and progesterone combined with HCG have also been used in other studies to induce PCOS models [47–49]. There were also studies modeling PCOS through torsion-detorsion and found that apoptosis caused by oxidative stress is an important factor in ovarian tissue damage [50]. Afterwards, they found that *Galega officinalis* extract and coenzyme Q10 could reverse this pathological change [51]. As PCOS is a highly heterogeneous disease, an animal model that can fully simulate the clinical characteristics of PCOS is not realistic. We suggest that appropriate modeling methods should be selected according to the purpose of the study.

Systematic review of preclinical studies is conducive to exploring the potential mechanism of CHM in ameliorating symptoms of PCOS. Our study included 2 publications with

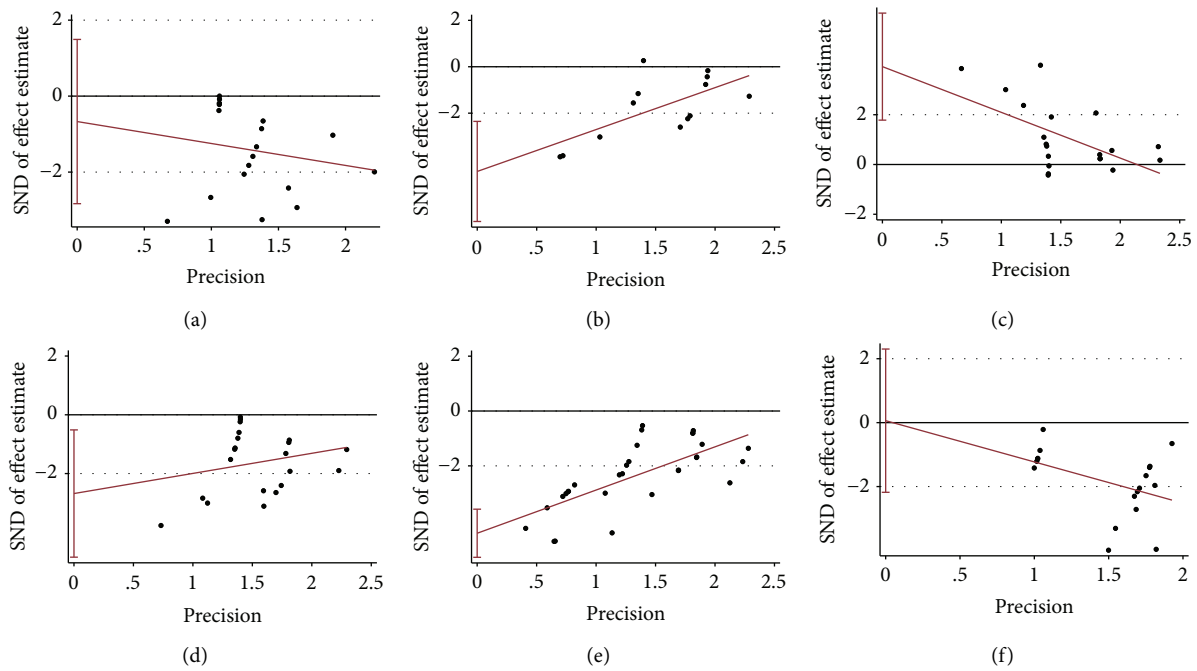

Figure 5: Publication bias assessment. Egger's test for (a) weight; (b) ovarian mass; (c) FSH; (d) LH; (e) testosterone; (f) HOMA-IR.

SGD as the intervention. The efficacy of SGD on decreasing ovarian mass was the best. SGD might reduce the phosphorylation of nuclear factor kappa-B (NF-kB) p65, suppress toll like receptor 4 (TLR4)/NF-kB signaling pathway, remodel gut microbiome structure, and protect gut barrier, which leads to ameliorate the inflammatory response in the ovary of PCOS rats [25, 33]. As the most effective formula to reduce high testosterone, BGC decreased the expression of P450c17α and increased the expression of P450arom to improve hyperandrogenism [30]. There were also studies on the effect of CHM on ovarian granule cell apoptosis through the PKR-like Reticulum Kinase (PERK)-ATK4- C/EBP homologous protein (CHOP) signaling pathway, PI3K/AKT/mTOR pathway, and P13K/AKT/Forkhead Box Protein O1A (FOXO1A) pathway [15, 23, 24, 31]. Furthermore, studies on the mechanism of CHM improving PCOS insulin resistance generally explored the glucose transporter 4 (GLU4) related pathways [27, 32].

PCOS is a very complex disease in the human body. Animal experiments can simplify the complex pathological process, and subtle research on various inducing factors can be carried out to recommend the best effect of the CHM formula on PCOS patients with different pathogeny characteristics in clinic. Compared with clinical trials, researchers can more accurately control the dose and method of administration in animal research, without considering the patient's compliance and other difficult problems in clinic. In the meanwhile, animal research is convenient to obtain materials, and the expression of molecular markers can be easily evaluated. This enabled our systematic review to summarize the mechanism of CHM in ameliorating symptoms of PCOS. Besides, our results pointed out that the efficacy of CHM prescriptions on various models was different, which indicated that the clinical application should also give attention to the advantages of individualized treatment of CHM.

The pooled sensitivity analyses showed the stability of the results. The Egger's bias test suggested the substantial presence of publication bias in ovarian mass, FSH, and testosterone, which indicated that the efficacy might be overestimated. Only English-language studies being included may account for the publication bias. Our initial plan was to include at least 10 articles in each result, but the reality is that some important outcomes were not assessed in the included articles. Even though the number of articles in some results did not meet the protocol, we still conducted the data integration. Our study also has the following limitations: First, the publications included were searched in English-language databases, so the databases in other languages were excluded. Moreover, gray literature and negative results were also lacking. Second, the number of studies assessing the pregnancy rate and litter size is too limited to conduct the meta-analysis, which means the effect of CHM on infertility in animal models could not be evaluated. Third, only one study used the mice model, so we could not conduct the subgroup analysis by species. Finally, although we tried to analyze more outcomes, the limited quantity of included studies made it unpractical.

5. Conclusion

To the best of our knowledge, this is the first systematic review and meta-analysis that evaluated the efficacy of CHM on PCOS in animal models. We concluded that CHM resulted in improvements in ovarian mass, weight, FSH, LH, testosterone, and HOMA-IR in animal models of PCOS. The improvement effects of different formulas are targeted. For instance, SGD reduced ovarian mass the most, and BGC

decreased testosterone levels the most. Furthermore, we suggest using PCOS modeling drugs that meet the research purpose when studying different mechanisms. Overall, the results should be interpreted with caution because of substantial heterogeneity and publication bias.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Jiacheng Zhang developed the search strategy, developed the manuscript, and drafted and revised the manuscript. Xiyan Xin and Haolin Zhang screened the articles, collected the data, and assessed the risk of bias. Yutian Zhu revised the manuscript and provided critical advice. Yang Ye and Dong Li conceived and designed the study, revised the manuscript, and supervised the whole study. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant no. 82074193).

Supplementary Materials

Supplementary File 1: Search strategy. Table S1: Study quality score report. Figure S1: Forest plot of the efficacy of CHM on ovarian mass. Figure S2: Forest plot of the efficacy of CHM on testosterone. Figure S3: Forest plots of the efficacy of different formulas. Figure S4: Sensitivity analysis. Figure S5: Forest plots of weight improvement by different PCOS induction drugs. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 6528264, 22 pages
https://doi.org/10.1155/2022/6528264

Research Article

Ethnogynaecological Knowledge of Traditional Medicinal Plants Used by the Indigenous Communities of North Waziristan, Pakistan

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Received 31 January 2022; Revised 2 June 2022; Accepted 8 July 2022; Published 4 August 2022

Academic Editor: Min Hu

Background. Since the beginning of civilization, medicinal plants have been used in human healthcare systems. Studies have been conducted worldwide to evaluate their efficacy, and some of the results have triggered the development of plant-based medications. Rural women in Pakistan frequently experience gynaecological disorders due to malnutrition and heavy physical work during pregnancy. Due to the low economic status, the remoteness of the area, and the lack of modern health services, herbal therapy for gynaecological disorders is common among the indigenous tribes of the study area. *Methods*. Field surveys were carried out from April 2018 to October 2020 to collect data regarding medicinal plants used for different gynaecological disorders. A semistructured questionnaire was used to collect ethnogynaecological data. *Results*. In total, 67 medicinal plant species belonging to 38 families are being used to treat 26 different gynaecological problems. The herbaceous growth form and the Lamiaceae family were recorded with the maximum number of plant species (42 species and 7 species, respectively). Leaves are the most highly utilized plant part, with 16 species. In the case preparation method, decoction was the dominant method (25 species, 36.76%). The informants reported the maximum number of species for the treatment of irregular menstrual flow as 11 species (15.28%). The highest relative frequency of citation (RFC) value was obtained for *Acacia modesta* (0.37), and the use value (UV) for *Tecomella undulata* (0.85). The highest informants' consensus factor (ICF) value (1.0) was obtained for emmenagogue and tonic

each after delivery. The highest consensus index (CI%) value was calculated for *Acacia modesta* (36.92%). The Lamiaceae had the highest family importance value (FIV) (98.46%). *Conclusion*. This is the first ever quantitative study focusing mainly on ethnogynaecological study conducted in the tribal areas of North Waziristan which highlights the importance of traditional herbal remedies for their basic medical requirements. The results of this study would serve as a baseline for advanced phytochemical and pharmacological screening, as well as conservationists for further studies.

1. Introduction

Ethnogynaecology is a new branch of ethnobotany, which mainly deals with the use of therapeutic plants for curing gynaecological disorders such as menses problems, abortion, lactation, infertility, gonorrhea, leucorrhoea, and delivery disorders [1, 2]. It has been documented that sexual and other women's basic healthcare problems are reported to account for 18% of the total worldwide diseases [3]. Medicinal plants used to treat gynaecological disorders such as menstrual pain, abortion, leucorrhoea, pregnancy, infertility, lactation, and delivery problems have been documented in some areas of this region's ethnic groups [4]. The tribal communities have been preparing medication from the available medicinal plant species, which are widely used to cure common women's ailments. The tribal communities depend on therapeutic plants because of their efficacy, lack of basic medical care facilities, and ethnic preferences [5]. The medicinal plants used in traditional remedies are mostly collected from the wild. Tribal people have diverse knowledge of traditional medicine based on local plants for basic medical care [6]. In tribal communities, traditional healers possess a lot of information about medicinal plants. In these regions, medicinal plants are important for the indigenous people, providing access to basic healthcare [7]. The traditional medicinal system acts as the principal supplier of primary healthcare services in the tribal areas because of the lack of modern healthcare facilities, the remoteness of the region, and a strong cultural belief in the efficacy of folk medicines [8].

The use of medicinal plants in everyday life has a long history and still has immense importance in aboriginal civilization [9]. In remote areas, therapeutic plants still play an important role [10] and are still used as the basic healthcare system. According to the literature, more than 50,000 flowering taxa have been used for medical purposes all over the globe [11]. Pakistan has diverse flora comprised of about 6000 flowering plant species [12, 13] and about 600 plant species have been identified with medicinal values [14]. About 80% of the inhabitants of remote areas of Pakistan are still dependent on medicinal plants [15]. Plant-derived medicines account for about 25% of all medicines available in the modern pharmacies, with many more artificial compounds isolated from plants.

In Pakistan, rural women frequently experience gynaecological disorders due to malnutrition, poor living standards, and hard physical work during pregnancy. A local woman, who is locally called "Dayiah," is found in each village and specializes in herbal therapy to relieve gynaecological disorders with local medicinal plants [16]. The highest use of the therapeutic plant in rural communities is due to the high price of allopathic medicine and its side effects [17]. A traditional way of life, as well as a lack of a suitable approach to modern health facilities, motivates rural women to consult with nearby midwives and indigenous healers [18].

There is very limited literature on ethnogynaecology [19], whereas many reports on ethnobotanical and ethnomedicinal knowledge are available across the globe [18, 20]. Some ethnomedicinal surveys have been conducted to study the role of herbal therapy in women's medical and reproductive health disorders [20, 21]. Similarly, little literature is available about medicinal plants used by pastoral women for the healing of gynaecological problems. There is very little work carried out in Pakistan and in the whole world [22, 23]. Moreover, due to modernization and the lack of interest of younger generations in indigenous knowledge, which is declining speedily, ethnoecological information may vanish if not properly recorded [24]. In today's society, allopathic medicines, anti-inflammatory medicines, surgery, and nonsteroidal analgesics are commonly used to treat gynaecological disorders. These remedies are effective but usually have side effects, particularly when medicines are used for a long time. Moreover, some medicines used during the entire pregnancy period can harm the embryo [20].

This study aimed to record different types of plant species used against various gynaecological problems encountered by the female inhabitants of the tribal district of North Waziristan, Pakistan. The area is dominated by the Wazir and Dawar tribes, with low financial status, poor infrastructure, no modern medical facilities, and a lack of modern resources [14]. Many women and men in the region seek healing from a traditional therapist for a variety of problems related to the female reproductive organs. Such traditional knowledge has not been reported before from the study area as no ethnoecological documentation has been done earlier. Hence, this survey aims to report the ethnomedicinal knowledge of indigenous herbal remedies for the cure of gynaecological disorders and to preserve this precious but fast-vanishing indigenous knowledge of the tribal communities of the study area.

2. Methods

2.1. Study Area. Tribal district North Waziristan, Khyber Pakhtunkhwa, Pakistan, is the hilly region that lies between 32°35′ and 33°20′ north latitudes and 69°25′ and 70°40′ east longitudes, with an altitude of 2143–7717 feet. North Waziristan falls under the Irano-Turanian Region [25]. The area is bounded by mountains that are connected with Koh-e-Sulaiman in the south and Koh-e-Sufaid in the north. North Waziristan is bounded on the south by the district of

South Waziristan; on the north by Kurram Agency, Hangu district, and Afghanistan; on the east by the district of Bannu; and on the west also by Afghanistan (Figure 1). The area is fertile and is irrigated by 3 rivers, namely, the Tochi, Kurram, and Katu rivers. The annual rainfall is 45 cm. The North Waziristan area contains 4,707 square kilometers (1,817 sq mi). There are two major tribes in the study area, that is, Wazir and Dawar. Pushto is the major language. The study area is one of the major war-affected areas of Pakistan. The total population in the conflict-affected area of North Waziristan is approximately 840,000. The region has been targeted with shelling and air raids, and at least 456,000 people, including nearly 200,000 children (42%), fled ahead of or during the ground assaults to safer parts of Pakistan and neighbouring Afghanistan.

2.2. Field Surveys and Medicinal Plants' Collection. The ethnogynaecological surveys were carried out in the tribal district (North Waziristan) from April 2018 to October 2020. Medicinal plants were collected during field visits [26, 27]. A collection number was given to each plant specimen with the help of tags. Plants were serially tagged and appropriately placed in the field presser. Snapshots of the collected plants were also captured [28, 29].

2.3. Questionnaires and Interviews. A semistructured questionnaire was used to collect the information regarding indigenous knowledge from the local informants and Hakeems of the study area [30–32]. Preference was given to elderly people and Hakeems. The collected specimens and photographs were further used in the interviews to recheck the information with other informants as well [28, 29]. A total of 130 local informants were interviewed, belonging to different age groups (35 years to 65 years), of which 105 were male and 25 were female, including housewives (daei/midwives and traditional healers) (Table 1) [33]. During the survey, local names, botanical names, folk uses, used parts, mode of preparation, mode of application (e.g., juice, paste, decoction, infusion, and powder), and growth/life form were documented by the local people of the study area. Through semistructured interviews [34, 35], knowledge about gender and age differences and occupation background and information about the herbal recipes for gynaecological disorders were documented [36].

2.4. Plant Identification and Preservation. The plant taxonomist Dr. Rahmatullah Qureshi identified the herbarium specimens and confirmed them with the help of available published literature [37]. These will be compared with identified specimens in the Herbarium of Pakistan Islamabad (ISL), Quaid-e-Azam University Islamabad. Medicinal plant species were also photographed at the time of collection [38, 39]. The collected plants' specimens were dried, pressed, poisoned with 1% $HgCl_2$ solution, and mounted on standard-sized herbarium sheets (11.5×17.5 inch). A voucher number was assigned and the voucher specimens were submitted to the herbarium of the Department of Botany, Hazara University, Mansehra, Pakistan, for future references.

2.5. Quantitative Data Analysis. Indigenous knowledge is quantitatively analyzed using different quantitative indices [40–42] such as relative frequency of citation (RFC), used reports (UR), use value (UV), informant consensus factor (ICF), consensus index (CI%), fidelity level (FL%), and family importance value (FIV).

2.5.1. Relative Frequency of Citation (RFC). The RFC value for indigenous therapeutic plants is based on the number of informants for each plant species. A relative frequency of citation (RFC) is obtained by dividing the frequency of citation (FC) by the total number of informants in the survey (N). RFC was calculated by using the following formula [43, 44]:

$$\mathrm{RFC} = \frac{FC}{N} \ (0 < \mathrm{RFC} < 1), \tag{1}$$

where FC is frequency of citation and N is total number of informants taking part in the survey ($N = 130$).

2.5.2. Use Value (UV). Use value (UV) of a species was determined by the following formula [45]:

$$\mathrm{UV} = \frac{U}{n}, \tag{2}$$

where U is number of use reports documented by the informants for a given medicinal plant and n is total number of informants interviewed for a specific medicinal plant.

2.5.3. Consensus Index (CI%). The percentage of local informants regarding their indigenous knowledge of therapeutic plants used to treat gynaecological problems was calculated by consensus index (CI%) [31]. The following formula was used:

$$\mathrm{CI} = \frac{n}{N} \times 100, \tag{3}$$

where "n" is the number of informants citing the medicinal plant species and "N" is the total number of respondents for the species during the survey.

2.5.4. Fidelity Level (FL%). The fidelity level (FL) is the percentage of informants who mention the utilization of particular medicinal plant species to cure specific ailments in the study area. The fidelity level (FL) is calculated by the following formula [46]:

$$\mathrm{FL}(\%) = \frac{Np}{N} \times 100, \tag{4}$$

where "Np" is the particular number of citations for a specific disease and "N" is the total number of respondents citing the plant species for any ailments.

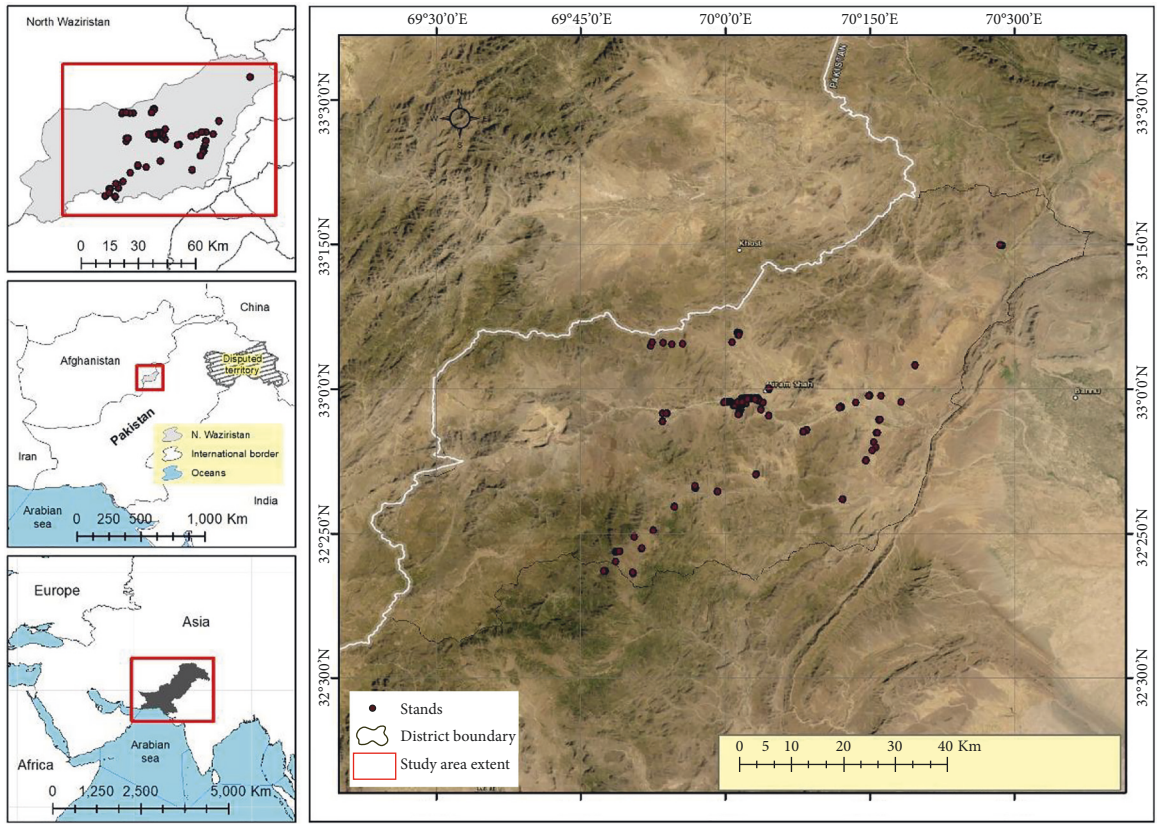

Figure 1: Map of the study area (North Waziristan, KP, Pakistan). The black dots indicate the visited sites for the study.

Table 1: Demographic information of the Informants.

Variable	Categories	No. of informants $N = 130$	Percentage (%)
Gender	Male	105	80.77
	Female (Dayiahs/midwives)	25	19.23
Informant category	Traditional healers	90	69.23
	Indigenous people	40	30.77
Occupation	Herbalists	76	58.46
	Housewives	25	19.23
	Professional	29	22.31
Age	35–50	15	11.54
	50–65	53	40.77
	Above 65	62	47.69
Education level	Illiterate	53	40.77
	Primary level	34	26.15
	Middle level	15	11.54
	Secondary level	12	09.23
	Undergraduate (Hakims)	9	06.92
	Graduate (Hakims)	7	05.38

2.5.5. Informant Consensus Factor (ICF). Informant consensus factor (ICF) was used to determine the informants' agreement on the reported treatment for any diseases group or ailment category [47]. The ICF value ranges from 0 to 1. Thus, the following formula was used:

$$\text{ICF} = \frac{\text{Nur} - Nt}{\text{Nur}} - 1, \quad (5)$$

where Nur is the number of useful reports in any disease category and Nt is the number of plant species used.

2.5.6. Family Importance Value (FIV). To determine the importance of a family, the family importance value (FIV) was applied [44] using the following formula:

$$\text{FIV} = \frac{\text{FC(family)}}{N} \times 100, \quad (6)$$

where FC is number of informants mentioning the family and N is total number of informants taking part in the survey ($N = 130$).

3. Results and Discussion

3.1. Informants' Demography. This study was conducted in the tribal district of North Waziristan, Pakistan. Inhabitants use medicinal plants for the cure of different gynaecological disorders. Demographic knowledge was acquired from the gender, age, education level, and practice of the informants. A total of 130 informants were interviewed, including 80.77% male and 19.23% female (Dayiahs). All the informants spoke Pushto. The dominance of male informants in the study area was greater as compared to females. There were certain cultural barriers due to which female informants could not talk with male interviewers outside of their families, but the investigated female informants gave their assent. Many of them were over 65 years old (47.69%), followed by 50–65 years old (40.77%) and 35–50 years old (11.54%). Participants were 76 herbalists, 29 professionals, and 25 housewives (Table 1). The majority of herbal healers in this study were males. These results are similar to the previous literature [48]. Based on educational facilities, the indigenous knowledge and use of therapeutic plants for the treatment of gynaecological disorders were more prevalent in the illiterate people, that is, 40.77%, and the same traditional knowledge was declining in the graduate level (5.38%) of the study area. Based on age, it was observed that the indigenous knowledge and use of medicinal plants remedies for gynaecological disorders were more prevalent in the elders. The same results were documented by other authors from nearby areas and other countries [49, 50]. The inherited traditional knowledge of therapeutic plants is transferred orally and verbally from their ancestors and passed from generation to another [24]. Noticeably, information and knowledge related to the traditional medication of gynaecological disorders are vanishing due to the death of older females (Dayiahs) in the community. Hence, there is a dire need to conserve this indigenous knowledge from extinction [5].

3.2. Indigenous Medicinal Plants' Diversity. The study area has a wealthy floral diversity. For their basic medical care needs, tribal people have varying knowledge of traditional medicine associated with medicinal plants. All medicinal plant species along with their qualitative analysis (botanical names, family name, parts used, mode of preparation, mode of application, and disease treated) and quantitative analysis (RFC, FL, ICF, UV, and UR) of each medicinal plant species were calculated and are presented in Table 2. In this study, 67 medicinal plants belonging to 38 families were recorded as being used to treat gynaecological disorders by the indigenous people of tribal district of North Waziristan, Pakistan. Approximately, 84% of the rural population depends on herbal therapeutic plants [51]. In rural areas of Pakistan, approximately 75% of the inhabitants are still reliant on traditional knowledge for their basic healthcare system [52], because there is no modern healthcare facility provided to them. Thus, most of the inhabitants are dependent on herbal remedies in the study area. The most dominant family was the Lamiaceae with 7 species, followed by Asteraceae and Rosaceae with 4 species each (Table 2). The family Lamiaceae is predominant in the study area similar to the results reported in the previous work [53].

3.3. Life Form of the Ethnomedicinal Flora. In terms of life forms, the most dominant life form used in gynaecological remedies was herbs (42 species, 62.69%), followed by shrubs (15 species, 22.39%), trees (7 species, 10.45%), ferns (2 species, 2.99%), and sedge (1 species, 1.49%) (Figure 2). The frequent use of herbs in herbal remedies has also been documented in other areas of the globe [54, 55]. Herbs often have a high content of bioactive compounds [56], are easily accessible, and have profuse growth in wild varieties. Similar to other studies carried out by [57], easy accessibility of herbaceous plants or therapeutic plants, valuable healing action, and reasonable cost of the healthcare system are the major factors for the preference and advancement of herbal medication in the economically backward rural communities [58].

3.4. Plant Parts Used in Herbal Remedies. Various plant parts are regarded as useful in various ailments. The indigenous communities of North Waziristan, Pakistan, use approximately all parts of the medicinal plants as remedies for gynaecological problems. The most highly utilized parts for herbal remedies observed were leaves (16 species, 22.54%), followed by the whole plant (15 species, 21.13%), roots (12 species, 16.90%), seed (7 species, 9.86%), fruits (6 species, 8.45%), aerial parts, gum and shoots (3 species, 4.23% each), bark and bulb (2 species, 2.82% each), and flower and rhizome (1 species, 1.41% each) (Figure 3). The collection of leaves and medication preparation from leaves are so easy as compared to the other plant parts. For these purposes, leaves are commonly used in folk remedies [59]. The removal of leaves from the medicinal plants can cause less harm as compared to the removal of other parts of the plant [60]. The high use of leaves in herbal remedies preparation is also reported in other study areas [61–63].

3.5. Preparation of Remedies. Medicinal plants were used by the indigenous people in diverse ways and in various recipes. A total of 14 modes of preparation were used in the indigenous communities. In the current study, decoction (25 species, 36.76%) is the dominant methodology used for the preparation of herbal remedies, followed by powder (22 species, 32.35%), juice (4 species, 5.88%), paste (3 species, 4.42%), herbal tea, poultice, raw, and smoke (2 species, 2.94% each) (Figure 4). Similarly, decoction and powder were reported as the most commonly used methods for preparing herbal remedies in other studies [64, 65].

Table 2: Medicinal plants with the quantitative analysis used for gynaecological disorders by the local communities of the tribal district of North Waziristan, Pakistan.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Abutilon indicum (L.) Sweet	Malvaceae	Zergulai	SR-13340	Shrub	Root	Powder + honey + milk	Leucorrhoea	The mixture of about one glass is taken once a day for seven days for the treatment of leucorrhoea.	40	0.31	20	0.50	30.77	57.50
Acacia modesta Wall.	Mimosaceae	Palusa	SR-13196	Tree	Gum	Powder + butter oil + milk	Backache after delivery, aphrodisiac	The mixture of about one cup is taken twice a day for 3–5 days to treat backache after delivery and used as an aphrodisiac.	48	0.37	37	0.77	36.92	100.00
Acacia nilotica (L.) Willd. ex Delile	Mimosaceae	Kekar	SR-13448	Tree	Bark	Decoction	Gonorrhea	Decoction of one medium size cup is taken for three days to cure gonorrhea.	27	0.21	14	0.52	20.77	62.96
Achyranthes aspera L.	Amaranthaceae	Ghoshkai	SR-13311	Herb	Leaves	Decoction	Labour pain	Decoction of leaves (half cup) is used to reduce excessive labour pain.	23	0.18	12	0.52	17.69	69.57
Adiantum capillus-veneris L.	Adiantaceae	Ebe betai	SR-13459	Ferns	Leaves	Decoction	Abnormal stoppage of menstruation	Decoction of leaves of about one medium size cup for 4-5 days is taken and used as menstruation additive.	24	0.18	16	0.67	18.46	50.00
Ajuga bracteosa Wall.	Lamiaceae	Varekai boti	SR-13425	Herb	Whole plant	Decoction	Abnormal stoppage of menstruation	Decoction of aerial parts of about 1 cup for three days is taken and used as menstruation additive.	20	0.15	13	0.65	15.38	55.00
Ajuga parviflora Benth.	Lamiaceae	Shengulai	SR-13413	Herb	Leaves, roots	Powder + milk	Amenorrhea	Powder (3 tablespoons) is given with one glass of warm milk for 6-7 days used to cure amenorrhea.	14	0.11	8	0.57	10.77	50.00

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Allium sativum L.	Alliaceae	Yeza	SR-13462	Herb	Bulb	Powder + curcumin powder	Easy delivery	Powder of 2 : 1 spoons is given to pregnant women with one glass of water used to stimulate uterine muscles for easy delivery.	25	0.19	13	0.52	19.23	56.00
Amaranthus spinosus L.	Amaranthaceae	Geta pakhe	SR-13326	Herb	Roots	Decoction	Excessive menstruation	Decoction of roots (one cup) is taken for 3 days and used to reduce menstrual flow.	21	0.16	11	0.52	16.15	57.14
Amaranthus viridis L.	Amaranthaceae	Surme	SR-13341	Herb	Leaves	Paste	Leucorrhoea	Leaves are cooked in oil and jaggery (gur) and the paste is taken for five days to treat leucorrhoea.	22	0.17	13	0.59	16.92	59.09
Androsace rotundifolia Hardw.	Primulaceae	Sergulai	SR-13424	Herb	Leaves	Juice	Irregular menstrual flow	Fresh juice (2 spoons) for 5–7 days is taken to regularize menstrual flow.	26	0.20	9	0.35	20.00	61.54
Berberis lycium Royle	Berberidaceae	Therkha	SR-13444	Shrub	Roots	Decoction	Gonorrhea	Decoction of roots (2 spoons) is taken for 7–10 days to cure gonorrhea.	45	0.35	34	0.76	34.62	100.00
Boerhavia diffusa L.	Nyctaginaceae	Pret boti	SR-13373	Herb	Aerial parts	Decoction	Irregular menstrual flow	Decoction of aerial parts (1 spoonful) is given twice a day for seven days to regularize menstrual flow.	39	0.30	28	0.72	30.00	61.54
Bupleurum falcatum L.	Apiaceae	Pest boti	SR-13443	Herb	Whole plant	Decoction	Irregular menstrual flow	Decoction of the whole plant (3 spoons) is taken once a day for fifteen days to regulate the menses.	28	0.22	13	0.46	21.54	64.29
Calendula arvensis M.Bieb.	Asteraceae	Zer gulai	SR-13367	Herb	Flower	Infusion	Painful menstruation	Infusion of flowers (10–15 ml) is taken twice a day to cure pain during menstruation.	25	0.19	10	0.40	19.23	48.00

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	Push boti	SR-13477	Herb	Aerial parts	Decoction	Irregular menstrual flow	Decoction of aerial pats (2 spoonful) is taken thrice a day for 3–5 days to regularize menstrual flow.	34	0.26	12	0.35	26.15	50.00
Carum carvi L.	Apiaceae	Zera	SR-13467	Herb	Seeds	Powder + butter oil	Expel impurities from the uterus	Powder of seeds (10 g) is mixed with butter oil, taken once a day for 5 days, and used to expel impurities from the uterus.	44	0.34	31	0.70	33.85	97.73
Chenopodium ambrosioides L.	Chenopodiaceae	Khersapaka	SR-13531	Herb	Leaves	Decoction	Painful menstruation, enhance milk flow	Leaves decoction (one cup) given twice a day for three days is recommended for painful menstruation. The same is given to nursing mothers to enhance the flow of breast milk.	41	0.32	27	0.66	31.54	56.10
Citrullus colocynthis (L.) Schrad	Cucurbitaceae	Maraginye	SR-13486	Herb	Fruit	Juice	Easy delivery	Fresh juice of fruit (two spoons) is given to women during childbirth and is used for easy and smooth delivery.	39	0.30	21	0.54	30.00	71.79
Cnicus benedictus L.	Asteraceae	Pest azghi	SR-13473	Herb	Aerial parts	Decoction + milk	Enhance milk flow	A decoction of aerial parts (one spoonful) is mixed with one glass of milk and given to nursing mothers to increase the flow of breast milk.	27	0.21	14	0.52	20.77	77.78
Cocculus pendulus (J.R. Forst. and G. Forst.)	Menispermaceae	Motiki boti	SR-13573	Shrub	Roots	Decoction	Amenorrhea	Decoction of roots (15 ml) is given for 7–10 days continuously to treat amenorrhea.	43	0.33	31	0.72	33.08	53.49

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Convolvulus arvensis L.	Convolvulaceae	Pervetia	SR-13215	Herb	Whole plant	Decoction	Irregular menstrual flow	Decoction of the plant (4 tea spoonful) is taken for 3-4 days to regulate menstrual flow.	18	0.14	10	0.56	13.85	44.44
Cydonia oblonga Mill.	Rosaceae	Bahi	SR-13520	Shrub	Fruit, seeds	Jame, powder	Nausea and vomiting, leucorrhoea	Fruit jam (two teaspoonful) is given to pregnant women once in the early morning on an empty stomach for 3 days to treat nausea and vomiting. Seeds powder (1 teaspoonful) is mixed with honey and given once a day for 7 days to treat leucorrhoea.	47	0.36	38	0.81	36.15	100.00
Cyperus rotundus L. K	Cyperaceae	Delgai	SR-13296	Sedge	Rhizome	Poultice	Enhance milk flow	A poultice of the rhizome is applied to the breast to increase the flow of breast milk.	33	0.25	9	0.27	25.38	63.64
Datura stramonium L.	Solanaceae	Berbaka	SR-13376	Shrub	Leaves	Poultice	Breast swelling	A poultice of fresh leaves is topically applied on a nursing mother's breast to cure the inflammation of breasts.	23	0.18	12	0.52	17.69	82.61
Dodonaea viscosa (L.) Jacq	Sapindaceae	Ghavajara	SR-13269	Shrub	Leaves	Decoction	Excessive menstruation	Decoction of leaves (two spoons) is taken twice a day for 3 days to control excessive menstruation.	43	0.33	27	0.63	33.08	79.07
Eclipta prostrate (L.)	Asteraceae	Thorkvanai	SR-13359	Herb	Whole plant	Herbal tea	Miscarriage	Herbal tea (1 tea spoonful) is given twice a day for 7 days to prevent miscarriage.	23	0.18	12	0.52	17.69	73.91

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Equisetum arvense L.	Equisetaceae	Bandkai	SR-13216	Ferns	Whole plant	Decoction	Gonorrhea	Decoction of plant (15–20 ml) is given once a day for 4-5 days and used to cure gonorrhea.	27	0.21	13	0.48	20.77	77.78
Erodium cicutarium L.	Geraniaceae	Not known	SR-13209	Herb	Whole plant	Herbal tea + jaggery	Irregular menstrual flow, enhance milk flow	Herbal tea (two teaspoonful) with jaggery is given twice a day to regulate menstrual flow. The same is given to nursing mothers to increase the flow of breast milk.	33	0.25	19	0.58	25.38	72.73
Euphorbia hirta L.	Euphorbiaceae	Bayavenia	SR-13529	Herb	Whole plant	Latex	Enhance milk flow	Latex (10 ml) is given once a day to the nursing mother to increase the flow of breast milk.	31	0.24	16	0.52	23.85	70.97
Fragaria nubicola (Hook.f.) Lindl.	Rosaceae	Jangli strawberi	SR-13429	Herb	Fruit	Raw	Irregular menstrual flow	Fruits (5–10) are taken twice a day for three days to regulate menstrual flow.	25	0.19	8	0.32	19.23	64.00
Fritillaria imperialis L.	Liliaceae	Geger Gul	SR-13383	Herb	Bulb	Powder + milk	Enhance milk flow	Bulb powder (one spoon) is given with one cup of milk to nursing mothers once a day for 7 days to increase the flow of breast milk.	25	0.19	11	0.44	19.23	52.00
Geranium wallichianum D. Don ex sweet	Geraniaceae	Varekai bote	SR-13389	Herb	Roots	Powder + milk	Leucorrhoea, tonic after delivery	Roots powder (1 teaspoonful) mixed with milk and sugar is given twice a day for 3–5 days to cure leucorrhoea and is also used as tonic after delivery.	41	0.32	23	0.56	31.54	78.05

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Gymnosporia nemorosa (Eckl. & Zeyh.) Szyszyl.	Celastraceae	Sagherzai	SR-13364	Shrub	Fruit	Powder + butter oil	Labour pain	Fruit powder (10–12 gm) mixed with butter oil is given to pregnant women during childbirth to reduce excessive labor pain.	29	0.22	11	0.38	22.31	72.41
Justicia adhatoda L. K	Acanthaceae	Bikarh	SR-13233	Shrub	Root, leaves	Paste + milk	Leucorrhoea	Roots paste (2 teaspoonful) mixed with one glass of milk is given twice a day for 15 days of and used to cure leucorrhoea.	33	0.25	16	0.48	25.38	66.67
Lepidium sativum L.	Brassicaceae	Bashke	SR-13320	Herb	Seeds	Powder + milk	Abnormal stoppage of menstruation	Seeds powder (5-6 g) mixed with one glass of milk is given once a day for 3–5 days and used as a menstruation additive.	20	0.15	9	0.45	15.38	65.00
Leucaena leucocephala (Lam.) de Wit	Mimosaceae	Pest kekar	SR-13358	Shrub	Seeds	Powder + honey	Menstrual cramps	Seeds powder (10 g) mixed with honey and taken twice a day for 4-5 days is recommended for menstrual cramps.	23	0.18	11	0.48	17.69	60.87
Melia azedarach L.	Meliaceae	Bakana	SR-13266	Tree	Fruit gum	Powder + cow's milk	Emmenagogue	Fruits gum powder (4-5 g) mixed with cow's milk and given to women once a day for 2-3 days is recommended for emmenagogue.	44	0.34	27	0.61	33.85	70.45
Mentha spicata L.	Lamiaceae	Velanai	SR-13283	Herb	Leaves	Juice	Easy delivery	Juice of leaves (one glass) is given to expectant mother to speed up child birth.	20	0.15	13	0.65	15.38	65.00

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Mentha arvensis L.	Lamiaceae	Sarkori Velanai	SR-13284	Herb	Whole plant	Powder	Antifertility	Powder of plant (2 spoons) mixed with one glass of water is given to women before the meeting and used for antifertility.	21	0.16	11	0.52	16.15	85.71
Mirabilis jalapa L.	Nyctaginaceae	Mazdergul	SR-13500	Herb	Roots	Powder + milk	Sexual tonic	Roots powder (1 spoonful) mixed with one glass of milk is taken during nighttime daily for 7 days and used as a sexual tonic.	36	0.28	16	0.44	27.69	80.56
Nasturtium officinale R.Br.	Brassicaceae	Mangore	SR-13317	Herb	Leaves, shoots	Juice	Produce temporary sterility	Juice of plant (one cup) is given to women daily for 3–5 days to produce temporary sterility.	28	0.22	14	0.50	21.54	78.57
Nepeta cataria L.	Lamiaceae	Khezbe	SR-13420	Herb	Whole plant	Decoction	Delayed menses	Decoction of plant (one cup) taken once a day for 5–7 days is recommended to delay menstruation.	18	0.14	10	0.56	13.85	72.22
Opuntia dillenii Haw.	Cactaceae	Sapre boti	SR-13168	Shrub	Fruit	Baking + honey	Gonorrhea	Fruit juice is baked and mixed with honey and given twice a day for 10 days to cure gonorrhea.	40	0.31	22	0.55	30.77	77.5
Oxalis corniculata L.	Oxalidaceae	Threw boti	SR-13254	Herb	Leaves	Chewing	Vomiting	The leaves are chewed to avoid vomiting during the early period of pregnancy.	37	0.28	19	0.51	28.46	59.46
Peganum harmala L.	Zygophyllaceae	Sponda	SR-13163	Herb	Whole plant	Smoke	Antiseptic	The smoke of the plant passed on to the women after childbirth is used as an antiseptic.	40	0.31	19	0.48	30.77	95.00

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Phyla nodiflora (L.) Greene.	Verbenaceae	Ebe betai	SR-13319	Herb	Roots	Decoction + honey	Infertility	Decoction of root (10–12 ml) with honey (2 spoons) is given to women for promoting sexual desire.	16	0.12	8	0.50	12.31	50.00
Pistacia integerrima J. L. Stewart ex Brandis.	Anacardiaceae	Shene	SR-13464	Tree	Gum	Powder + milk	Gonorrhea	Gum powder (8 g) mixed with milk and sugar is given once a day for 12 days to cure gonorrhea.	43	0.33	21	0.49	33.08	90.70
Portulaca oleracea L.	Portulacaceae	Parkhorai	SR-13169	Herb	leaves	Cooked	Excessive menstruation	Leaves are cooked in oil and black pepper and this paste is given for 3-4 days to control excessive menstruation.	31	0.24	16	0.52	23.85	74.19
Potentilla erecta (L.) Raeusch.	Rosaceae	Dhania ghonde	SR.13432	Herb	Whole plant	Powder + curd	Excessive menstruation	Powder of plant (10 g) mixed with curd is taken daily for 3 days to control excessive menstruation.	25	0.19	11	0.44	19.23	60.00
Prunus domestica L.	Rosaceae	Manra	SR-13521	Tree	Fruit	Raw	Vomiting	The unripe fruit is given to pregnant women to avoid vomiting during the early period of pregnancy.	25	0.19	17	0.68	19.23	72.00
Ricinus communis L.	Euphorbiaceae	Arind	SR-13132	Shrub	Seed	Powder	Abortion	Powder of seeds (15–20 g) is given with water to pregnant women for 3 days at the initial stage to induce abortion.	37	0.28	18	0.49	28.46	72.97
Solanum surattense Burm. f.	Solanaceae	Kurkundai	SR-13396	Herb	Whole plant	Decoction + jaggery	Conception	Decoction of the plant (one cup) mixed with jaggery is given for 5 days to promote the chance of pregnancy in females.	43	0.33	27	0.63	33.08	90.70

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Tagetes erecta L.	Asteraceae	Zenda gula	SR-13260	Herb	Roots	Decoction	Irregular menstruation	Decoction of roots (10–12 ml) is taken once a day for 3-4 days to regulate menstruation.	25	0.19	13	0.52	19.23	56.00
Tamarix aphylla (L.) H. Karst.	Tamaraceae	Ghaz	SR-13215	Tree	Leaves	Smoke	Antiseptic	The smoke passed on to women after childbirth is used as an antiseptic.	42	0.32	23	0.55	32.31	95.24
Tecomella undulata (Roxb.) Seeman.	Bignoniaceae	Rawdana	SR-13378	Shrub	Bark	Decoction + sugar	Lecucorroea	Decoction of the bark (one cup) mixed with sugar is given twice a day for 7 days to cure leucorrhoea.	46	0.35	39	0.85	35.38	97.83
Teucrium stocksianum Boiss.	Lamiaceae	Malgai	SR-13274	Herb	Shoots	Powder + milk	Conception, miscarriage	Powder of shoot (8–10 g) mixed with milk is taken once a day for 5 days to increase chances of fertilization and to prevent miscarriage.	20	0.15	11	0.55	15.38	90.00
Thymus serpyllum L.	Lamiaceae	Pestekai	SR-13451	Herb	Whole plant	Decoction + sugar	Irregular menstruation	Decoction of the plant (one cup) mixed with sugar is given once a day for 3-4 days to regulate menses.	15	0.12	8	0.53	11.54	60.oo
Trachyspermum ammi (L.) Sprague	Apiaceae	Sperkai	SR-13206	Herb	Seeds	Powder	Irregular menstruation	Powder of the seeds (15–20 g) is taken with water twice a day for 3-4 days to regulate menstruation.	41	0.32	21	0.51	31.54	95.12
Trianthema portulacastrum L.	Azoiaceae	Mardor betai	SR-13339	Herb	Whole plant	Decoction	Abortion	Decoction of the plant (one glass) is given twice a day to pregnant women in the early period of pregnancy to induce abortion.	45	0.35	27	0.60	34.62	77.78

Table 2: Continued.

Plants species	Family name	Vernacular name	Voucher no.	Habit	Parts used	Mode of preparation	Gynaecological use	Mode of application	FC	RFC	UR	UV	CI%	FL%
Tribulus terrestris L.	Zygophyllaceae	Markhiri	SR-13236	Herb	Leaves	Decoction + sugar	Gonorrhea	Decoction of leaves (one cup) mixed with sugar is taken once a day for 5–7 days to cure gonorrhea.	17	0.13	9	0.53	13.08	88.24
Urtica dioica L.	Urticaceae	Sezankai	SR-13128	Herb	Whole plant	Powder + cow's milk	Leucorrhoea	Powder of the plant (12–15 g) mixed with one glass of cow's milk is taken twice a day for 15 days to cure leucorrhoea.	19	0.15	8	0.42	14.62	63.16
Verbena officinalis L.	Verbenaceae	Bachawai	SR-13293	Herb	Whole plant	Decoction	Miscarriage	Decoction of the plant (one cup) is given once a day for 5 days to prevent miscarriage.	23	0.18	11	0.48	17.69	60.87
Vitex negundo L.	Verbenaceae	Marwandai.	SR-13171	Shrub	Shoots	Decoction + honey	Irregular menstruation	Decoction of the shoots (two teaspoonful) mixed with honey is taken once a day for 3-4 days to regulate menstrual flow.	26	0.20	14	0.54	20.00	65.38
Withania somnifera (L.) Dunal	Solanaceae	Sre dane	SR-13230	Shrub	Roots	Powder + butter oil	Sexual tonic	Powder of roots (6–8 g) mixed with butter oil is taken once a day during nighttime for 10 days to stimulate sexual desire.	46	0.35	36	0.78	35.38	84.78
Ziziphus mauritiana Lam.	Rhamnaceae	Bara	SR-13198	Tree	Seeds	Paste	Leucorrhoea	The paste made from seeds is given twice a day for 15 days to cure leucorrhoea.	21	0.16	11	0.52	16.15	76.19
Ziziphus nummularia (Burm. f.) Wight and Arn.	Rhamnaceae	Karkana	SR-13179	Shrub	Roots	Powder	Abortion	Powder of the roots (8–10 g) is given with water twice a day to pregnant women in the early stage of pregnancy to induce abortion.	37	0.28	21	0.57	28.46	70.27

RFC: relative frequency of citation, FC: frequency of citation, UR: used reports, UV: use value, FL%: fidelity level, FIV: family importance value.

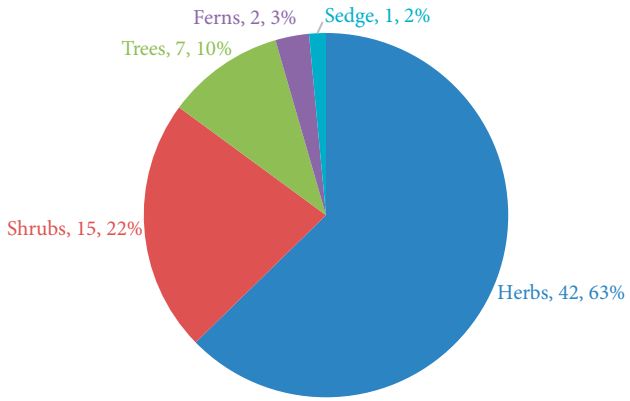

Figure 2: The proportion of various plant life forms.

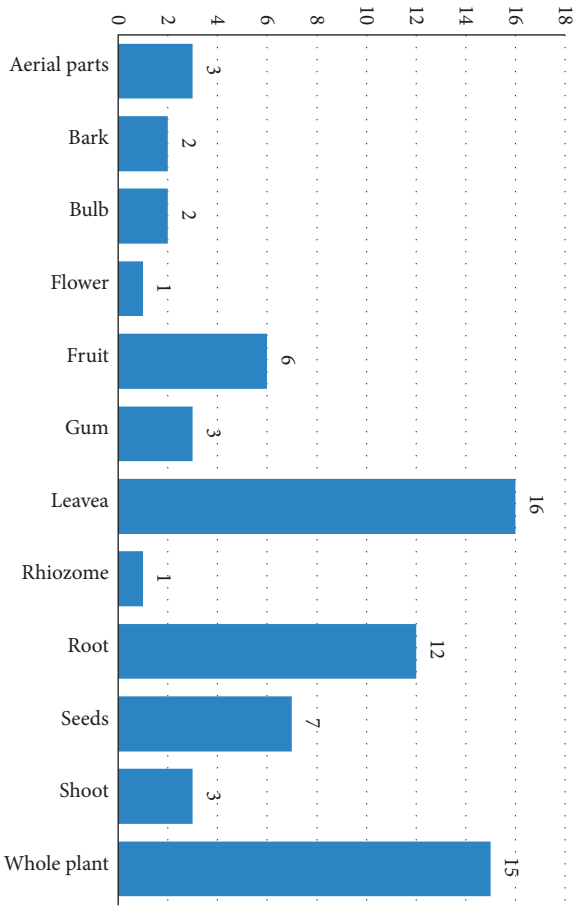

Figure 3: Plant parts are used in herbal remedies. The blue bar shows the "number of species."

3.6. Mode of Administration. In this study, the dominant modes of administration/application were orally advised (62 species, 92.54%), followed by inhaling and topical (2 species, 92.54% each), and chewing (1 species, 1.49%) (Figure 5). The majority of oral administration was also reported in other study areas [66, 67].

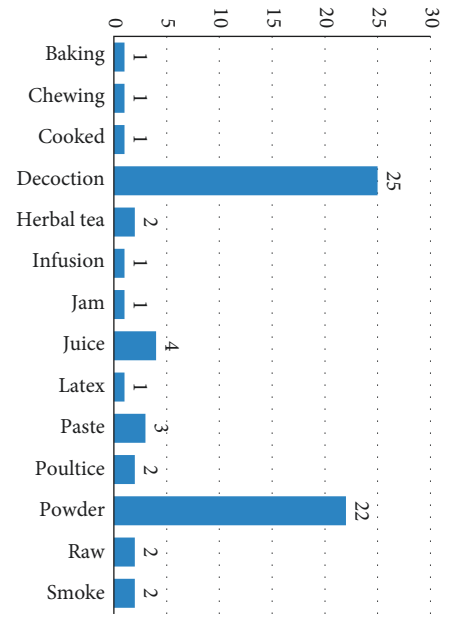

Figure 4: Mode of herbal drug preparation. The blue bar shows the "number of species."

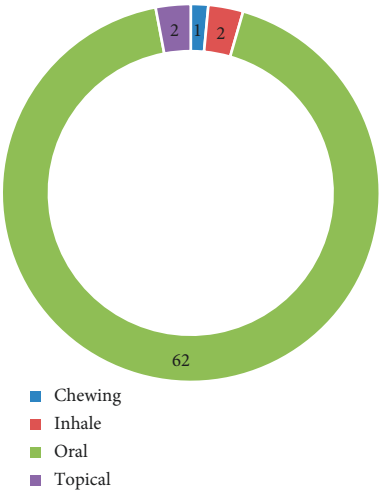

Figure 5: Mode of application/administration. The blue bar shows the "number of species."

3.7. Indigenous Plants Used for the Treatment of Gynaecological Disorders. Tribal people have a wide range of knowledge about traditional medicine based on local plants for basic medical care [6]. During this ethnogynaecological study, 26 gynaecological disorders were documented, which were treated by using 67 medicinal plants (Table 3). The common gynaecological disorder in the study area was irregular menstrual flow, which was treated by using 11 plant species (15.28%), followed by leucorrhoea (8 species, 11.11%), enhanced milk flow and gonorrhea (6 species, 8.33% each), excessive menstruation (4 species, 5.56%), abnormal stoppage of menstruation, abortion, easy delivery, miscarriage, and vomiting (3 species, 4.17% each).

Table 3: Gynaecological diseases treated by using indigenous plants.

Sr. no.	Diseases	Number of species	Percentage
1	Abnormal stoppage of menstruation	3	4.17
2	Abortion	3	4.17
3	Amenorrhea	2	2.78
4	Antifertility	1	1.39
5	Antiseptic	2	2.78
6	Backache after delivery	1	1.39
7	Breast swelling	1	1.39
8	Conception	2	2.78
9	Delayed menses	1	1.39
10	Easy delivery	3	4.17
11	Emmenagogue	1	1.39
12	Enhance milk flow	6	8.33
13	Excessive menstruation	4	5.56
14	Expel impurities from uterus	1	1.39
15	Gonorrhea	6	8.33
16	Infertility	1	1.39
17	Irregular menstrual flow	11	15.28
18	Labour pain	2	2.78
19	Leucorrhoea	8	11.11
20	Menstrual cramps	1	1.39
21	Miscarriage	3	4.17
22	Painful menstruation	2	2.78
23	Produce temporary sterility	1	1.39
24	Sexual tonic	2	2.78
25	Tonic after delivery	1	1.39
26	Vomiting	3	4.17

3.8. Quantitative Analysis. The recorded data were analyzed through different statistical indices like RFC, UV, CI%, ICF, FL%, and FIV.

3.8.1. Relative Frequency of Citation (RFC). A relative frequency of citation was used to assess the most commonly used therapeutic plants [68] for gynaecological disorders. In this study, the RFC ranged from 0.11 to 0.37 (Table 2). Based on RFC values, the most valuable medicinal plant having a high degree of RFC was *Acacia modesta* (0.37), followed by *Cydonia oblonga* (0.36), *Berberis lycium, Tecomella undulata, Trianthema portulacastrum,* and *Withania somnifera* (0.35). The lowest RFC value was calculated for *Ajuga parviflora* (0.11). Those therapeutic plant species having the highest RFC value should be further analyzed pharmaceutically and phytochemically to identify their bioactive compounds for medicinal discovery [69, 70].

3.8.2. Use Value (UV). According to [45], the use value indexation is a quantitative technique of ethnobotany that correlates the importance of plant species among aboriginal communities with regard to their uses. The use value in our documented data ranged from 0.27 to 0.85 and the use reports (URs) ranged from 9 to 39 (Table 2). The highest use value was reported for *Tecomella undulata* (0.85), followed by *Cydonia oblonga* (0.81), *Withania somnifera* (0.78), *Acacia modesta* (0.77), and *Berberis lycium* (0.76). The lowest use value (UV) was recorded for *Cyperus rotundus* (0.27). It was observed that the maximum use values were due to the higher number of use reports (URs) in the study area. The highest used values of documented therapeutic plants might indicate their indigenous professional expertise, which leads to a preference option for the disorder [71]. Medicinal plants with the lowest UV do not mean that they are not medicinally important, but it is shown that the traditional knowledge about these medicinal plants is limited [72]. Therapeutic plants for which the use value (UV) is high due to their frequent distribution in the research area and the inhabitants are well known for their medicinal value [35].

3.8.3. Consensus Index (CI%). The percentage of informants having traditional indigenous knowledge of medicinal plant species used for illness control (in this study, gynaecological disorders) was determined using a consensus index (CI%) [73], which indicates the citation by percent of informants [74]. The consensus index (CI) value ranges from 10.77% to 36.92% (Table 2). The maximum CI value was obtained for *Acacia modesta* (36.92%), followed by *Cydonia oblonga* (36.15%), *Tecomella undulata,* and *Withania somnifera* (35.38%). The lowest consensus index (CI) value was calculated for *Ajuga parviflora* (10.77%). CI indicates an agreement on the fact that *Acacia modesta* and *Cydonia oblonga* are the most important and well-known therapeutic plants used for the treatment of gynaecological disorders in North Waziristan.

3.8.4. Fidelity Level (FL%). Fidelity level (FL%) is used to determine the medicinal plant species that are most preferred by indigenous people for the cure of any specific ailment [46]. The therapeutic plants with the highest healing

Table 4: Informant consensus factor (ICF) value for various diseases categories.

Sr. no.	Use categories	Nur	*Nt*	Nur − *Nt*	Nur_{-1}	ICF
1	Amenorrhea	57	2	55	56	0.98
2	Antiseptic	82	2	80	81	0.99
3	Breast inflammation and lactation	213	7	206	212	0.97
4	Delivery problems	84	3	81	83	0.98
5	Emmenagogue	44	1	43	43	1.00
6	Gonorrhea	199	6	193	198	0.97
7	Induce abortion	119	3	116	118	0.98
8	Labour pain and backache	100	3	97	99	0.98
9	Leucorrhoea	269	8	261	268	0.97
10	Menstrual problems	620	23	597	619	0.96
11	Prevent miscarriage	109	4	105	108	0.97
12	Sexual problems	147	5	142	146	0.97
13	Tonic after delivery	41	1	40	40	1.00
14	Vomiting stoppage	109	3	106	108	0.98

effects have the maximum fidelity level of 100%. The medicinal plant species that were mentioned by a single informant were not considered for the FL level study. In this study, FL ranged from 44.44% to 100% (Table 2). It is a fact that the higher the plant's utilization is, the higher the FL value will be. In this study, the highest FL was determined for *Acacia modesta* (backache after delivery), *Berberis lycium* (gonorrhea), and *Cydonia oblonga* (leucorrhoea) (100%), followed by *Carum carvi* (97.73%) for expelling impurities from the uterus and *Tamarix aphylla* (95.24%) for antiseptic, while the lowest FL was recorded for *Convolvulus arvensis* (44.44%) for irregular menstrual flow. The highest value of fidelity level (FL) determined the choice of informants to cure the specific disease [75].

3.8.5. Informant Consensus Factor (ICF). The informant consensus factor (ICF) establishes the even sharing of informants' information regarding the medicinal plants, which validates that all the local people in the research area use plants for the treatment of the same ailment in same or different methods. In other words, the ICF value explains the cultural consistency in the use of a group of medicinal plants to treat a specific ailment [76]. To determine the informants' consensus factor (ICF), various diseases were grouped into 14 different disease categories based on taxa and use reports (Table 4). In this study, the ICF values ranged from 0.96 to 1.0. The highest ICF value was reported for emmenagogue and tonic after delivery (1.0), followed by antiseptic (0.99), and the lowest ICF value was reported for menstrual problems (0.96). Similar results were reported by [77] demonstrating that emmenagogue disorder has the highest ICF values.

3.8.6. Family Importance Value (FIV). The family importance value increases with the increase in the frequency of citations of all species. In this work, the most important family, based on the frequency of citations, was Lamiaceae with an FIV value of 98.46%, followed by Rosaceae (93.85%), Apiaceae (86.92%), Solanaceae (86.15%), Asteraceae (76.92%), and Mimosaceae (75.38%). Convolvulaceae has the lowest family importance value, with 13.85% (Table 5).

Table 5: Family importance value (FIV) of medicinally important families.

Sr. no.	Family name	No. of species	FC (family)	FIV
1	Acanthaceae	1	33	25.38
2	Adiantaceae	1	24	18.46
3	Alliaceae	1	25	19.23
4	Amaranthaceae	3	66	50.77
5	Anacardiaceae	1	43	33.08
6	Apiaceae	3	113	86.92
7	Asteraceae	4	100	76.92
8	Azoiaceae	1	45	34.62
9	Berberidaceae	1	45	34.62
10	Bignoniaceae	1	46	35.38
11	Brassicaceae	3	82	63.08
12	Cactaceae	1	40	30.77
13	Celastraceae	1	29	22.31
14	Chenopodiaceae	1	41	31.54
15	Convolvulaceae	1	18	13.85
16	Cucurbitaceae	1	39	30.00
17	Cyperaceae	1	33	25.38
18	Equisetaceae	1	27	20.77
19	Euphorbiaceae	2	68	52.31
20	Geraniaceae	2	74	56.92
21	Lamiaceae	7	128	98.46
22	Liliaceae	1	25	19.23
23	Malvaceae	1	40	30.77
24	Meliaceae	1	44	33.85
25	Menispermaceae	1	43	33.08
26	Mimosaceae	3	98	75.38
27	Nyctaginaceae	2	39	30.00
28	Oxalidaceae	1	37	28.46
29	Portulacaceae	1	31	23.85
30	Primulaceae	1	26	20.00
31	Rhamnaceae	2	58	44.62
32	Rosaceae	4	122	93.85
33	Sapindaceae	1	43	33.08
34	Solanaceae	3	112	86.15
35	Tamaraceae	1	42	32.31
36	Urticaceae	1	19	14.62
37	Verbenaceae	3	65	50.00
38	Zygophyllaceae	2	57	43.85

Medicinally important plant species of the families Asteraceae, Apiaceae, Lamiaceae, and Rosaceae are mentioned as

important in various pharmacological works [78, 79]. The highest FIV value percentage reveals that the plants of a specific family are commonly used in treating various disorders, as reported by informants.

3.9. Status of Medicinal Plants. According to local residents, the population of most medicinal plants has decreased over the last few decades. Threatened and endangered species of the study area are *Berberis lycium*, *Fritillaria imperialis*, *Gymnosporia nemorosa*, *Pistacia integerrima*, and *Tecomella undulata*. Excessive and injudicious use, overgrazing, improper harvesting practices such as digging out the entire plant, market pressure, and deforestation are also contributing factors. Medicinal plants are collected from the study area, transported to a small market by locals, and then exported to major cities. Locals also use shrubby species and trees as fuel sources, which have a negative impact on medicinal plant populations. Forests are necessary for the survival of several therapeutic plant species. As a result, the area's medicinally important plants are decreasing. Such flora need preservation through sustainable use, appropriate management, and conservation. A regional awareness campaign regarding the state of indigenous flora, sustainable plant harvesting, and the conservation of valuable therapeutic plants will lead to better outcomes. Local inhabitants, local stakeholders, and plant collectors should be aware of the conservation of plant resources in the region, and the indigenous people should be involved in conservation practices.

3.10. Novelty and Future Impacts. This study was compared with previously published literature of neighbouring areas and distant areas of utilization of medicinal plants for ethnogynaecological disorders [18, 80–86]. The comparative study between previously reported medicinal plants showed that some medicinal plants have the same or different medicinal uses, while some were documented for the first time and others were not previously documented. The following 9 species were reported for the first time to cure gynaecological diseases: *Acacia modesta* (aphrodisiac), *Cnicus benedictus* (enhance milk flow), *Cocculus pendulus* (amenorrhea), *Cydonia oblonga* (leucorrhoea), *Cyperus rotundus* (enhance milk flow), *Peganum harmala* (antiseptic), *Prunus domestica* (vomiting), *Tamarix aphylla* (antiseptic), and *Tecomella undulata* (leucorrhoea) (Table 2). Many ethnomedicinal studies have similar medicinal uses of therapeutic plants for the treatment of various ailments all over the globe. This study adds some new therapeutic plant uses, which may provide baseline data for phytochemical and pharmacological screening for the detection of new drugs in future studies. The discovery of drugs from therapeutic plants links an interdisciplinary approach to joining ethnomedicinal, pharmacological, botanical, and natural methods. However, any medicinal plants in this study area are not subjected to detailed pharmacological screenings.

4. Conclusion

This study focuses on pastoral women's health and healing. In rural areas, modern health facilities are insufficient or not available. Rural people (midwives, traditional healers) have indigenous knowledge of herbal remedies for treating gynaecological disorders. In the research area, 67 therapeutic plants are used to treat 26 different types of gynaecological disorders. Leaves are the dominant part used in the preparation of herbal remedies for gynaecological disorders. Menstrual problems were the most prevalent ailment category treated using 26 therapeutic plants in the study area. Decoction and powder were reported as the most commonly used methods for preparing herbal remedies, which clearly shows the consistency with other studies as well [53, 54]. The highest use value was reported for *Tecomella undulata* (0.85), followed by *Cydonia oblonga* (0.81) and *Withania somnifera* (0.78). It was observed that the medicinal plants having maximum UV were due to their higher number of use reports (URs) in the study area. The literature reveals that the therapeutic plants with higher UV are because of their frequent distribution in the research area and the inhabitants are well known for their medicinal value [62], which leads them to be the preferred option for the particular ailment [59]. The cultural consistency in the use of a group of medicinal plants to treat a specific ailment group was explained using ICF [47], through which the consistency of our results was found in accordance with Sadeghi et al. [20]; they reported that emmenagogue disorder has the highest ICF values. Some medicinal plants, like *Berberis lycium*, *Fritillaria imperialis*, *Gymnosporia nemorosa*, *Pistacia integerrima*, and *Tecomella undulata* are under extreme pressure as a result of the indiscriminate collection by locals. We believe that forest protection and floral habitat conservation are critical. For this, the government and nongovernmental organizations (NGOs) must design appropriate programmes with the participation of local people who must be educated about the need to maintain precious forest resources and participate in forestation for future generations. This survey provides a baseline for future clinical and pharmacological studies in the field of gynecology. Therefore, it is necessary to focus on the medicinal uses of the reported plants [48]. Detailed clinical and pharmacological trials are needed to find out the bioactive components for the treatment of the gynaecological disorder.

Data Availability

The figures and tables supporting the results of this study are included within the article, and the original datasets are available from the first author or the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest in this article.

Acknowledgments

The authors are thankful to the local community members of the study area for sharing their valuable information. The

authors extend their appreciation to the Researchers Supporting Project no. RSP-2021/190, King Saud University, Riyadh, Saudi Arabia.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1281336, 12 pages
https://doi.org/10.1155/2022/1281336

Research Article

Curative Effect of Heat-sensitive Moxibustion on Primary Dysmenorrhea: A Meta-Analysis

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Received 22 April 2022; Accepted 6 July 2022; Published 30 July 2022

Academic Editor: Min Hu

Background. Primary dysmenorrhea (PD) refers to functional dysmenorrhea, typically characterized by cyclical, pronounced lower abdominal pain and seriously affects a woman's work and quality of life. Some studies have reported that heat-sensitive moxibustion (HSM) is expected to alleviate the clinical symptoms. This systematic review aimed to evaluate the current evidence regarding the efficacy and safety of HSM on PD. *Methods*. 7 databases including PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Wan Fang Data Knowledge Service Platform (Wan Fang Data), and China Science and Technology Journal Database (VIP) were searched for clinical randomized controlled trials. Meanwhile, Revman 5.3 software was used to evaluate the methodological quality of the included literature. The confidence interval (CI) of either relative risk or mean difference was set to 95%. Besides, the heterogeneity of the research results is tested by I^2. *Results*. 19 studies were ultimately included in this meta-analysis. All of them were declared as random controlled trials. 18 studies reported the total effective rate of the test group and the control group, which was significantly higher (RR: 0.92; 95% CI: 0.85,0.99; $P = 0.031 < 0.05$) than the control group. It is demonstrated that the VAS score of the test group, totally 9 studies included, was significantly lower (SMD: −0.98; 95% CI: −1.15, −0.81; $P < 0.001$). The meta-analysis of 6 studies showed the symptom score of the test group was significantly lower (SMD: −0.67; 95% CI: −0.87, −0.47; $P < 0.001$). There were the CMSS results of 3 studies which were significantly lower (SMD: −0.88; 95% CI: −1.13, −0.62; $P < 0.001$). Combined with the results of subgroup analysis, compared with the control group, the test group had advantages in the VAS score, symptom score, and CMSS score. *Conclusions*. The result has revealed the effectiveness and feasibility of HSM in treating PD, especially in improving the total effective rate and reducing the VAS score, symptom score, and CMSS score.

1. Introduction

Primary dysmenorrhea (PD) refers to functional menstrual pain, which is distinguished from secondary dysmenorrhea with pelvic organic lesions [1,2]. The typical symptom of PD is periodic and obvious lower abdominal pain that occurs around menstruation [3]. Possible concomitant symptoms include involved pain in the waist and sacrum, diarrhea, nausea and vomiting, fatigue, mood disorders, and syncope in severe cases. [4] The pathogenesis of PD is closely related to the increased secretion of prostaglandin F2α (PGF2α) and prostaglandin E2(PGE2) in the late luteal phase, as well as the contraction of the smooth muscle and the blood vessels [5]. PD is a common gynecological disease. A retrospective analysis based on large community samples [6] indicates that the prevalence of PD rages from 16% to 91% among women of childbearing age. Other studies suggest that the worldwide prevalence of PD may be higher, ranging from 45% to 95%

[1]. PD has a general and obvious adverse effect on women's daily life, work, and study [7]. The treatment of PD includes drug therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs), hormones, as well as complementary and alternative therapies, such as moxibustion, acupuncture, and local hot compress [3]. Due to their high benefit, NSAIDs are recommended as the clinical first-line drugs of PD and have been widely used at present [5]. However, a systematic review [8] shows that NSAIDs used for PD may cause certain adverse reactions, including gastrointestinal symptoms (such as nausea and vomiting) and nervous system disorders (such as dizziness, headache, drowsiness, and exertion). Besides, NSAID resistance in PD is also common and worth paying attention to [9]. Thus, it may be of practical significance to find a treatment with definite curative effect and satisfactory safety to reduce the dosage of NSAIDs or replace them.

uterine microcirculation, so as to relieve pain. In addition, a double-arm-designed RCT [11] reported that the curative effect of moxibustion was similar to that of NSAIDs (ibuprofen-sustained release capsule) in the treatment of PD, and there was no significant adverse event. Heat-sensitive moxibustion (HSM) is a moxibustion therapy invented by Professor Chen RX, and it focuses on "heat-sensitive" sensation of acupoints as well as Deqi of moxibustion [12, 13]. HSM pays great attention to standardizing the clinical practice of moxibustion, and meanwhile it pays great attention to the individual differences of patients [12]. According to a prospective cohort study based on propensity score match [14], HSM stimulates heat-sensitized acupoints with a better effect than traditional moxibustion as a treatment of PD. In addition, a case [15] reported that by standardizing the moxibustion temperature, dose, and treatment time, HSM effectively relieved menstrual pain. However, as a complementary and alternative therapy for PD, the clinical efficacy and safety of HSM still need to be systematically studied. Therefore, this study aims to provide as much evidence as possible through meta-analysis for the feasibility of HSM in treating PD.

2. Methods

2.1. Inclusion Criteria. The population-intervention Comparators-Outcomes-Study design (PICOS) framework was used as the eligibility criteria for the review as follows.

2.1.1. Selection of Studies. All randomized controlled trials (RCTs) investigating HSM combined with other therapies in the treatment of PD were not limited by language or publication status.

2.1.2. Selection of Participants. The study subjects were female patients with clear clinical diagnostic criteria and all included patients were clearly diagnosed with PD.

2.1.3. Types of Interventions. The experimental group was treated with HSM for intervention, and the control group was treated with non HSM, including NSAIDs, acupuncture therapy, moxibustion, progesterone, and traditional Chinese medicine. Studies that did not meet the above inclusion criteria were excluded. In addition, the following exclusion criteria were applied: ①non-randomized controlled trial research literature; ②participants were non-primary dysmenorrhea patients; ③literature without original data or incomplete research data; and ④literature on interventions that did not meet the inclusion criteria.

2.2. Types of Outcome Measures. According to the author's definition, we found that the commonly used evaluation indicators include the total effective rate, cox menstrual symptom scale (CMSS), visual analogue scale (VAS) score, and symptom score [16].

2.3. Data Sources and Search Strategy. Computer searches of PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Wan Fang Data Knowledge Service Platform (Wan Fang Data), and China Science and Technology Journal Database (VIP) were conducted, all from the establishment of the database to January 2022. The search strategy involved the use of the following keywords: "Heat-sensitive Moxibustion," "thermal moxibustion," "Dysmenorrhea" " dysmenorrhoea," "HSM," "Menstrual Pain," "Primary dysmenorrhea," etc. Taking PubMed as an example, the search terms and strategies are as follows: (((thermal moxibustion[Title/Abstract]) OR (Heat-sensitive Moxibustion[Title/Abstract])OR (HSM[Title/Abstract]))) AND (Dysmenorrhea[mesh] OR dysmenorrhea [tiab] OR "Pain, Menstrual"[tiab] OR "Menstrual Pain"[tiab] OR "Menstrual Pains"[tiab] OR "Pains, Menstrual"[tiab] OR "Menstruation, Painful"[tiab] OR "Menstruations, Painful"[tiab] OR "Painful Menstruation"[tiab] OR "Painful Menstruations"[tiab] OR "Primary dysmenorrhea"[tiab]) AND (((clinical[tiab] AND trial[tiab]) OR "clinical trials as topic"[mesh] OR "clinical trial"[pt] OR random ∗ [tiab] OR "random allocation"[mesh] OR "therapeutic use"[sh]))). The search strategies of other databases follow their search rules.

2.4. Literature Screening and Data Extraction. Two researchers conducted literature screening independently in strict accordance with inclusion and exclusion criteria, and managed and identified the retrieved literature by Note Express software. Excel software was used to establish the database of literature information extraction, including research types, number of cases, diagnostic criteria, intervention and treatment, methodology and curative effect of standard, outcome indicators, and adverse reactions. Finally, the results were cross-checked, and disputes were resolved through discussion or seeking the opinions of the third party.

2.5. Quality Assessment. According to the Cochrane system assessment handbook that provides the standard, we

Table 1: The basic characteristics of the included studies. T: trial group; C: control group; NA: not reported; ①Total effective rate; ②VAS score; ③Symptom score; and ④CMSS score.

Trail	Sample Size (T/C)	Age (y), Mean ± SD or Median (Range)		Duration		T	C	Main Outcomes	Follow-up time/ month
		T	C	T	C				
Guan, 2021	47/46	20.73 ± 5.46	21.41 ± 5.92	5.43 ± 1.22m	5.19 ± 1.38m	HSM	Traditional Chinese medicine	①④	—
Lin, 2021	30/31	23.70 ± 2.78	24.03 ± 2.63	7.60 ± 2.58	8.48 ± 2.57	HSM	Sustained-Release ibuprofen capsules	①②③	3
Zhang, 2020	32/32	18.56 ± 2.35	17.22 ± 3.39	12.46 ± 4.35 m	11.96 ± 4.25m	HSM	Moxibustion	①②④	3
Li, 2020	30/30	23.5	25.1	22.8	4.6	HSM	Sustained-Release ibuprofen capsules	①②	—
Zhu, 2020	31/31	22.97 ± 4.04	21.97 ± 3.63	4.26 ± 2.68	3.09 ± 2.63	HSM	Traditional Chinese medicine	①	3
Wang, 2020	30/30	24.57 ± 3.88	23.73 ± 2.92	7.27 ± 3.86	6.90 ± 3.62	HSM	Traditional Chinese medicine	①②	3
Han, 2019	30/30	NA	NA	NA	NA	HSM	Traditional Chinese medicine	①②③	—
Wang, 2019	30/30	24.4	24.4	4m-13 y	4m-13y	HSM	Traditional Chinese medicine	①②	3
Li, 2019	40/40	22.93 ± 3.41	22.65 ± 3.27	9.43 ± 3.59m	9.06 ± 3.62m	HSM	Progesterone	①④	1
Ou, 2017	30/30	NA	NA	NA	NA	HSM	Moxibustion	①	—
Ma, 2016	35/35	30.0 ± 4.5	30.2 ± 5.0	2.5 ± 0.5	3.0 ± 0.5	HSM	Sustained-Release ibuprofen capsules	①	—
Xie, 2016	40/40	19.3 ± 1.2	19.2 ± 1.3	10.7 ± 2.3 m	10.9 ± 2.5m	HSM	Moxibustion	①	—
Lu, 2015	40/40	14-30	15-29	8m-7 y	6m-6.5y	HSM	Sustained-Release ibuprofen capsules	①②	—
Zhang, 2014	61/56	22.76 ± 4.26	23.30 ± 4.51	6.76 ± 2.26	7.03 ± 2.71	HSM	Moxibustion	②④	3
Zhong, 2014	30/30	23.774 ± 2.582	22.900 ± 2.537	6.67 ± 2.264	7.000 ± 1.894	HSM	Moxibustion	①②③	3
Li, 2013	20/20	20-42	20-42	1-5y	1-5y	HSM	Moxibustion	①	—
Nie, 2010	47/46	19.25 ± 4.03	19.24 ± 3.76	3.02 ± 1.38	2.93 ± 1.27	HSM	Acupuncture	①③	—
Rao, 2009	30/30	22.07 ± 4.50	21.47 ± 4.8	3.65 ± 2.75	3.51 ± 2.99	HSM	Sustained-Release ibuprofen capsules	①③	—
Zhang, 2008	33/32	NA	NA	NA	NA	HSM	Moxibustion	①③	—

adopted Revman5.3 for the methodological quality evaluation of the literature. Risk items include random sequence, allocation hiding, blindness, integrity of outcome data, risk of selective reporting bias, and other biases. If each of the 7 items is assessed as low risk, the study bias risk is assessed as low risk. If one or more entries are assessed as high risk, the study is assessed as high risk.

2.6. Statistical Analysis. Stata 15.1 software was used to perform the meta-analysis. If relative risk (RR) is used for dichotomous variables, the confidence interval (CI) is set at 95%. Continuous variables were represented by STD mean difference (SMD), and confidence interval (CI) was set at 95%. Heterogeneity of the research results was tested by I^2. If $I^2 \leq 50\%$, the outcome data of the fixed effects model (FE) were selected for analysis; if $I^2 > 50\%$, the outcome data of the random effects model (RE) were selected for reference analysis. Sensitivity analysis was used to analyze the sources of heterogeneity and to assess the stability of the meta-analysis results.

3. Result

3.1. Search Results. Acting by the search strategy, 194 references were identified. After excluding duplicate studies, 83 studies were scanned based on their abstracts and titles. Then, 29 articles were evaluated by full text. After full-manuscript assessment, 10 records were excluded with the following reasons: not RCT ($n = 6$), lack of outcomes ($n = 1$),

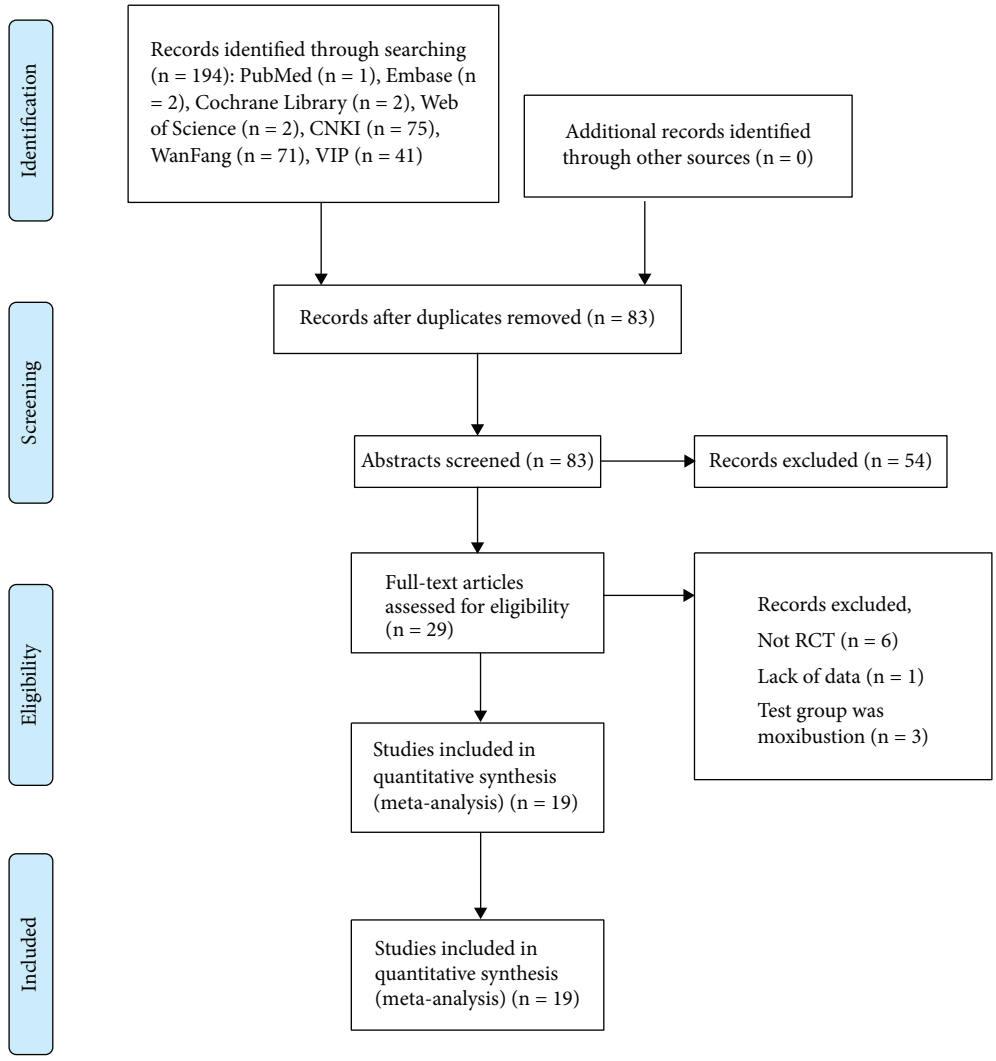

Figure 1: The inclusion process of literature.

and the test group was manual acupuncture treatment ($n = 3$). Eventually, 19 studies [17–35] were included in this meta-analysis (Table 1). The PRISMA statement flow chart shows this process (Figure 1).

3.2. Heat-Sensitive Acupoints. The heat-sensitive acupoints and their frequency and positions were included in this meta-analysis (Tables 2 and 3). Data analysis of the 19 heat-sensitive acupoints collated from the 19 research studies included in this study for the treatment of primary dysmenorrhea involved 14 heat-sensitive points (Baliao (BL31, BL32, BL33, BL34) contained 4 acupuncture points, containing Ciliao (BL32)). The top 4 heat-sensitive points used in frequency were Guanyuan (CV4), Sanyinjiao (SP6), Zigong (EX-CA1), and Ciliao (BL32).

3.3. Risk of Bias Assessment. Nineteen included studies [17–35] involved two-arm designs, and 9 trials reported proper generation methods (random number table) with a low risk of bias [17–19, 21–23, 25, 31, 33] (Figures 2 and 3). Seven trials did not describe the randomization procedure clearly [20, 24, 26, 27, 32, 34, 35]. Three trials reported generation methods (order of treatment or therapeutic measures) with a high risk of bias [28–30]. Three trials reported the blinding of participants and personnel [18, 33, 35]. Two trials reported any allocation concealment [18, 31]. Incomplete outcome data, selective reporting, and other biases had a low risk of bias. None of the trials reported the blinding of outcome assessment.

3.4. Primary Outcomes

3.4.1. Total Effective Rate. Eighteen studies [17–29, 31–35] reported the total effective rate of the test group and the control group. The meta-analysis showed that the total effective rate of the test group was significantly higher (RR: 0.92; 95% Cl: 0.85,0.99; $P = 0.031 < 0.05$, $I^2 = 0\%$, Figure 4) than the control group. $I^2 = 0\%$ showed that the meta-analysis of total effective rate has high stability. Subgroup analysis (Supplement Figure 1) was performed for the type of control intervention (Traditional Chinese medicine,

Table 2: The heat-sensitive acupoints of the included studies. NA: not reported.

Trail	Heat-Sensitive Acupoints
Guan, 2021	Guanyuan (CV4)
Lin, 2021	Guanyuan (CV4), Zhongji (CV3), Zigong (EX-CA1), Qihai (CV6), Sanyinjiao (SP6),
Zhang, 2020	Guanyuan (CV4), Zhongji (CV3), Zigong (EX-CA1)
Li, 2020	Sanyinjiao (SP6)
Zh-, 2020	Guanyuan (CV4), Zigong (EX-CA1), Ciliao (BL32), Sanyinjiao (SP6)
Wang, 2020	Guanyuan (CV4), Zigong (EX-CA1), Ciliao (BL32), Sanyinjiao (SP6), Shenque (CV8)
Han, 2019	Guanyuan (CV4), Zigong (EX-CA1)
Wang, 2019	NA
Li, 2019	Guanyuan (CV4), Zhongji (CV3), Zigong (EX-CA1), Guilai (ST29), Ciliao (BL32), Sanyinjiao (SP6)
Ou, 2017	Guanyuan (CV4), Zhongji (CV3), Zigong (EX-CA1), Sanyinjiao (SP6), Ciliao (BL32)
Ma, 2016	Guanyuan (CV4), Sanyinjiao (SP6), Ciliao (BL32)
Xie, 2016	Guanyuan (CV4)
Lu, 2015	Guanyuan (CV4), Zhongji (CV3), Sanyinjiao (SP6)
Zhang, 2014	Guanyuan (CV4)
Zhong, 2014	Guanyuan (CV4), Zigong (EX-CA1), Ciliao (BL32), Sanyinjiao (SP6)
Li, 2013	Guanyuan (CV4), Zigong (EX-CA1), Sanyinjiao (SP6), Qihai (CV6), Diji (SP8), Shiqizhui (EX-B 8), Bajiao (BL31, BL32, BL33, BL34)
Nie, 2010	Guanyuan (CV4), Zhongji (CV3), Sanyinjiao (SP6)
Rao, 2009	Guanyuan (CV4), Zhongji (CV3), Sanyinjiao (SP6), Ciliao (BL32)
Zhang, 2008	Guanyuan (CV4), Sanyinjiao (SP6)

Table 3: Frequency and position of the heat-sensitive acupoints.

Number	Heat Sensitive Acupoints	Frequency (times)	Percentage%	Position
1	Guanyuan (CV4)	17	27.87	Abdomen
2	Sanyinjiao (SP6)	13	21.31	lower extremities
3	Zigong (EX-CA1)	9	14.75	Abdomen
4	Ciliao (BL32)	8	11.67	Sacrum
5	Zhongji (CV3)	7	13.11	Abdomen
6	Qihai (CV6)	2	3.28	Abdomen
7	Shenque (CV8)	1	1.64	Abdomen
8	Baliao (BL31, BL32, BL33, BL34)	1	1.64	Sacrum
9	Diji (SP8)	1	1.64	lower extremities
10	Shiqizhui (EX-B 8)	1	1.64	Sacrum
11	Guilai (ST29)	1	1.64	Abdomen

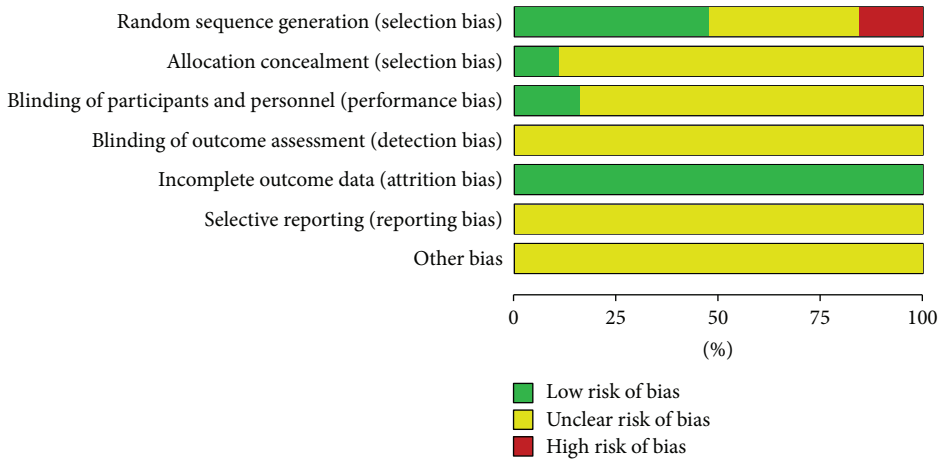

Figure 2: Risk of bias assessment in studies.

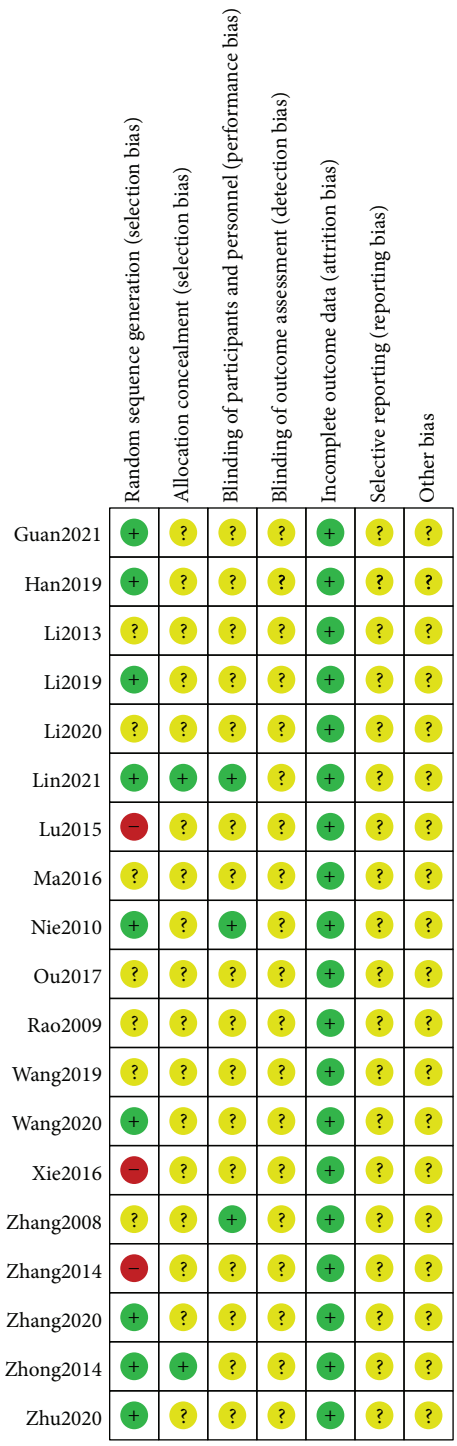

Figure 3: Risk of bias assessment for each included study in the review.

Ibuprofen, Moxibustion, Progesterone, and Acupuncture). The meta-analysis showed that the result of control intervention was low heterogeneity ($I^2 = 0\%$), and the total effective rate of the test group was not significantly higher than the control group ($P > 0.05$).

3.4.2. VAS Score. Nine studies [18–20, 22–24, 29–31] reported the VAS score of the test group and the control group. The meta-analysis showed that the VAS score of the test group was significantly lower (SMD: -0.98; 95% Cl: -1.15,-0.81; $P < 0.001$, $I^2 = 86.2\%$, Figure 5) than the control group. The results of all these trials showed high heterogeneity, and thus a sensitivity analysis was conducted (Figure 6), which showed that the included trail [31] had a more significant impact on the results. A careful review of the included trail [31] found that the intervention in the control group was ginger-partitioned moxibustion or patients were limited to cold-dampness coagulation syndrome. The remaining eight studies were used to analyze the VAS score and get new results (SMD: −0.79; 95% Cl: −0.97,−0.62; $P < 0.001$, $I^2 = 31.1\%$, Figure 7). Subgroup analysis (Supplement Figure 2) was performed for the type of control intervention (Traditional Chinese medicine, Ibuprofen, and Moxibustion). The meta-analysis showed that the result of control interventions (Ibuprofen, Moxibustion) was high heterogeneity ($I^2 = 62.1\%$ and $I^2 = 94.9\%$), the result of control interventions (Traditional Chinese medicine) was low heterogeneity ($I^2 = 0\%$), and the VAS Score of the test group was significantly higher than the control group. ($P < 0.05$).

3.4.3. Symptom Score. Six studies [20, 23, 31, 33, 34] reported the symptom score of the test group and the control group. The meta-analysis showed that the symptom score of the test group was significantly lower (SMD: -0.67; 95% Cl: -0.87,-0.47; $P < 0.001$, $I^2 = 0.0\%$, Figure 8) than the control group. Subgroup analysis (Supplement Figure 3) was performed for the type of control intervention (Traditional Chinese medicine, Ibuprofen, Moxibustion, and Acupuncture). The meta-analysis showed that the result of control interventions was low heterogeneity ($I^2 = 0\%$), and the symptom score of the test group was significantly higher than the control group ($P < 0.05$).

3.4.4. CMSS Score. Three studies [19, 25, 30] reported the CMSS score of the test group and the control group. The meta-analysis showed that the CMSS score of the test group was significantly lower (SMD: −0.88; 95% Cl: −1.13,−0.62; $P < 0.001$, $I^2 = 0.0\%$, Figure 9) than the control group. Subgroup analysis (Supplement Figure 4) was performed for the type of control intervention (Moxibustion, Progesterone). The meta-analysis showed that the result of control interventions was low heterogeneity ($I^2 = 0\%$), and the CMMS score of the test group was significantly higher than the control group ($P < 0.05$).

3.4.5. Publication Bias. The funnel plot (Figure 10) of the total effective rate was symmetrically distributed. What's more, Eggr's test showed no potential publish bias ($P = 0.976$).

4. Discussion

Primary dysmenorrhea [36] is defined as dysmenorrhea that occurs in the absence of pelvic pathology. Dysmenorrhea

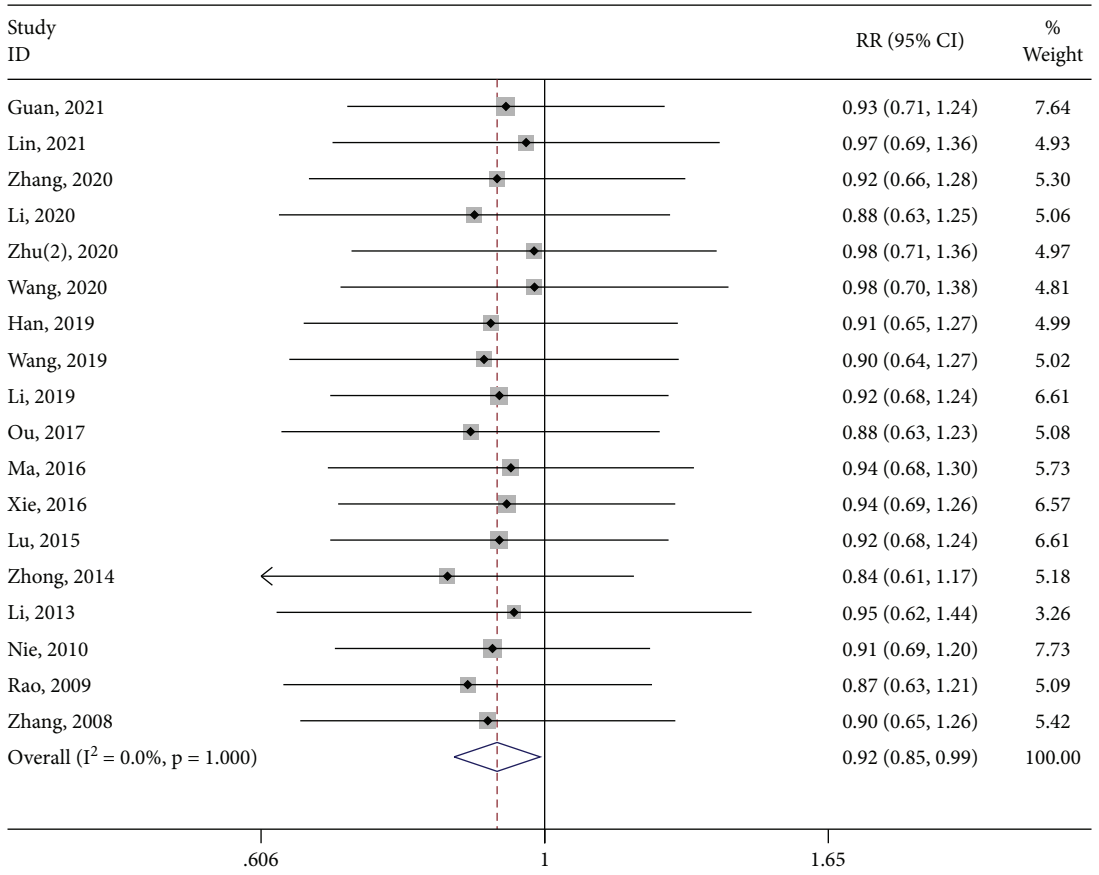

Figure 4: Forest plot of the total effective rate.

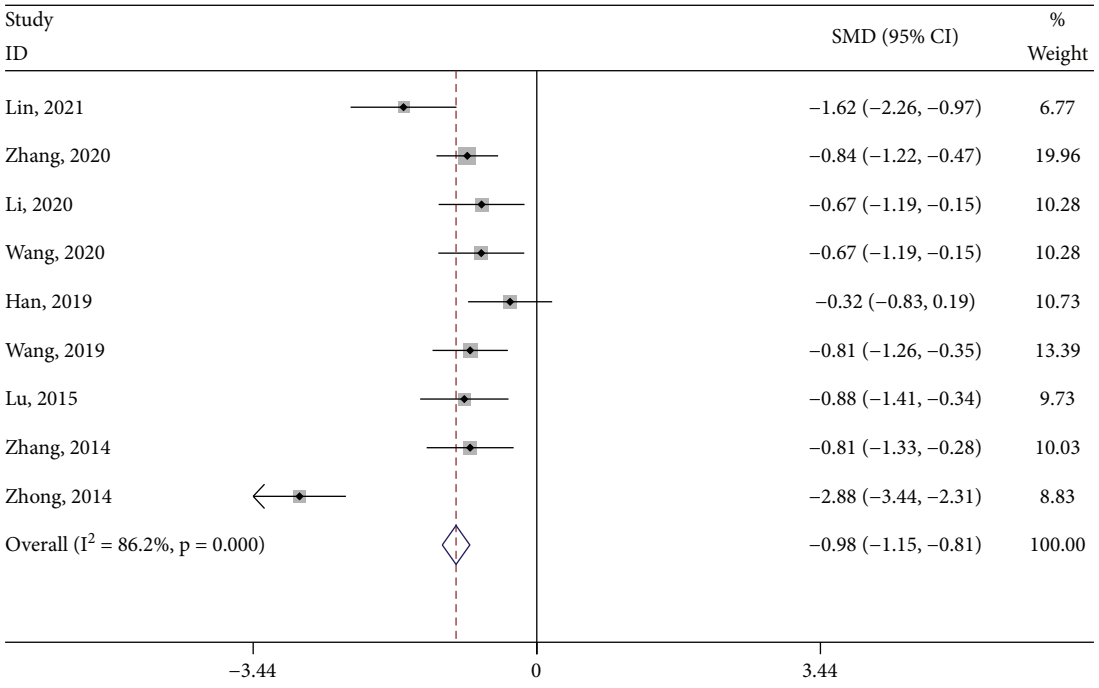

Figure 5: Forest plot of the VAS Score.

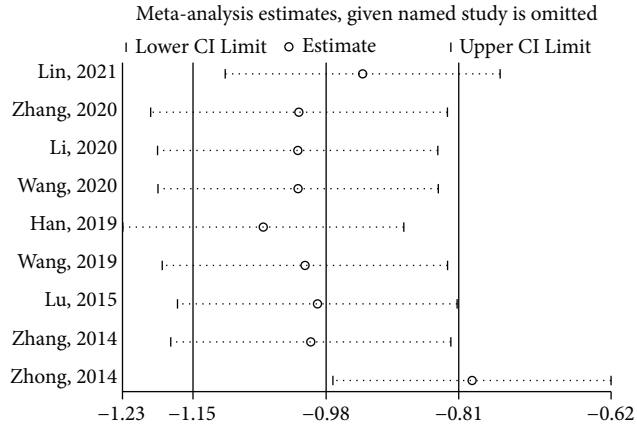

Figure 6: Sensitivity analysis of the VAS Score for each included study in the review.

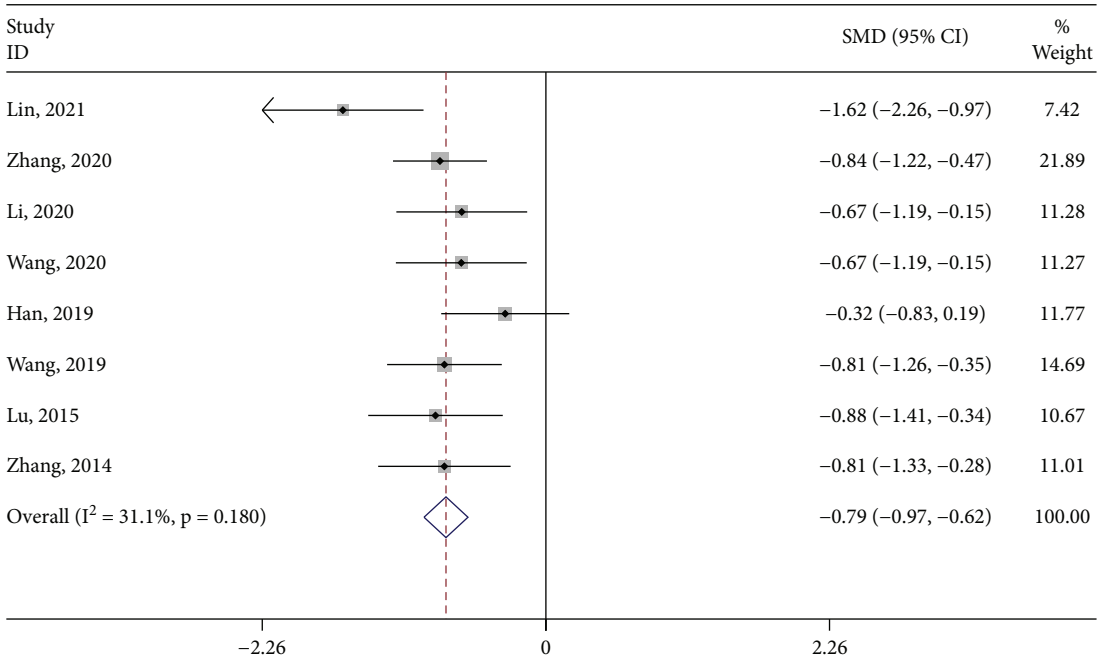

Figure 7: Forest plot of the VAS Score after excluding one trail.

usually begins after the ovulatory cycle is established during puberty. The associated pain is caused by the excessive release of prostaglandins (PGs) during the shedding of the endometrium. High levels of PGs during menstruation cause excessive contraction of the uterine smooth muscle, leading to hypoxia and ischemia, producing a painful sensation. NSAIDs can be used as a first-line treatment for PD; it is uncertain that NSAIDs are the best clinical choice for the treatment of PD [37]. These drugs reduce uterine tone and contractility by blocking cyclooxygenase to reduce prostaglandin synthesis, thereby relieving prostaglandin-induced spastic contractions of the uterus and bringing relief from dysmenorrhea. However, long-term administration can cause serious gastrointestinal, cardiovascular, skeletal, and renal adverse effects [38]. Therefore, it is important to seek new therapies with few adverse effects and low side effects for the treatment of PD. Some studies have reported that HSM is expected to alleviate the clinical symptoms. Therefore, this study aimed to provide as much evidence as possible for the feasibility of HSM in the treatment of PD through a meta-analysis.

HSM as one of the suspension therapies [12] is a new therapy to improve the efficacy of moxibustion, which uses the moxa heat generated by burning moxa velvet to apply moxibustion to heat-sensitive acupuncture points, stimulating the body's acupuncture points to produce moxibustion sensation. When moxibustion is applied to heat-sensitive acupoints, patients experience unusual feelings (referred to as "heat-sensitive sensations"), including penetrating heat, expanding heat, heat transfer, distant heat, deep heat, and other feelings unrelated to heat (such as soreness, bloating, and numbness) [39]. The heat-sensitive

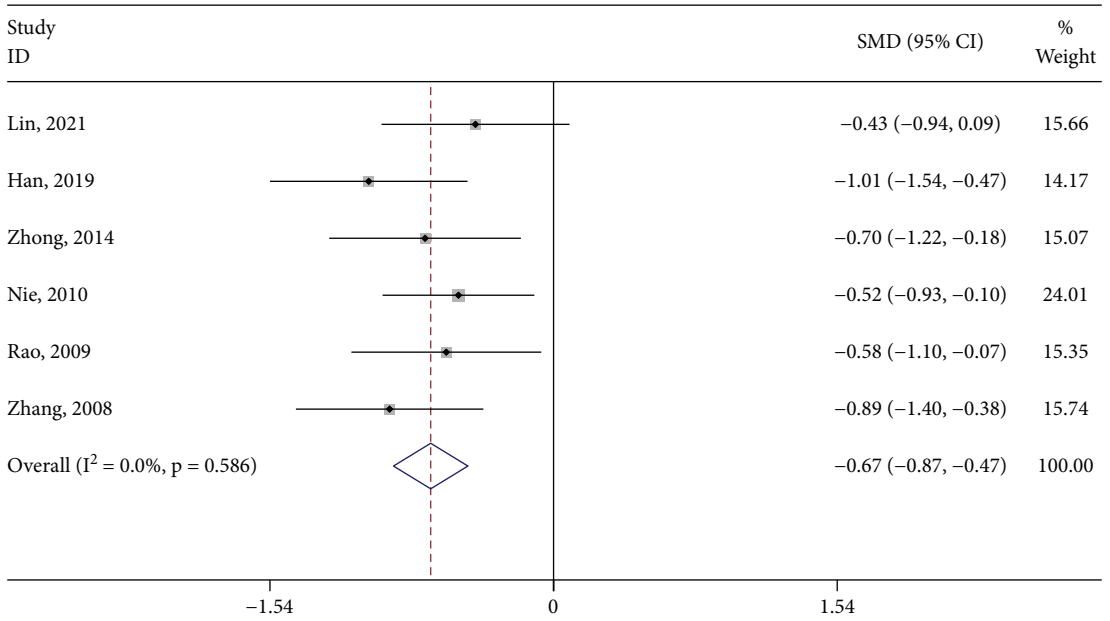

Figure 8: Forest plot of the symptom score.

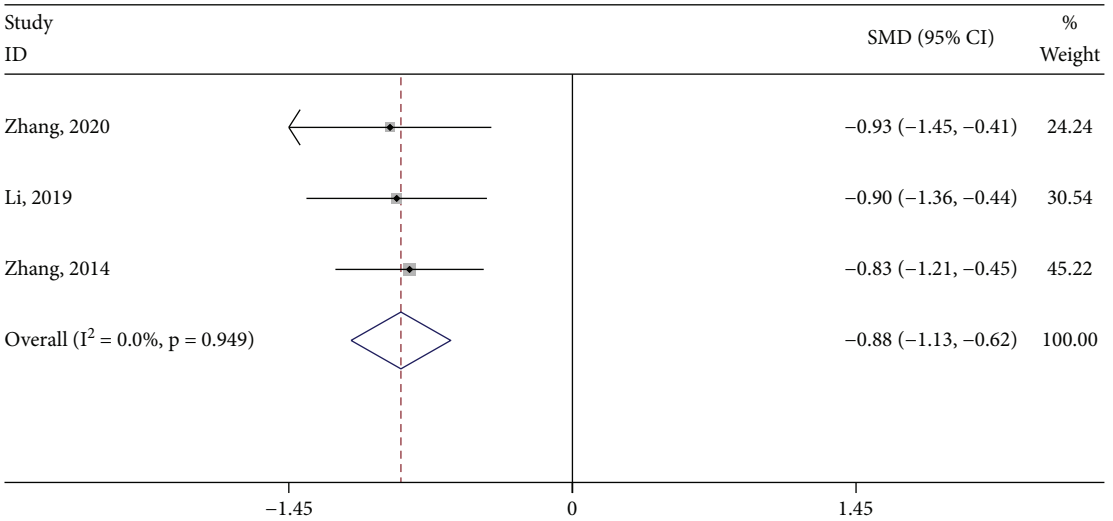

Figure 9: Forest plot of the CMSS score.

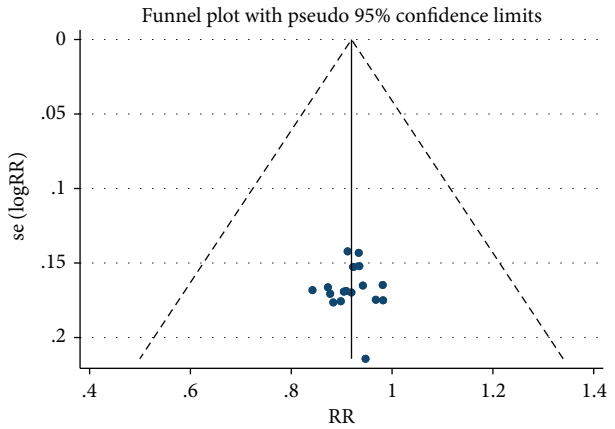

Figure 10: Funnel plot of the total effective rate.

phenomenon of moxibustion is one of the manifestations in the activated functional activities of meridian of Chinese medicine just like the arrival of the qi caused by acupuncture stimulation. It is also a sign of activation of the human endogenous functional regulative system [40]. It is a kind of external therapy for internal disorders, directly acting on the pathogenesis, strengthening the antipathogenic qi [41]. The current clinical and experimental research indicate that moxibustion improves the body's immunity and effectively inhibits inflammatory responses [42]. In addition, a study [10] on the mechanism of moxibustion found that moxibustion plays a therapeutic role through its four mechanisms of action: heat, light, moxa smoke, and drug effects. The mechanism of moxibustion treatment for primary dysmenorrhea focuses on adjusting endocrine hormones, regulating immune function and neuro-related factors, and improving uterine microcirculation. Another study [43] concluded that it can also diffuse inhibitory substances in the cerebral cortex, reduce the excitability of the nervous system, and exert sedative and analgesic effects. Compared to oral medications such as NSAIDs and contraceptives, HSM as an external treatment is safer and cheaper, as mugwort is a very common and inexpensive herbal remedy. In conclusion, compared to other treatments, heat-sensitive moxibustion has obvious advantages in terms of efficacy, safety, patient acceptance, and low treatment cost.

The results of this meta-analysis showed that the overall efficiency of HSM for PD was better than the control groups, and HSM was also better than other therapies in terms of improving the CMSS scores, VAS scores, and symptom scores. Six studies [19, 26, 28, 30, 32, 35] suggest that HSM is more effective than traditional moxibustion, four studies [18, 20, 27, 29] suggest that HSM is more effective than oral ibuprofen in the treatment of PD, one study [33] suggests that HSM is more effective than acupuncture combined with massage, and one study [31] suggests that HSM is more effective than ginger moxibustion in the treatment of PD. Combined with the results of subgroup analysis, compared with the control group, HSM had advantages in VAS score, symptom score, and CMSS score. It can be seen that HSM has obvious advantages in the treatment of PD and is worth promoting in the clinics, and clinicians can prefer HSM for PD according to the actual situation. This study included a total of 19 research studies and only 5 had safety analyses that mentioned adverse effects. None of the HSM groups had any adverse effects, which may mean that HSM is safe overall.

There are some limitations in this study. First of all, the 19 RCTs included involved 1325 patients. The overall sample size was not very large. Secondly, the frequency and duration of treatment regimen interventions were not uniform in this study, which affected the accuracy of the results to some extent. Besides, the included studies were all observed for immediate efficacy, and the long-term efficacy of HSM on PD needs to be studied in a progressive manner.

5. Conclusions

The findings of this study suggest that heat-sensitive moxibustion was an effective intervention for reducing the VAS score and CMSS score, reducing the dysmenorrhea symptom score, and improving the total effective rate. HSM avoids the disadvantages of systemic medication, which finds it difficult to reduce the disease, and is simple and easy to implement, which is worth further clinical exploration and promotion. This study provides a reference for clinicians in the treatment of primary dysmenorrhea in order to provide data support for the future widespread use of HSM as a community or family self-treatment modality. However, due to the possibility that the randomization bias was high, it is necessary to use a large sample size, multicenter, low bias risk clinical research and basic medical research in the future based on strict control of the research design.

Data Availability

All available data are included in this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Subgroup analysis of the total effective rate was performed on Supplemental Figure 1. Subgroup analysis of the VAS score was performed on Supplemental Figure 2. Subgroup analysis of the symptom score was performed on Supplemental Figure 3. Subgroup analysis of the CMSS score was performed on Supplemental Figure 4. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7705194, 15 pages
https://doi.org/10.1155/2022/7705194

Research Article

He's Yangchao Recipe Ameliorates Ovarian Oxidative Stress of Aging Mice under Consecutive Superovulation Involving JNK- And P53-Related Mechanism

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Received 14 March 2022; Accepted 21 May 2022; Published 8 July 2022

Academic Editor: Yong Wang

Objective. To evaluate the effects of He's Yangchao Recipe (HSYC) on ameliorating ovarian oxidative stress of aging mice under consecutive superovulation. *Methods*. An 8-month-old C57BL/6 female mouse was chosen to establish an aging model under ovarian hyperstimulation. Mice were randomly separated into four groups: *R*1 as the control group, *R*4 as the model group, NR4 with N-acetyl-L-cysteine (NAC) administration, and TR4 with HSYC administration. Oocyte collection, in vitro fertilization, and embryo culture were performed. The serum hormone levels were measured by enzyme-linked immunosorbent assays (ELISA); the reactive oxygen species (ROS) level of oocytes, the number of growing follicles, corpus luteum, ovulated oocytes, and developing embryos at each stage, along with the proportions of fragmented oocytes and abnormal mitochondria in granulosa cells (GCs) and the apoptosis rate of GCs were calculated; the mRNA and protein levels of JNK, P53, BAX were detected by real-time PCR and the Simple Western System. *Results*. HSYC enhanced estradiol, progesterone, and inhibin-B levels and increased growing follicle and corpus luteum and ovulated egg counts compared to the *R*4 group ($P < 0.05$), whereas it decreased the proportions of fragmented oocytes ($P < 0.01$); Meanwhile, embryos from mice subjected to four superovulation cycles with HSYC treated had a higher hatching potential. The ROS level of oocytes is downregulated by HSYC ($P < 0.01$) and the percentage of abnormal mitochondrial in ovaries of the TR4 group was also significantly declined compared to the R4 group ($P < 0.05$); the most TUNEL-positive cells proportion was detected in the *R*4 group; nevertheless, HSYC effectively attenuated this detrimental effect ($P < 0.05$). The mRNA and protein expressions of JNK and P53 in ovary tissues were reduced in the TR4 group while these genes were upregulated by repeated superovulation ($P < 0.05$). *Conclusions*. HSYC exerted promising effects on promoting the diminished ovarian reserve and decreased oocyte quality induced by both aging and consecutive ovarian superovulation, potentially via the ROS/JNK/p53 pathway.

1. Introduction

Aging, a hotspot arising intense discussion, is considered the main trigger that causes gradual depletion of ovarian reserve and a reduced ability to produce oocytes competent for fertilization in females. Altogether with a progressive reduction of the follicles, woman aging also involves a compromised competence of the embryos [1]. A large retrospective study showed that females over the age of 42 had a live-born baby rate of only 1%, but those under 35 had a rate of 26% [2]. The aging phenomena can be attributed to increased reactive oxygen species (ROS) levels and cumulative oxidative stress in somatic cells [3, 4]. Moreover, mitochondria are the most remarkable targets of ROS, and the dysfunction of mitochondria would induce nondisjunction of the chromosomes, gestation failure, and decreased embryonic viability [5, 6].

Procedures for superovulation and assisted reproductive technologies (ART) are highly successfully and widely used

as clinical approaches to treating couples with infertility issues. Though repeated superovulation is considered safe, previous studies have demonstrated that it has some detrimental effects on female reproduction. Several studies showed that ovarian hyperstimulation can lead to pregnancy loss and delayed puberty in female offspring, decreased serum hormone levels, impaired mitochondrial function in ovarian cells, and reduced the oocyte and embryo quality [7–10].

Nowadays, the number of elderly women seeking ART help is gradually increasing. One research studying the intrinsic fertility of the human oocyte showed that natural cycles have higher intrinsic fertility per oocyte than hyperstimulated cycles [2]. It's also been reported that in *in vitro* fertilization (IVF), maternal age is among the strongest predictors of success [11].

It has been demonstrated that traditional Chinese medicine (TCM) is a promising alternative form of treatment for gynecological endocrinology dysfunctions [12–14], with significant efficacy and reduced side effects through various herbal combinations [15]. He's Yangchao Recipe (HSYC, with China Patent Application number of 201710902472X) is a special herbal prescription that originates from the ancestral experience of He's School Doctrine of Gynecology, which is one of the first studios of inheritance of academic schools of traditional Chinese medicine approved by the National Administration of TCM, and it is also the intangible cultural heritage of Hangzhou [16]. Results from previous studies of our research group have already shown that HSYC effectively increased the anti-Müllerian hormone (AMH) level and the growing follicle counts, as well as reducing serum FSH and improving ovarian reserve in patients with diminished ovarian reserve (DOR) [17, 18]. Animal experiment shows that high-dosage HSYC could notably improve ovarian reserve and alleviate the further development of DOR in mice [19].

To elucidate the potential mechanism of the therapeutic effect of HSYC, an 8-month-old C57BL/6 female mouse (represents humans at ages 38–47 according to The Jackson Laboratory) undergoing consecutive superovulation was chosen as a model to explore the regulatory effect of HSYC on protecting ovarian functions and oocytes/embryos quality after repeated superovulation. Figure 1 shows the workflow of the study. Our results demonstrated that consecutive superovulation can compromise oocyte quality and embryo development competence, increase oxidative stress and granular cell apoptosis along with damaging mitochondrial functions, but these detrimental effects from the oxidative insult were attenuated by HSYC, probably involving ROS/JNK/P53 signaling pathway.

2. Materials and Methods

2.1. He's Yangchao Recipe. The HSYC recipe used in this study was provided by the Department of Pharmacy, Hangzhou Hospital of Traditional Chinese Medicine, and authenticated by Zhejiang University of Traditional Chinese Medicine. After the material herbs of HSYC being soaked for 30 min, HSYC was decocted with distilled water and heated for 1 h after boiling, and the mixture was then filtered. HSYC was concentrated with rotary evaporators, boiled into a thick slurry, and then was sealed and refrigerated at −20°C for later use. HSYC consists of eight traditional Chinese herbs (Table 1) as follows: 10.3% *Paeonia lactiflora* Pall; 15.5% *Cuscuta chinensis* Lam; 15.5% *Cistanche salsa* (C.A.Mey.) Beck; 10.3% *Angelica sinensis* (Oliv.) Diels; 15.5% *Rubus chingii* Hu; 12.4% Pueraria lobata (Willd.) Ohwi; 10.3% *Asparagus cochinchinensis* (Lour.) Merr; and 10.3% *Platycladus orientalis* (Linn.) Franco.

2.2. UPLC-ESI-MS/MS. Mix the samples with vortex after thawing and 1000 ul of them was centrifuged (12000 r/min, 4°C) for 10 min, and then filtered and stored in a sample flask. UPLC-ESI-MS/MS implement conditions are as follows: the column involved in UPLC was Agilent SB-C18 (1.8 μm, 2.1 mm $*$ 100 $*$ mm); pure water and 0.1% formic acid mobile phase was used for solvent *A*, and acetonitrile with 0.1% formic acid for solvent *B*; A gradient program starts with conditions under 95% *A*, and 5% *B* and lasted for 9 min, and subsequently, a linear gradient was programmed to 5% *A*, 95% *B* and lasted for 1 min. The ratio of the *B* phase is reduced to 5% again in 10.00–11.10 min and remained for 14 min, and then the effluent was connected to an ESI-Q TRAP-MS; AB4500 Q TRAP UPLC/MS/MS system was used to acquire triple quadrupole (QQQ) scans; Analyst 1.6.3. software (AB Sciex) was used to analyze the results.

2.3. Animals and Ethical Approval. Sixty 8-month-old female C57BL/6 mice (28–33 g), together with five nine-week-old male mice (18–20 g) for in vitro fertilization, were purchased from Beijing Charles River Laboratory Animal Company (Certificate No. SCXK 2019-0009, Beijing, China). Mice were housed in a barrier facility at the Animal Experimental Research Center of Zhejiang Chinese Medical University with constant temperatures, humidity, and daylight (12 hours light/12 hours darkness). All of the experimental procedures were supervised by the Institutional Animal Care and Use Committee of the Zhejiang Chinese Medical University (Approval number: IACUC-20201123-01), and the research was conducted in accordance with ARRIVE guidelines [20].

2.4. Repeated Superovulation Procedures. The 8-month-old female mice were randomly separated into four groups. R4 mice were the ones that underwent four superovulatory cycles. In addition, R4 mice taking N-acetyl-L-cysteine (NAC) (Sigma-Aldrich, St. Louis, USA) were termed as NR4 mice, and the remained ones taking traditional Chinese herbal prescription HSYC were termed as TR4 mice. The selection for dosage of NAC and HSYC was based on the dosage used in clinical trials and conversion of human to mouse dose. NAC dosage was determined to be 870 mg/kg [21] and 29.1 g/kg for HSYC [19] via intragastric administration daily. A dose of 10 IU of pregnant mare serum gonadotropin (PMSG; Nanjing

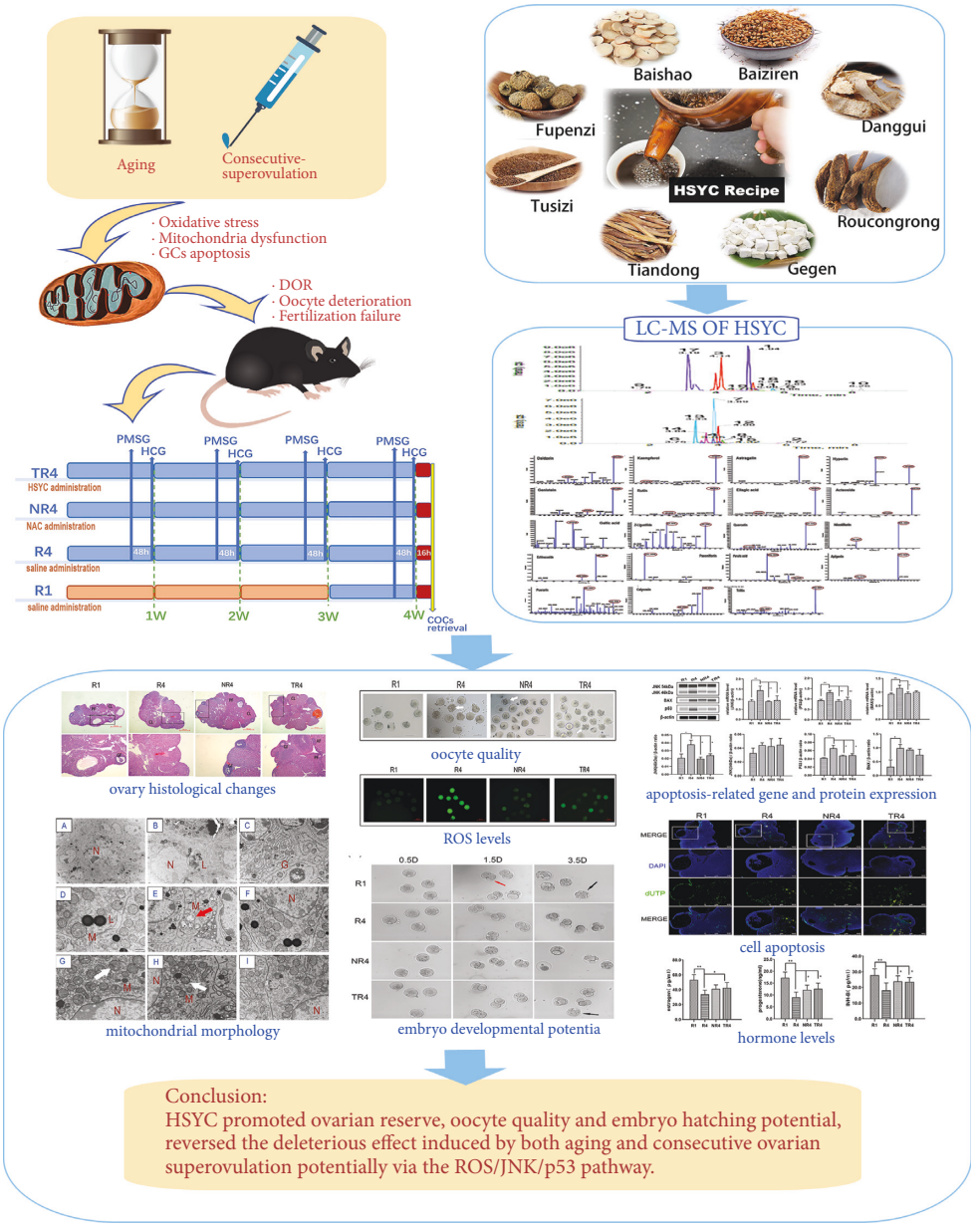

Figure 1: A workflow of this study.

Table 1: Composition of HSYC.

Chinese name	English name	Family	Plant part	Crude herb (g)
Baishao	*Paeonia lactiflora* pall	Paeoniaceae	Root	10
Tusizi	*Cuscuta chinensis* lam	Convolvulaceae	Seeds	15
Roucongrong	*Cistanche salsa* (C.A.Mey.) beck	Orobanchaceae	Stem	15
Danggui	*Angelica sinensis* (Oliv.) diels	Apiaceae	Root	10
Fupenzi	*Rubus chingii* Hu	Rosaceae	Fruit	15
Gegen	*Pueraria lobata* (Willd.) Ohwi	Leguminosae	Root	12
Tiandong	*Asparagus cochinchinensis* (Lour.) Merr.	Asparagaceae	Root	10
Baiziren	*Platycladus orientalis* (Linn.) Franco	Cupressaceae	Seed	10

Aibei Biotechnology Company, Nanjing, China) was administered to the female mice for repeated superovulation, followed 48 h later by 10 IU of human chorionic gonadotropin (HCG; Nanjing Aibei Biotechnology Company, Nanjing, China) every other week for 4 weeks. Mice injected with PMSG only once were set to be *R*1 mice. Figure 2 shows the superovulation injection protocols in the present study.

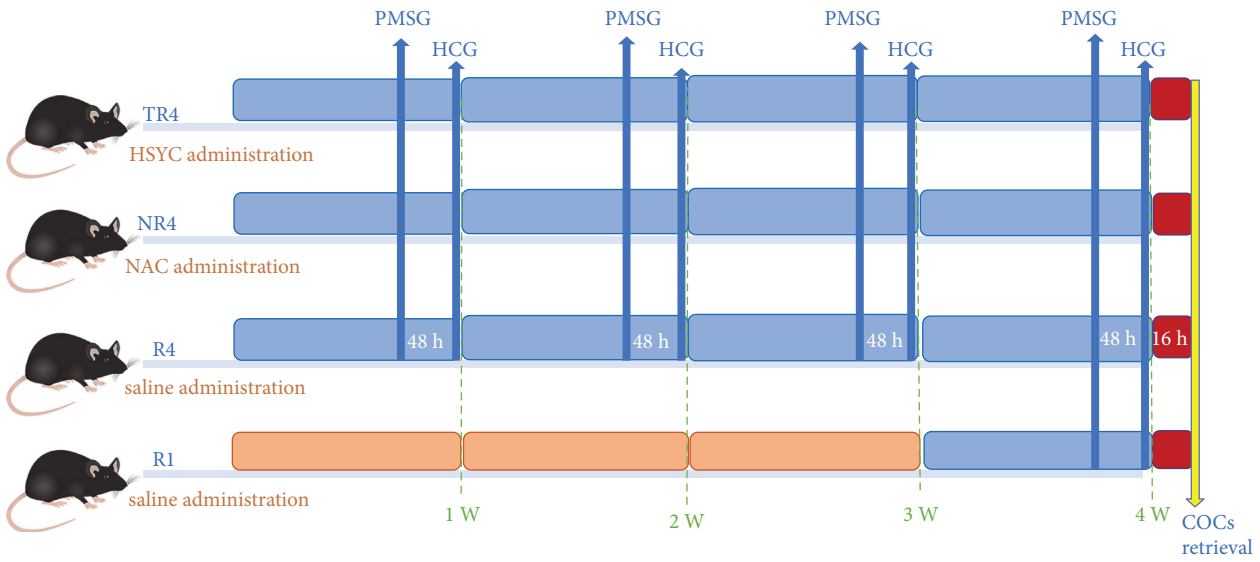

Figure 2: The superovulation treatment protocol in the present study.

2.5. Oocyte Collection, In Vitro Fertilization, and Embryo Culture. After the female mice were sacrificed by CO_2 inhalation, the ampulla of the oviduct was obtained and placed in a test tube containing Hepes buffer, and the bulge of the ampulla of the oviduct was cut through with the needle to allow the cumulus-oocyte complexes (COCs) to slide out. The COCs from 9 mice per group were placed into the prepared Hepes containing hyaluronidase, and the number and morphology of oocytes were observed under the Stereo Microscope after 5–10 minutes of digestion. These oocytes were then collected for the subsequent experiments. The COCs from another 6 mice per group were placed in HTF fertilized drop in an incubator. The eggs were picked up and washed after 6 hours, moved to the M2 medium with mineral oil, and put into an incubator containing 5% CO_2, whose temperature was set to 37°C, and continue to culture. Observe and record the condition of embryos at 0.5 d, 1.5 d, and 3.5 d, respectively. In addition, ovaries were collected in cryopreservation tubes, placed into liquid nitrogen, and then transferred to −80°C for future analysis.

2.6. ROS Assessment. Intracellular ROS levels were evaluated by the dichlorodihydrofluorescein diacetate (DHFC-DA) probe method. Oocytes were incubated with 10 mM DHFC-DA (Beyotime Biotechnology, Shanghai, China) in PBS for 20 minutes at 37°C in a dark interior. Images were captured by an inverted fluorescent microscope (Zeiss Axio Observer.A1).

2.7. Enzyme-Linked Immunosorbent Assay (ELISA). The levels of serum estrogen, progesterone, and INH-B were detected using the ELISA kits (Vankew, Shanghai, China), as instructed by the manufacturer.

2.8. Histopathology and Electron Microscopy. The ovary tissues were fixed in 4% paraformaldehyde after mice were sacrificed and then went through paraffin embedding and serial sections. 4 μm thick ovary sections were stained with hematoxylin and eosin (*H* & *E*) on a glass slide. Images of the ovaries' structure and follicles counts were taken by microscope (Motic AE2000). Immediately after dissection, the ovary tissue was fixed with glutaraldehyde and osmic acid, dehydrated, and transferred to a resin mixture. 100 nm thick ovary sections were stained with uranyl acetate and lead citrate. Images were photographed by transmission electron microscope (HITACHI-H7650).

2.9. TUNEL Staining of Ovarian Cells. Apoptosis of ovarian granulosa cells was observed by a One-Step TUNEL Apoptosis Assay Kit (Beyotime Biotechnology, Shanghai, China). The paraffin sections of ovaries were digested with proteinase K for 30 min and then incubated in a TUNEL test reagent composed of enzyme terminal deoxynucleotidyl transferase (TdT) and fluorescein-dUTP for 1 h in a dark interior at 37°C, and mounted in fluorescence decay-resistant medium containing DAPI. The digital images were recorded using a fluorescence microscope (Leica DM2500B).

2.10. Protein Expression Analysis. To extract the total protein from the mice's ovarian tissues, we used a total protein extraction reagent (KeyGen Biotech, Jiangsu, China) according to the manufacturer's protocol. The protein concentration was assayed using the BCA Protein Assay kit (KeyGen Biotech, Jiangsu, China). The Simple Western (WesProteinSimple, San Jose, CA, USA) system was used for protein quantification (JNK, Bax, and p53). The antibodies used in the above protein determination are all from Cell Signaling Technology (CST, Danvers, MA, USA). Then, the signal intensities were quantified and analyzed using Compass Software (ProteinSimple).

2.11. Real-Time PCR. RNA samples were prepared using TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, Shiga, Japan), and a reverse transcriptase reaction was performed using PrimeScript RT Master Mix (TaKaRa, Shiga, Japan). SYBR Premix Ex TaqTM II (Perfect Real Time) (TaKaRa, Shiga, Japan) was used for the detection of the specified genes. The conditions for the reverse transcription reaction are as follows: 37°C 15 min⟶85°C 5 s⟶4°C. The reverse transcription reaction system was added to tubes used for fluorescence quantitative reaction, and the amplification reaction conditions are as follows: predenaturation: 95°C 3 min; 40 cycles (95°C 10 s, 60°C 30 s); dissolution curve: starting from 55°C, increasing by 0.5°C each 30 s until 95°C, and circulate once. All reaction information is collected by ABI StepOnePlus™ Real-Time PCR System, and normalized expression was calculated as relative fold change using the formula $2^{-\Delta\Delta CT}$. Table 2 shows the primer sequences used in the quantitative real-time PCR experiment, and β-actin was considered as the internal reference gene.

2.12. Statistical Analysis. The experimental data were analyzed by Prism GraphPad software, and all results were indicated by mean ± SD. One-way analysis of variance (ANOVA) was used to analyze differences among the groups. P values < 0.05 were commonly considered to be statistically significant, and the number of asterisks indicates the following levels of statistical significance: $^{**}P < 0.01$, $^{*}P < 0.05$.

3. Results

3.1. The Main Bioactive Components in HSYC by LC-QQQ-MS. LC-MS was implemented to identify the main bioactive components in HSYC, and qualitative analysis was performed to elucidate the components of the HSYC based on secondary spectrum information and the local database MWDB (Metware database). As shown in Table 3, Figures 3 and 4, the main active substances are daidzein, kaempferol, astragalin, hyperin, genistein, rutin, ellagic acid, acteoside, gallic acid, Z-ligustilide, quercetin, nicotiflorin, echinacoside, paeoniflorin, ferulic acid, apigenin, puerarin, calycosin, and trillin.

3.2. HSYC Improves Reproductive Endocrinology Dysfunction in Mice. Superovulation following decreased ovarian function is usually accompanied by decreased levels of estrogen, progesterone, and INH-B, as is shown by the difference between groups $R1$ and $R4$ in Figure 5. However, HSYC regulated hormone levels, with progesterone, estrogen, and INH-B levels increased ($P < 0.05$).

3.3. HSYC Reduces ROS Levels in Oocytes of Repeated Superovulation Mice. We examined oocytes' ROS levels retrieved from the oviduct and observed that R4 oocytes had dramatically higher ROS levels than R1 oocytes ($P < 0.01$), while ROS levels were significantly reduced in HSYC-treated oocytes and NAC-treated oocytes ($P < 0.01$; Figures 6(d) and 6(e)). The above results suggest that consecutive superovulation increased ROS levels while HSYC administration could resist oxidative stress.

Table 2: Primer sequences of the target genes.

Gene	Primer	Sequences(5′ to 3′)
JNK	Forward primer	AGTGACAGTAAAAGCGATGGTC
	Reverse primer	AGCACAAACAATTCCTTGGGC
p53	Forward primer	CCCCTGTCATCTTTTGTCCCT
	Reverse primer	AGCTGGCAGAATAGCTTATTGAG
Bax	Forward primer	TGAAGACAGGGGCCTTTTTG
	Reverse primer	AATTCGCCGGAGACACTCG
β-Actin	Forward primer	CATCCGTAAAGACCTCTATGCCAAC
	Reverse primer	ATGGAGCCACCGATCCACA

3.4. HSYC Increases the Quantity and Quality of Oocytes. We observed the oocytes retrieved from oviducts under the microscope to determine whether HSYC could influence the quantity and quality of oocytes (Figure 6(a)). As shown in Figure 6(b), fewer eggs per oviduct from superovulated mice were retrieved compared to the $R1$ group ($P < 0.01$), but HSYC could increase the egg counts ($P < 0.01$). We also evaluated the effects of HSYC treatment on oocyte quality in mice after application of HSYC for one month, and the proportions of fragmented oocytes were markedly decreased in HSYC-treated mice and NAC-treated mice compared to the $R4$ group ($P < 0.01$, Figure 6(c)).

3.5. HSYC Treatment Ameliorated the Histological Changes in Ovaries. We observed pathologic changes in the ovary to elucidate the mechanism by which the number of ovulated oocytes increased by HSYC after superovulation. Contrary to the $R4$ group, significantly increased numbers of developing follicles were observed in the HSYC group as well as the total follicles (Figure 7(d)). While the number of the corpus luteum increased in all three groups after the fourth cycle of ovulation compared to the $R1$ mice, it showed a more significant incline in the TR4 group ($P < 0.01$) than in the NR4 group ($P < 0.05$) (Figure 7(b)). Hemorrhagic corpus luteum, characterized by corpus luteum infiltrated with erythrocyte, was occasionally observed in ovaries in the present study, presumably indicating recent ovulation (Figure 7(a)). However, there was no difference in the percentage of Hemorrhagic CLs in the prevalence of this phenotype among the four groups (Figure 7(c)). Administration of HSYC had a considerable effect on the histological changes in the ovary during repeated ovulation.

Table 3: The RT, MW, MS-MS fragment ions, DP and CE of HSYC.

Component	Classification	Precursor ion (m/z)	Product ion (m/z)	MW (Da)	Ionization model	DP (V)	CE (eV)	RT (min)
Daidzein	Isoflavones	255.07	137.00	254.06	[M + H]+	40	40	4.94
Kaempferol	Flavonols	285.04	151.00	286.05	[M − H]−	-40	-30	5.72
Astragalin	Flavonols	449.11	287.06	448.10	[M + H]+	50	30	4.14
Hyperin	Flavonols	463.09	300.00	464.10	[M − H]−	-60	-40	3.88
Genistein	Isoflavones	271.06	215.07	270.05	[M + H]+	20	40	5.65
Rutin	Flavonols	609.15	301.00	610.15	[M − H]−	-40	-40	3.75
Ellagic acid	Tannin	301.00	185.02	302.01	[M − H]−	-60	-40	3.89
Acteoside	Phenolic acids	623.20	461.17	624.21	[M − H]−	-50	-30	4.03
Gallic acid	Tannin	171.03	107.01	170.02	[M + H]+	50	30	1.79
Z-Ligustilide	Others	191.11	117.07	190.10	[M + H]+	20	20	8.26
Quercetin	Flavonols	303.05	137.02	302.04	[M + H]+	50	50	5.08
Nicotiflorin	Flavonoid	593.15	285.04	594.16	[M − H]−	-50	-30	4.00
Echinacoside	Phenolic acids	785.25	623.20	786.26	[M − H]−	-50	-50	3.33
Paeoniflorin	Monoterpenoids	479.16	121.03	480.16	[M − H]−	-50	-30	3.64
Ferulic acid	Phenolic acids	193.05	134.01	194.06	[M − H]−	-20	-20	4.12
Apigenin	Flavonoid	271.06	153.01	270.05	[M + H]+	50	30	5.63
Puerarin	Isoflavones	417.12	297.07	416.11	[M + H]+	50	30	3.19
Calycosin	Flavonoid	285.08	225.06	284.07	[M + H]+	50	30	5.24
Trillin	Steroidalsaponins	577.37	271.21	576.36	[M + H]+	50	30	4.75

MW: molecular weight, DP: declustering potential, CE: collision energy, and RT: retention time.

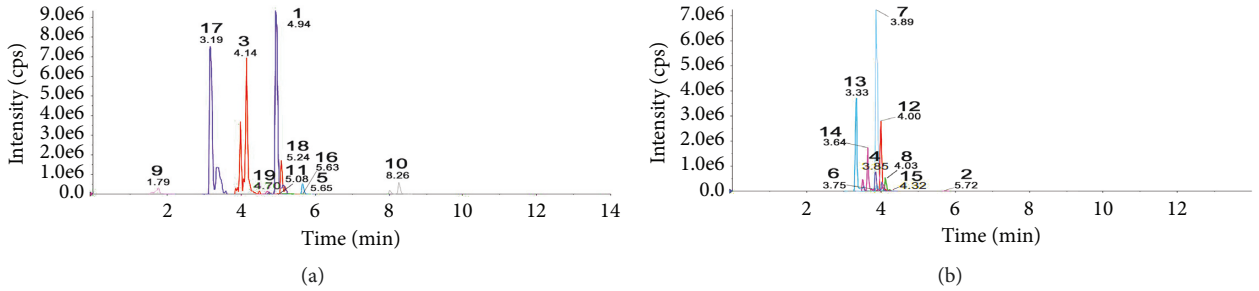

Figure 3: MRM chromatograms in (a) negative and (b) positive modes. Identification: 1, daidzein; 2, kaempferol; 3, astragalin; 4, hyperin; 5, genistein; 6, rutin; 7, ellagic acid; 8, acteoside; 9, gallic acid; 10, Z-ligustilide; 11, quercetin; 12, nicotiflorin; 13, echinacoside; 14, paeoniflorin; 15, ferulic acid; 16, apigenin; 17, puerarin; 18, calycosin and 19, trillin.

3.6. HSYC Improved the Mitochondrial Morphology in Ovary. We use electron microscopy to view ultrastructural changes in the ovaries (Figures 8(a)–8(i)). What we observed is that there were marked differences among four groups in mitochondrial morphology. In the *R*1 group, most of the mitochondria were normal, the structures of which were regular, round, oval, or rod-shaped, with clear and complete crista, nevertheless mitochondria of granulosa cells in ovaries suffering from superovulation showed abnormal and damaged structures, being swollen and ruptured with aggregation, extrusion, and fusion, with cristae being vacuolated and barely visible. The percentage of abnormal mitochondrial (mitochondria with vague cristae and vacuolated mitochondria) of the TR4 group is significantly declined than the *R*4 group ($P < 0.05$, Figures 8(j) and 8(k)).

3.7. A Positive Effect of HSYC on Reducing Cell Apoptosis. The percentage of apoptotic granulosa cells in the antral follicles of ovaries after consecutive superovulation was estimated in the four groups (Figure 9(a)). TUNEL-positive cells occurred more in the *R*4 group than in the *R*1 group, suggesting the aggravation of apoptosis due to ovarian hyperstimulation, whereas HSYC significantly decreased the percentage of TUNEL-positive cells ($P < 0.05$, Figure 9(b)). HSYC showed a positive effect on the reduction of ovarian cell apoptosis of HSYC.

3.8. HSYC Inhibited JNK and p53 mRNA Expression. The JNK and p53 mRNA increased significantly in the *R*4 group versus that in the *R*1 group, while HSYC could reduce the JNK and p53 mRNA expression, as well as NAC, did ($P < 0.05$, Figures 10(b) and 10(c)). HSYC did not show a capacity in affecting the BAX mRNA expression while NAC did ($P > 0.05$, Figure 10(d)).

3.9. HSYC Inhibited JNK and p53 Protein Expression. Consecutive superovulation in the *R*4 model group significantly increased JNK (46 kDa) and p53 expression in the ovary compared to the *R*1 group. However, HSYC significantly decreased JNK (46 kDa) and p53 protein expression compared with the *R*4 group, as presented in

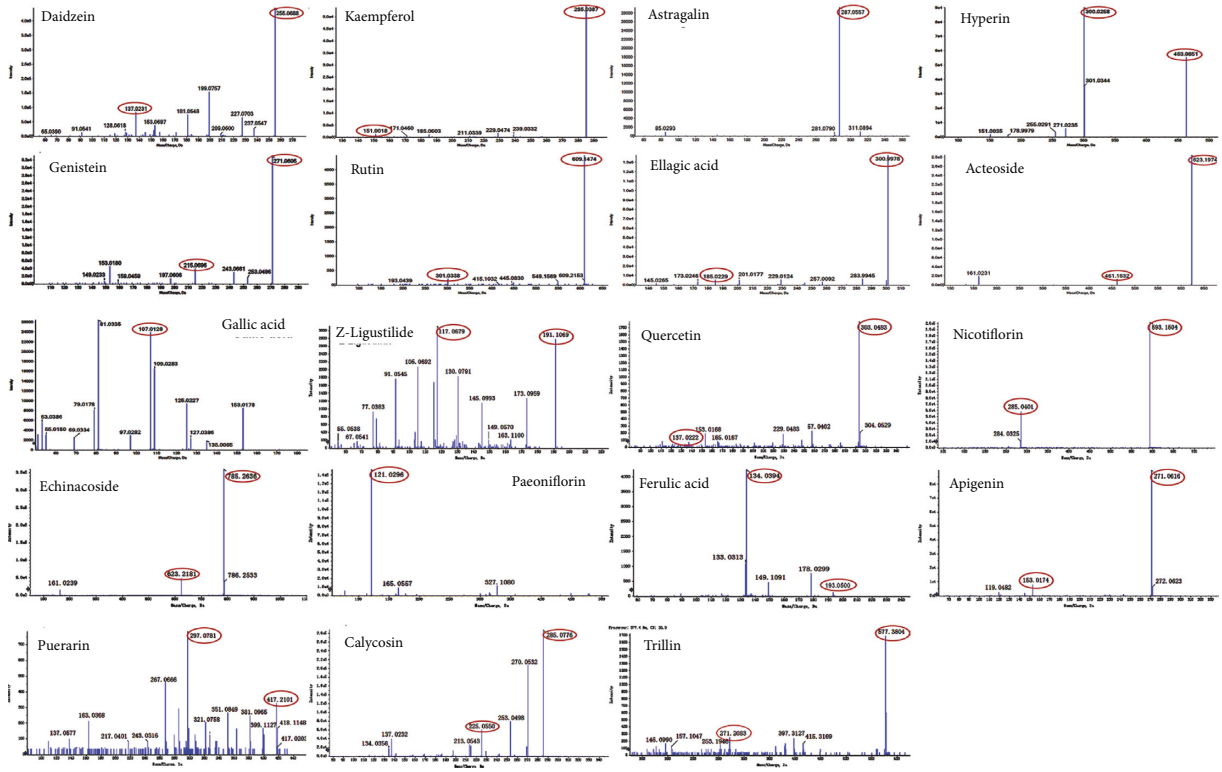

Figure 4: The product ion scan spectra of 19 bioactive compounds in HSYC.

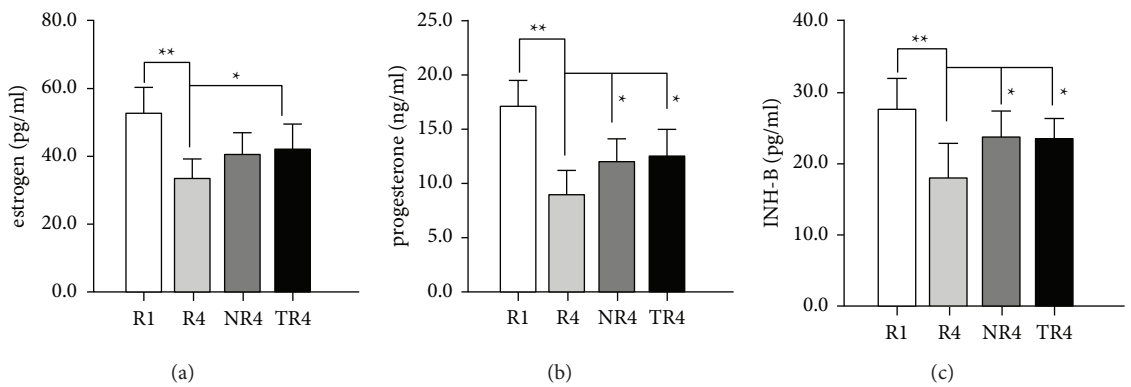

Figure 5: The effect of HSYC on improving reproductive endocrinology dysfunction. (a–c) The serum content of estrogen, progesterone, and INH-B level of mice in different groups, respectively.

Figures 10(f) and 10(g) ($P < 0.05$). The results above measured by WES are consistent with the mRNA expression measured by RT-PCR. Nevertheless, the JNK (54 kDa) and BAX protein expressions did not show significant statistical differences among the four groups (Figures 10(e) and 10(h)). Representative band images of the target proteins are shown in Figure 10(a).

3.10. HSYC Improved on Developmental Potential of Embryos. As shown in Figure 11, repeated superovulation resulted in a significant decline in embryos quantity obtained after four superovulation cycles, and the number of zygotes obtained from mice COCs after in vitro fertilization was markedly higher in TR4 than that in the *R*4 group ($P < 0.05$), whereas NR4 group has a more significant higher zygote count than in the *R*4 group ($P < 0.01$). The number of 2-cell stage embryos retrieved from the TR4 mice is as well higher than that in the *R*4 group ($P < 0.05$). The difference between the morula counts of the TR4 group and the *R*4 group showed statistical significance ($P < 0.05$). Embryos from mice subjected to four superovulation cycles with HSYC treated had a higher hatching potential compared with all other groups without HSYC administration.

4. Discussion

The aging phenomena can be attributed to increased ROS levels and accumulated oxidative damage in somatic cells

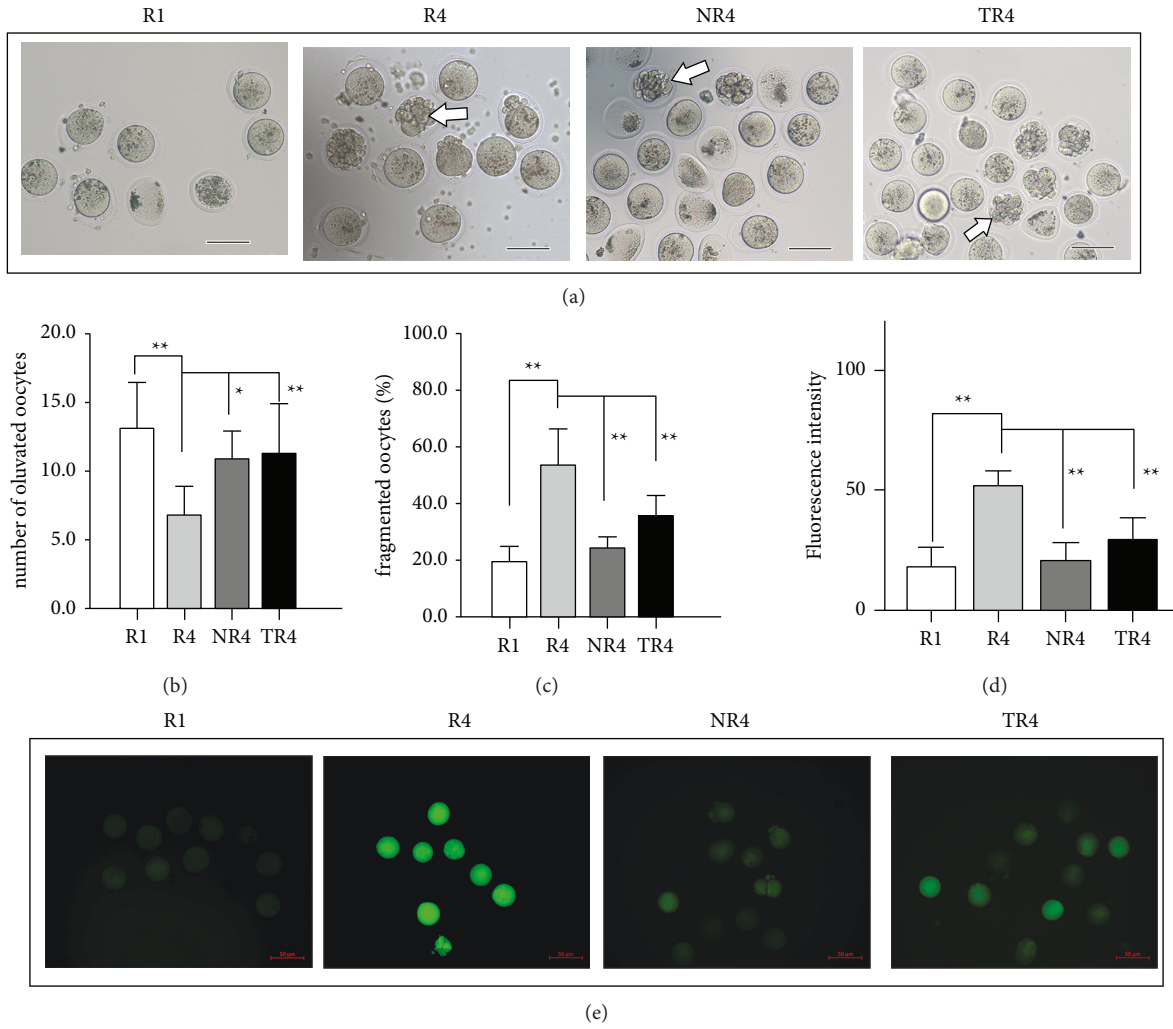

Figure 6: Effect on the quantity and quality of oocytes ovulated into the oviducts of HSYC (a–c) and different ROS levels in oocytes from mice undergoing repeated superovulation with or without intervention (d, e). (a) Representative images of morphologically normal and fragmented oocytes. White arrow: oocytes with obvious cytoplasmic fragments. (e) Representative fluorescence images of ROS; scale bars are 50 μm. (b) Graph showing the proportion of morphologically abnormal and fragmented oocytes in different groups. (c) Graph showing the average number of oocytes ovulated per mouse ($n = 9$ per group, 18 oviducts). (d) Quantitative fluorescence intensities of ROS staining in oocytes from the *R*1 ($n = 32$), *R*4 ($n = 36$), NR4 ($n = 38$), and TR4 ($n = 42$) groups, and n denotes the number of oocytes for each group. ROS: reactive oxygen species.

and the affection of oxidative stress to oocytes has been well-demonstrated [5, 22, 23]. ROS are not only constantly generated but also eliminated in the mitochondria thus maintaining redox balance and homeostasis, and when the redox balance is broken due to aging, ROS are accumulated [24]. Mitochondria, which is crucial for controlling cell survival and death, is the most remarkable target of ROS. The accumulation of spontaneous mitochondria damage was due to increased ROS in oocytes, and mitochondria dysfunction would induce chromosomal nondisjunction, pregnancy loss, or decreased embryo viability [5, 6].

Previous studies have suggested that consecutive superovulation can harm fertility and fecundity in mice, whose AMH expressions, along with the concentrations of estrogen and progesterone significantly decreased [25]; Results of recent studies also suggest that multiple superovulations affect mitochondrial function in cumulus cells, inducing apoptosis and mitochondrial DNA (mtDNA) damage as well as altering histone modifications in early embryos, so as to decrease ovarian functions, reduce the oocyte and embryo quality and delay embryonic development [8–10]. Increased cytoplasmic fragmentation, abnormal mitochondrial distribution, and spindle damage were also observed in oocytes ovulated from mice that underwent superovulation [26]. And to be more precise, it seems that repeated superovulation induces strong oxidative stress and damage to all reproductive organs of female mice, which results in subsequent negative effects mentioned above [27, 28]. Women of late childbearing age ovulate fewer eggs beyond a loss of ovarian reserve [29]. Meanwhile, there are often difficulties for these women to respond to ovarian stimulation when undergoing IVF [30],

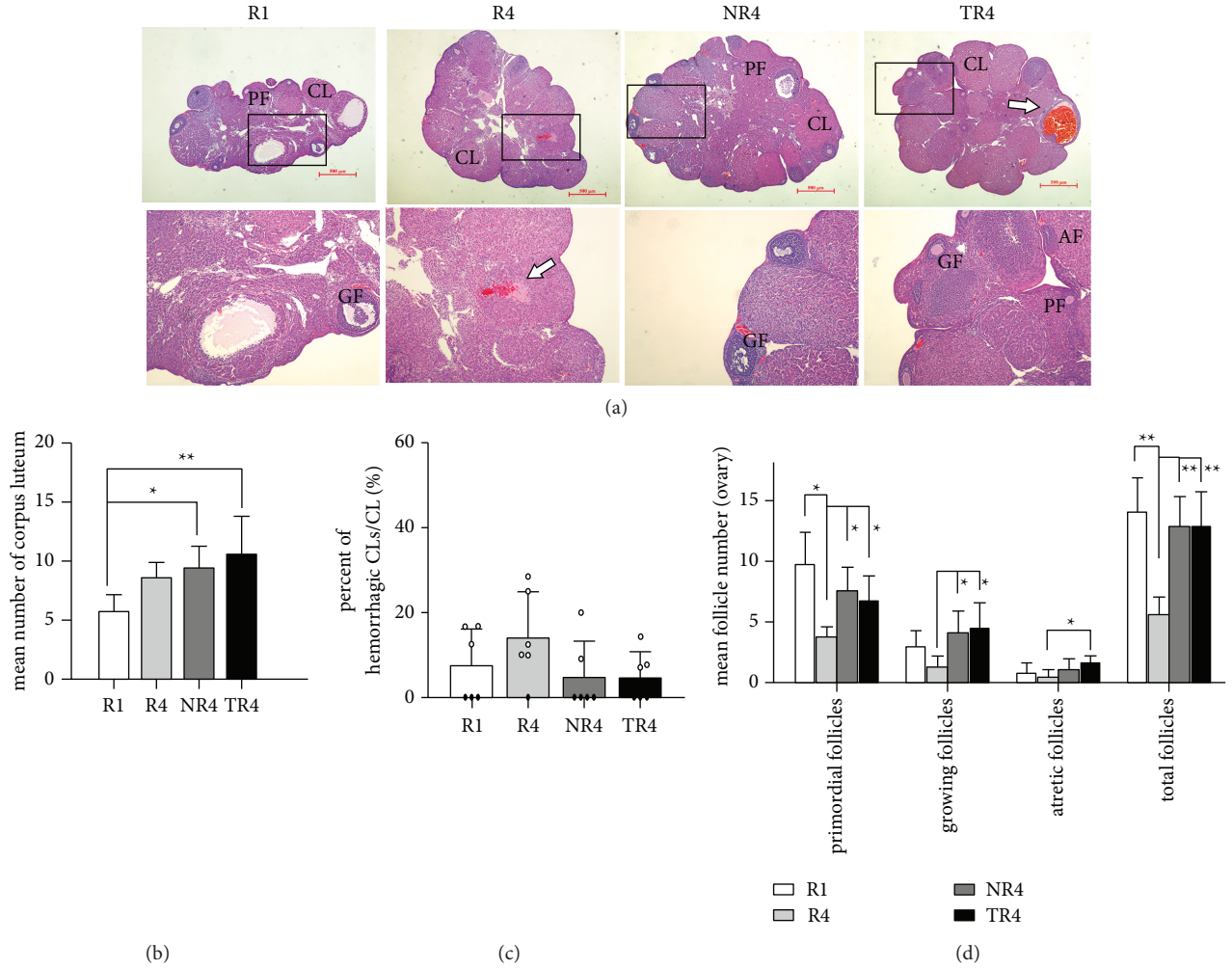

Figure 7: Effect on follicles after 28 days of HSYC administration. (a) Follicles observed after *H* & *E* staining. PF: primary follicles; GF: growing follicles; AF: atretic follicles.CL: corpus luteum. White arrow: hemorrhagic CLs. Scale bars, 500 μm and 200 μm. (b, c) The number of CLs and the percent of hemorrhagic CLs in each group. (d) The numbers of follicles at different developmental stages of maturation.

and when pregnancy does occur to them, they will have a higher incidence of aneuploid blastocysts and unexplained recurrent abortion [31].

In this present study, we can see that as previously reported, consecutive superovulation in aged mice caused the declined serum estrogen, progesterone, and INH-B expressions, followed by reduced growing follicles, indicating impaired ovarian functions. Aging together with superovulation, contributes to the oxidative stress and ROS accumulation in the ovary, leading to the dysfunction of mitochondria of granulosa cells and the accelerated apoptosis in ovarian cells. Increased oocyte cytoplasmic fragmentation has also been observed. As is demonstrated by some research, the average number of ovulated oocytes of 4-week-old female C57BL/6 mice undergoing superovulation could reach to 42.7, while the mice aged 8 months (30 weeks) could only ovulate 16.2 oocytes [32]. Although the number of follicles after super-stimulation in 8-month-old mice is less than that of mice at their young age, the condition of continuous super-stimulation reduces ovulation simultaneously.

In contrast to the model group without drug intervention, the results of the present study revealed the efficacy of HSYC in improving ovarian function, fertility, and embryonic development. Under oxidative stress, HSYC lessened the apoptotic rate of granulosa cells (GCs) and the ROS Level of oocytes, along with an escalation in serum hormone expressions and growing follicles in ovaries. The normal morphological rate of mitochondria also increased, followed by the enhanced embryonic development potential. The Corpus luteum of the three groups after 4 times superstimulation increased greatly, resulting in an augment in ovarian volume, while the amount of corpus luteum in the TR4 group was significantly larger than that in the *R*4 group, which may be due to the exhausted ovarian reserve after *R*4 group experienced 4 hyperstimulation without in time intervention, and we can draw this conclusion from the different ovulated follicle counts between *R*4 group and TR4 group.

The c-jun N-terminal kinase (JNK) has a necessarily close connection with oxidative damage close [33]. It has been well-demonstrated that ROS are potent inducers of

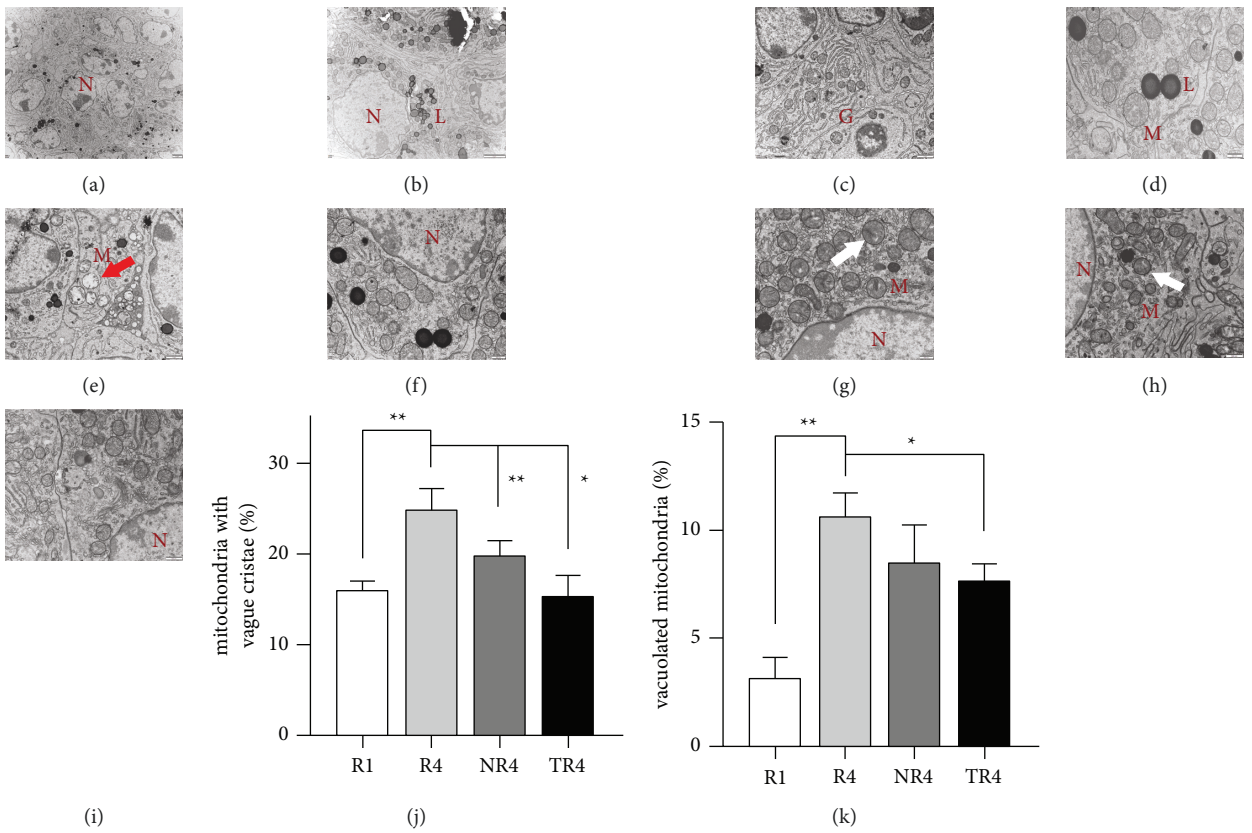

Figure 8: Effect on ultrastructural changes of the ovarian granulosa cells of HSYC. (a–e) Normal ultrastructure in control group mice ovaries. (f–i) Representative images of mitochondria around the granulosa cells in each group, (f) *R*1 group, (g) *R*4 group, (h) NR4 group, and (i) TR4 group. (G: golgi complex; L: lipid; M: mitochondrial; N: nucleus of granulosa cells; white arrow: abnormal mitochondria; red arrow: vacuolated mitochondria). (j, k) The percentage of mitochondria with vague cristae and vacuolated mitochondria in all mitochondria.

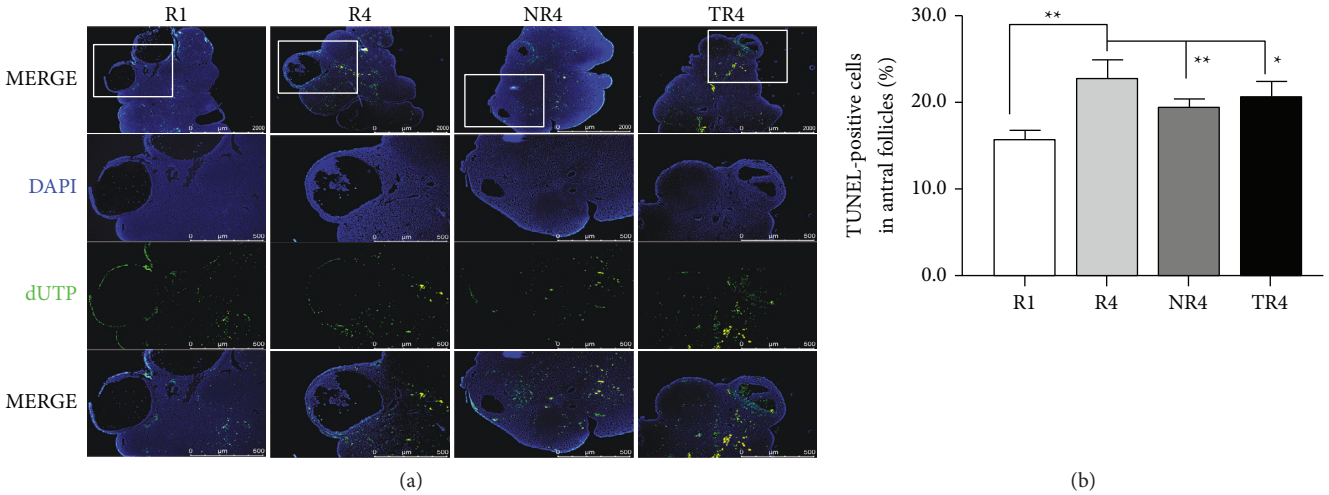

Figure 9: Effect of HSYC on ovarian cell apoptosis. (a) Apoptotic nuclei and total nuclei show green and blue fluorescence respectively. Scale bar: 500 μm. (b) The percentage of TUNEL-positive granulosa cells in the antral follicles.

JNK, and there are some studies suggesting that the proapoptotic effect of JNK activation in ROS-mediated apoptosis is closely related to the mitochondrial pathway and the p53 pathway. As is shown in the present study, the increased JNK and P53 mRNA along with corresponding proteins expression induced by ovarian super-

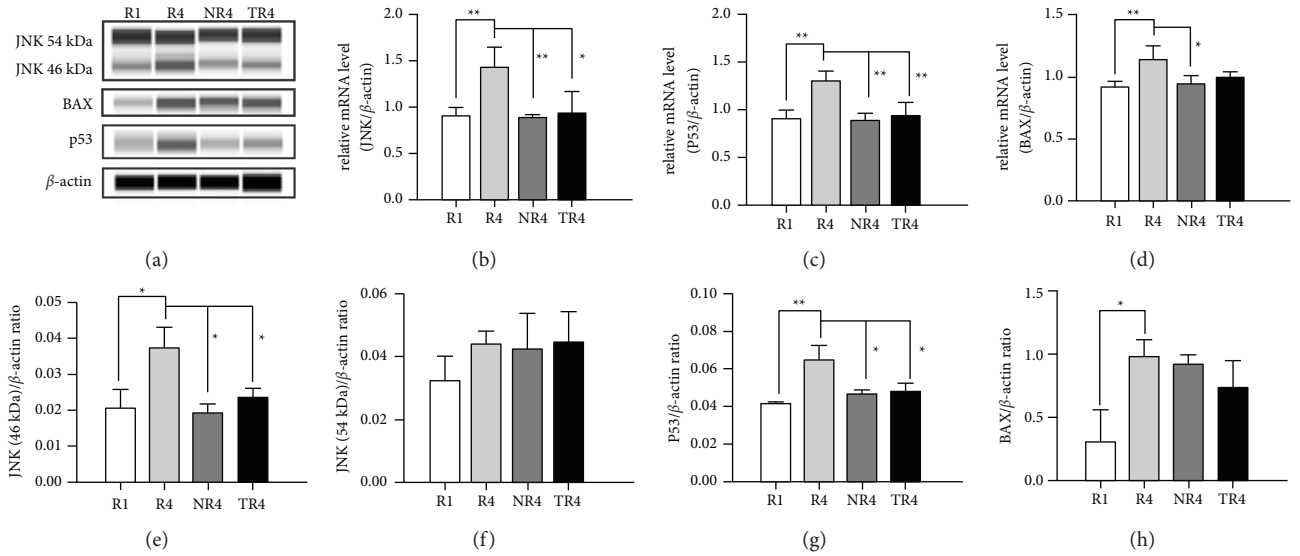

FIGURE 10: The effects of HSYC on superovulation-induced changes of protein JNK, p53, BAX, and mRNA levels of JNK, p53, and BAX. (a) Representative band images of the three proteins, JNK, P53, and BAX. (e–h) Relative protein levels of JNK, P53, and BAX. (b–d) Relative mRNA expression levels of JNK, P53, and BAX. The data represent at least three independent experiments.

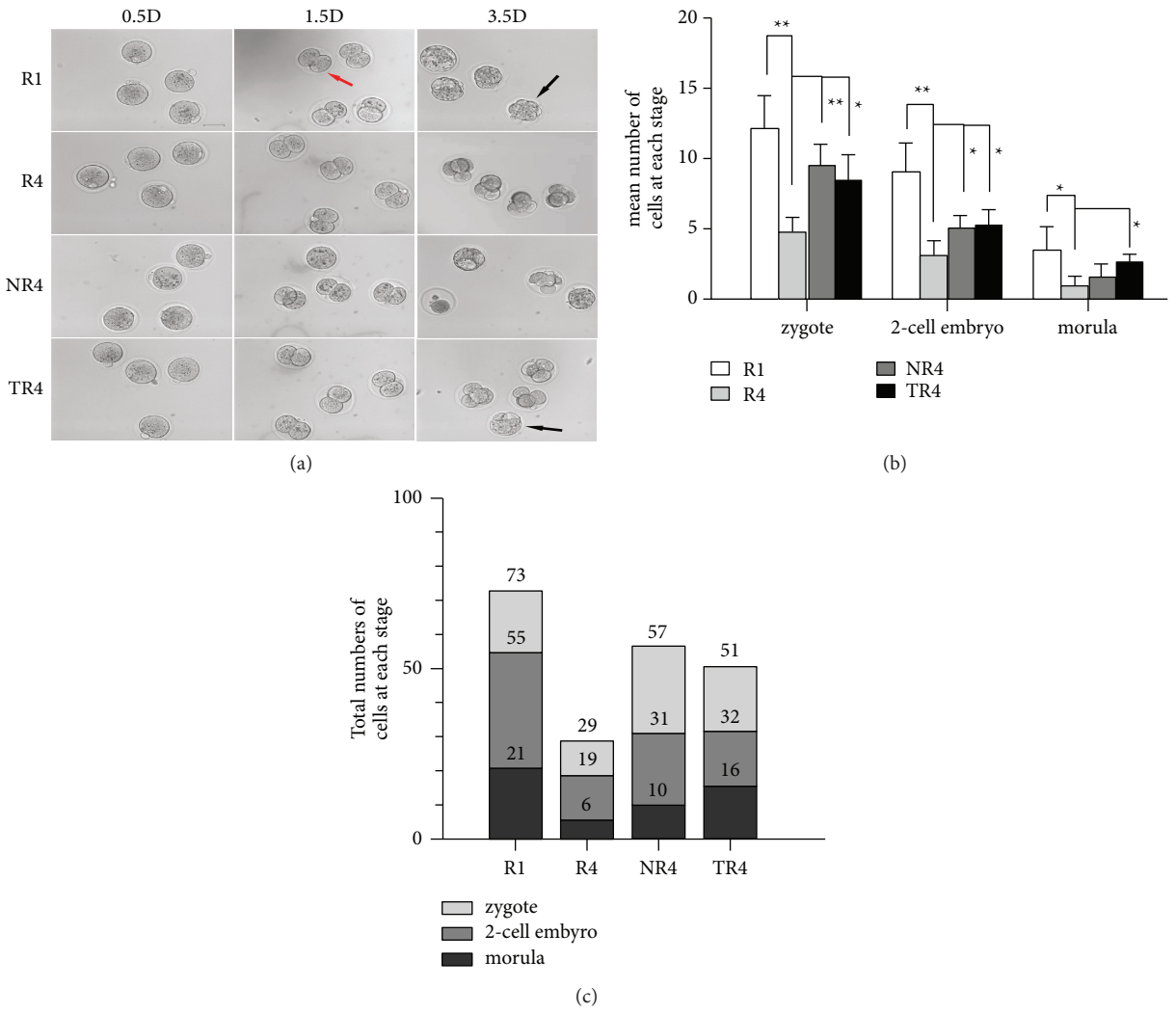

FIGURE 11: Effect on the quality and quantity of embryos of HSYC. (a) Representative images of embryos, were acquired on 0.5 D, 1.5 D, and 3.5 D in vitro embryo culture. The red arrow indicates two-cell stage embryos, and the black arrowhead refers to morula. Scale bars = 50 μm. (b) The total amount and the average number of embryos at different stages in each group.

stimulation and aging are all attenuated by HSYC. Meanwhile, JNK could mediate mitochondrial translocation of the proapoptotic gene BAX and thus activate proapoptotic Bcl-2 family members [34]; nevertheless, the change of BAX expression among *R*4, NR4, and TR4 groups did not show statistical significance in this present experiment.

The main components of HSYC have a long history of application in the historical process of traditional medicine. *Cuscuta chinensis Lam* is used for kidney tonifying and could prevent habitual abortion and improve excessive cold in female reproductive organs [35]. *Pueraria lobata (Willd.) Ohwi* exhibits phytoestrogen-like activities, regulates the endocrine system, and has beneficial effects on menopausal metabolic dysfunction due to the high levels of isoflavones it contains [36]. Danggui, the name of which in English is *Angelica sinensis* (*Oliv*.) *Diels*, is used to invigorate the blood circulation in menstrual disorders [37], while Baishao (*Paeonia lactiflora Pall*) is used to nourish blood and regulate menstruation according to the basic theory of TCM. Danggui–Baishao, which is recognized as an herb pair, is used to nourish blood and can significantly enhance the proliferation of granulosa cells in rat ovaries [38]. And research shows that TCM treatment involving kidney tonifying and blood activating methods has been proven effective in patients with primary ovarian insufficiency (POI) [39].

Bioactive compounds in traditional Chinese medications are rich in antioxidants, mainly including flavonoids, phenols, and polysaccharides. Flavonoids are potent antioxidants protecting plants from unfavorable environmental conditions [40]. Furthermore, HSYC is a great source of flavonoids, which act as antioxidants and regulate ROS homeostasis [41]. The main component are as follows: daidzein, kaempferol, astragalin, hyperin, genistein, rutin, ellagic acid, acteoside, gallic acid, Z-ligustilide, quercetin, nicotiflorin, echinacoside, paeoniflorin, ferulic acid, apigenin, puerarin, calycosin, and trillin.

Daidzein, Astragalin, Hyperin, and Genistein could lift the estrogen and progesterone levels of rats with impaired ovarian function. Moreover, Daidzein could elevate total antioxidant capacity in rats, attenuate ROS-induced toxicity by antioxidant action in ovarian cells [42, 43]; Kaempferol could maintain follicular survival, increase active mitochondria levels, prevent the H_2O_2-induced compromise of mitochondrial membrane potential (MMP) and ROS generation [44, 45]. Astragalin could enhance ovarian reserve and reduce ovarian GCs apoptosis in aged female rats via the Bcl-2 relative pathway [46]. Hyperin could increase proliferation and cell viability in H_2O_2 stimulated GCs, reverse the increased MDA level and decrease SOD, GSH-Px, and CAT, thus frequently used Chinese herbs like *Cuscuta Chinensis* Lam which contain Hyperin could improve ovarian functions through these effects [47, 48]. Genistein has antioxidant activity against radiation-mediated oxidative stress and Cyclophosphamide-induced ovarian toxicity through improving ovarian histology and immunostaining of ovarian iNOS, thus reversing ovarian apoptosis [49, 50], and meanwhile, it could elevate MMP of GCs, followed by a decline in the levels of intracellular mitochondrial superoxide and the apoptotic rate [51]. Ellagic acid could revoke ROS by manipulating oxidative biomarkers within the ovarian cells and exhibits a significant antioxidant capacity in aging and could protect the embryo DNA and development from the oxidative insult [52, 53]. The addition of acteoside during in vitro maturation could improve the rate of blastocyst formation Improve and mitochondrial morphology with decreased ROS level [54, 55]. Acteoside could also attenuate the drop of the MMP in the Chinese hamster ovary cell line (CHO) treated with H_2O_2 [56]. Rutin treatment before cisplatin could reduce apoptosis to preserve the normal follicles, decrease ROS levels, increase GSH levels and enhance mitochondria functions in ovaries. Rutin could also ameliorate the ischemia-reperfusion (I/R)-induced ovarian injury in rats via its possible antioxidative effects [57, 58]. Gallic acid could restrain granulosa cells apoptosis by inhibiting the expression of proapoptotic genes in mouse ovaries [59].

The relative comparative advantage of TCM compared to modern medicines is that TCM stresses compatibility, and the bioactive components in TCM act via multiple targets, while the mode of modern medicines is "one drug for one target" [60]. HSYC can not only resist oxidative stress and improve ovarian function but also regulate hormone levels. From the perspective of TCM theory, HSYC could tonify the liver and kidney, nourish essence and blood, soften the liver and benefit heart Qi, regulating body functions with a holistic concept. While resisting oxidative stress, it also improves symptoms such as insomnia, lassitude, nervousness, hot flashes, and night sweats. Herein, HSYC takes effect through multiple targets and multiple links, highlighting the unique advantages of Chinese traditional medicine.

5. Conclusions

The fertility of aging mice decreases and repeated superovulation could cause oxidative stress to damage ovarian function, and further reduce the number and quality of eggs. The LC-MS results indicate that HSYC contains ample and certain antioxidant bioactive compounds. The present study revealed that the TCM formula, HSYC, has exerted promising effects in promoting ovarian reserve, oocyte quality, and embryo hatching potential, and could reverse the deleterious effect induced by both aging and consecutive ovarian superovulation, potentially via the ROS/JNK/p53 pathway.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ying Zhao and Yun Chen contributed equally to this work.

Acknowledgments

This work was financially supported through grants from the Nature Science Foundation Project of Zhejiang Province (grant number LQ19H270008), Zhejiang Traditional Chinese Medicine Modernization Special Project (grant number 2021ZX012), Zhejiang Basic Public Welfare Research Program (LGF22H27009), and Key Medical Discipline Project of Hangzhou (grant number 2020SJZDXK02).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3854117, 17 pages
https://doi.org/10.1155/2022/3854117

Review Article

Acupuncture for Female Infertility: Discussion on Action Mechanism and Application

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Received 31 January 2022; Accepted 11 June 2022; Published 4 July 2022

Academic Editor: Min Hu

A higher incidence of female infertility has been reported with an unexpectedly early appearance in recent years. The female infertility treatment and application of assisted reproductive technology have recently gained immense interest from scientists. Many studies have discussed the beneficial effects of acupuncture on female infertility. With advancements in science and medical technology, acupuncture-related research has increased in investigating its effectiveness in treating female infertility. This review focuses on a compilation of research in recent years on acupuncture for female infertility treatment and the exploration of the underlying mechanism. For this purpose, literature was searched using various search engines like PubMed, Web of Science, and Google Scholar. The search was refined by only focusing on recent studies on acupuncture effectiveness and mechanism in female infertility and evaluating pregnancy outcomes.

1. Introduction

The reproduction cycle continued to work, but the primary focus was on arranging life commodities for the family in ancient times due to limited resources. The advancement in science and technology and the modernization of civilization led to an increase in the pace of people's lives. Their roles gradually diversified, which translated into an increase in the burden of responsibilities. Similarly, fertility seems no longer a "must" thing to ponder upon with the increasing enrichment of human spiritual culture. Although stupendous progress in human civilization is inevitable and cannot be segregated from spiritual beliefs, fertility problems demand attention.

An estimated 8 to 12% of couples of childbearing ages suffer infertility globally [1], and the incidence rate of female infertility is reported at 15% [2], making it the third primary disease after cancer and cardiovascular diseases. In addition, female infertility affects the women's psychological well-being and results in disturbed intrafamily harmony. With an increasing incidence rate of female infertility each year, it has become one of the urgent problems to be solved and has aroused the widespread concern of researchers. Infertility is a condition characterized by the failure to establish a clinical pregnancy after 12 months of regular unprotected sex [3]. Many factors, behaviors, and pathologies can hinder the pregnancy process and lead to infertility, except for artificial contraception [4].

Furthermore, increased age has also been regarded as one of the important contributing factors to female fertility [5]. The rate of female infertility incidence among younger women has increased in recent years [6]. Due to unhealthy lifestyles and environmental factors, the incidence of reproductive and endocrine diseases in female infertility is gradually growing, such as premature ovarian insufficiency (POI), polycystic ovary syndrome (PCOS), chronic endometritis (CE), and endometrial polyps [7]. They all hinder the occurrence of pregnancy from different nodes and lead to adverse pregnancy outcomes.

The recent increase in female infertility cases has also promoted the development and application of assisted reproductive technology, such as artificial insemination (AI),

in vitro fertilization-embryo transfer (IVF-ET), and intracytoplasmic sperm injection (ICSI). However, the current success rate of AI and ET technology is reported to be 15% [8, 9] and 30 to 40% [10], respectively. Therefore, further improving the success rate of assisted reproductive technology is also the hotspot and focus of current research. The combined application of complementary and alternative medicine (CAM) and assisted reproductive technology may become a solution to improve the clinical pregnancy rate.

Acupuncture is an alternative auxiliary therapy that has existed for thousands of years and is widely used in clinical practice. There is much research evidence to support the therapeutic effect of acupuncture [11]. Traditional acupuncture is comprised of inserting a "needle" into specific parts (i.e., topically disinfected acupoints), followed by manipulation to enhance the sense of acupuncture after the needle enters the skin and reaches the site of action [12]. Later, with the progress of science and technology, to make the effect of acupuncture more obvious and lasting, a series of similar treatment methods have been derived, such as electroacupuncture (EA), acupoint catgut embedding (ACE), and transcutaneous electrical acupoint stimulation (TEAS) [13]. The working principle of EA, ACE, and TEAS is the same as that of traditional acupuncture, with only difference in the action parts and operating devices.

Being a novel subject, the development of assisted reproductive technology has gained much interest in recent years. The clinical efficacy of acupuncture in female infertility has undoubtedly aroused the interest of many reproductive doctors, and the research on acupuncture in female infertility has gradually increased, which provides a lot of evidence basis for the application of acupuncture in female infertility. This review summarizes the current application of acupuncture in treating female infertility. It further explores acupuncture's potential mechanism and application potential as an auxiliary and alternative therapy option for treating female infertility. This paper is also envisaged to promote research on acupuncture further and optimize the clinical treatment plan.

2. Causes of Female Infertility

Understanding female infertility requires prior knowledge and understanding of the pregnancy cycle. The pregnancy cycle starts with sperms entering the vagina and traveling upwards through the cervix into the uterus. The sperms then travel to fallopian tubes, meeting the ova and causing its fertilization. The fertilized egg follows the movement of cilia in the lining of the fallopian tube toward the uterine cavity. Once in the uterine cavity, it is implanted in the endometrium; that is, the embryo is injected to complete the pregnancy [14]. Thus, successful conception requires sperms auspiciously reaching the cervix, sperms-ova meet-up in the fallopian tubes, unhindered fertilization, successful traveling of the zygote into the uterine cavity, and successful embryo implantation. Female infertility can be caused due to the following reasons.

2.1. Abnormal Cervical Mucus. Abnormal cervical mucus will affect the penetration of sperm [15], making it much more difficult for them to enter the cervical opening. Under physiological conditions, cervical mucus can prevent pathogenic microorganisms from entering the uterus and infecting the endometrium [16], thus making it naturally viscous. During ovulation, to facilitate sperm entering the uterus, mucus permeability is selectively regulated under the biochemical interaction of a series of proteins and molecules [17, 18], primarily the expression levels of the trefoil factor family (TFF) peptide [15], resulting in reduced viscosity of the cervical mucus [19, 20]. This process is highly dependent on the regulation of sex hormones [21], so cervical mucus at different periods of the menstrual cycle has different mucin concentrations, which is the main reason why cervical mucus becomes thinner during ovulation [22]. The TFF is a polypeptide containing the P domain, which maintains the mucosal barrier. There are three types of human TFF, that is, TFF1, TFF2, and TFF3. TFF3 is secreted by gelatin forming mucin (GFM) cells that produce mucin [23]. It has been shown that TFF expression changes with the menstrual cycle, where the expression of TFF1 and TFF2 decreases during the ovulation period, while the expression of TFF3 significantly increases [24].

Cervical inflammation caused by pathogenic microorganism infection can also cause abnormal cervical mucus. Moreover, due to the infiltration of inflammatory secretions, patients often show sweeping changes in the amount, color, and quality of secretions [25]. At the same time, pathogens of reproductive tract infection like *Chlamydia trachomatis* and Neisseria gonorrhoeae [26, 27] are usually transmitted through sexual intercourse, which can directly lead to infertility by affecting sperm motility and impeding sperm entry.

2.2. Oocyte Quality. Oocyte quality is an independent factor in successful pregnancy [28, 29]. Each follicle comprises an oocyte with encapsulated granulosa cells and follicular membrane cells [30]. The average development and maturation of oocytes depend on the "nutrients" provided by granulosa cells and theca cells, such as steroid hormones, growth factors, and cytokines [31]. Studies have shown that mitochondria in granulosa cells are the core of steroid hormones required for follicular development [32]. Degraded granulosa cells are associated with mitochondrial swelling, the leading cause of oocyte apoptosis and follicular atresia [33]. Abnormal follicular development is not conducive to fertilization, fertilized egg implantation, and embryonic development [34]. Relative to it, the diseases leading to infertility mainly include POI and, more seriously, premature ovarian failure (POF) [35].

Increasing age will inevitably lead to ovaries aging [36], causing a gradual decrease in the number of follicular cells as well as the follicular pool in females, resulting in a decline in levels of inhibin B (INH-B) and anti-Müllerian hormone (AMH) secreted by granulosa cells in follicles [37], which are indicative of the declining function of ovaries, resulting in a reduction in the responsiveness of follicles to reproductive

hormones [5]. "Fewer available follicles" and "decreased ovarian secretion function" are irreversible factors that contribute to female infertility [38].

In addition, bad living habits can also affect the quality of follicles by disrupting the average metabolic level [39], which is also the main reason for the gradual younger onset of POI/POF. For example, Zhang et al. [40] reported that women with BMI >29 kg/m^2 showed lower fertility due to adverse effects of inferior follicular development on oocyte quality. Similarly, spicy food, improper sleep, and mental stress cause chronic inflammation in the body [41, 42], which result in reactive oxygen species (ROS) production and accumulation in granulosa cells to induce oxidative stress [43], which will further damage the function of organelles such as granulosa mitochondria and endoplasmic reticulum [44, 45], thus impairing the fertilization ability and normal development of oocytes [46], thus leading to infertility and adverse pregnancy outcomes. Similarly, the inflammatory-induced macrophage infiltration and significant secretion of proinflammatory factors increase the incidence of ovarian fibrosis [47–49], which compromises the ova's normal development and destroys the ovary's secretory function.

2.3. Ovulatory Dysfunction. Ovulatory dysfunction is the leading cause of infertility. The hypothalamic-pituitary-ovary (HPO) axis is essential in controlling female reproduction and the key to timely ovulation [50, 51]. World Health Organization (WHO) divides ovulatory dysfunction into three categories [52]: type I is gonadotropin-induced hypogonadism, mainly including hypothalamus and pituitary dysfunction, accounting for about 10% of ovulatory dysfunction; type II is HPO axis dysfunction, often due to some endocrine diseases, such as polycystic ovary syndrome (PCOS) and obesity; and type III is ovarian insufficiency. Ovulatory dysfunction mainly includes irregular ovulation and nonovulation, and such patients often presented with menstrual thinning or amenorrhea [53]. Hence, female infertility caused by ovulatory dysfunction often suggests a disordered reproductive hormone balance in patients.

PCOS is the most common ovulatory dysfunctional disease in women, accounting for approximately 80% of anovulatory infertility [54]. Research statistics show that PCOS affects 7 to 10% of women of childbearing age [55]. In recent years, it has been reported to be an endocrine disease caused by genetic, endocrine, and environmental factors [56]. Furthermore, abnormal metabolism results in persistently "high luteinizing hormone (LH)" status in patients, resulting in ovulatory dysfunction. Lifestyle adjustment is the first-line treatment for PCOS [53]. Therefore, a healthy lifestyle is essential for adjusting endocrine metabolism. In addition, Chavarro et al. [57] showed that following a nutritional diet plan benefits female fertility. Therefore, most cases of female infertility caused by ovulatory dysfunction can be prevented by changing diet and lifestyle.

Hyperthyroid hormones, caused by hyperprolactinemia (HPRL) and hypothyroidism, can interfere with the pulsed secretion of gonadotropin-releasing hormone, hinder the normal feedback regulation of HPO axis, and lead to ovulatory dysfunction. High thyroid-stimulating hormone (TSH) status induced by hyperprolactinemia and hypothyroidism can interfere with the pulsed secretion of GnRH and hinder the normal feedback regulation of the HPO axis, leading to ovulatory dysfunction [53, 58]. Furthermore, hypothalamic-pituitary failure (HPF) directly results in GnRH and/or gonadotropin (Gn) deficiency like idiopathic gonadotropic hypogonadism (IGH), panhypopituitarism, and Langerhans cell histiocytosis [50, 59].

Psychological stress-induced ovulation disorder infertility has received extensive attention in recent years. A series of neuroendocrine signal transduction produced by psychological stress will eventually be manifested as the continuous activation of the hypothalamus-pituitary-adrenal gland (HPA) axis [60], translating into the reduced release of central GnRH [61], disrupting the menstrual cycle, and inhibiting ovulation. Chronic stress directly acts on the hypothalamus to produce more corticotropin-releasing hormone (CRH) [62], while activation of the HPA axis results in more cortisol. Bolt's study has shown that cortisol and estradiol have the same coactivator to "fine-tune" transcription [63], and there is a significant overlap between glucocorticoids and estrogen-controlled signaling molecular networks [64], which indicates that elevated glucocorticoids will antagonize estrogen effects and disrupt regular ovulation. In a randomized controlled trial by Michopoulos [65], ovulation resumed in six of eight women treated with cognitive-behavioral therapy (CBT).

2.4. Tubal Obstruction. The anatomical-based tubal obstruction will directly hinder the sperm and egg combination, causing fertilization to fail. According to statistics, about 30% of the world's infertile women have tubal lesions [66]. Moreover, other common pathogenic contributing factors are Neisseria gonorrhoeae, *Chlamydia trachomatis*, endometriosis, and surgery [26, 67], leading to tubal infertility. Cilia in the fallopian tube become active during ovulation to transport the fertilized egg. However, pathological conditions such as tubal inflammation and hydrosalpinx result in cilia destruction, thereby reducing the ciliary movement [68], thus preventing the ova and sperm combination for successful fertilization and increasing the risk of infertility or ectopic pregnancy.

2.5. Endometrial Damage. Embryo implantation is the result of the interaction between blastocyst and endometrium. The key to its success lies in embryo quality and endometrium conditions. This process cannot be avoided even with assisted reproductive technology, so a good endometrium is necessary for a successful pregnancy. Endometrial receptivity (ER) is commonly used to evaluate the ability of an embryo's endometrial receptivity [69] and is closely related to the time of embryo implantation [70]. In most women, the endometrial implantation window is approximately five to seven days after ovulation [71]. Still, various pathological factors will shorten or shift the implantation window, which is not conducive to embryo implantation.

CE is a significant factor that damages the normal function and is often neglected due to the lack of apparent clinical manifestations [72]. Various "stimuli" promote changes in the number of white blood cells, cytokine production, and growth factors in the endometrial microenvironment [73], such as plasma cell infiltration and tissue edema [74], thus exacerbating their adverse effects on ER. In addition, the endometrium is regularly subjected to sex hormones under the regulation of the HPO axis, resulting in periodic growth changes. This growth pattern of the endometrium is of pivotal importance for a successful pregnancy, which CE compromises. Studies [73, 75] showed that antiapoptotic expressions, such as estrogen receptor, progesterone receptor, Ki-67 cell proliferation marker, B-cell lymphoma-2 (BCL-2), and BCL-2-associated *X* (BAX), were increased in endometrial epithelial cells and stroma of CE patients, leading to endometrial polyps and endometriosis [76]. Thus, it can be concluded that the immune disorder in the endometrial microenvironment is an essential factor leading to endometrial lesions. Endometrial polyps, endometriosis, adenomyosis, fibroids, and other space-occupying lesions can directly damage the endometrium and hinder embryo implantation. Surgical resection is the preferred treatment [77], but as an intrauterine operation, surgery has the possibility of compromising fertility, such as intrauterine adhesions [78].

In addition, CE has also been found to change the pattern of uterine contractions during ovulation and the luteal phase [79]. Under physiological conditions, in the proliferative phase, the uterus tends to contract from the fundus to the cervix, which is beneficial for menstrual discharging; in the ovulation and luteal phase, the retrograde contraction from the cervix to the fundus of the uterus is the primary process, which is conducive to the migration of sperm to the fallopian tube. However, CE reduces the probability of retrograde contractions during ovulation and the luteal phase by 3.3 folds [80], which will reduce the likelihood of successful pregnancy. It is also associated with additional discomfort symptoms, such as dysmenorrhea and pelvic pain [72, 81].

3. Acupuncture

Acupuncture is regarded as a multidimensional procedure that can prove being helpful in treating many diseases with minimal invasiveness using needles. It has been practiced for thousands of years, yet scientists started investigating its effects after the 18th century [82]. With the increasing application of acupuncture in clinical practice, researchers also developed a strong aptitude for researching acupuncture to treat various ailments. After decades of clinical practice and development, acupuncture was reported to possess significant clinical effects [11]. During early times, acupuncture was only realized through doctors' manipulations [83], which had high requirements for doctors' acupuncture skills. With the development of science and technology and the continuous integration of modern technology and medical technology, various acupuncture-derived therapies were introduced to strengthen the effect of acupuncture and facilitate clinical treatment, like EA and ACE.

3.1. Traditional Acupuncture. Traditional acupuncture involves inserting needles into meridians and acupoints [12]. To enhance the sense of needling, doctors often use auxiliary manipulation after needling the skin, such as repeatedly lifting and inserting and rotating the needling back and forth [83]. Primary scientific studies have shown [84] that manual stimulation of acupuncture needles can activate muscle afferent nerves on the one hand and exert its effect by locally generating electrical signals on the other. Manually operated acupuncture needles effectively regulate peripheral and central neural pathway activity [85]. When acupuncture is inserted into specific parts, the conduction through meridians will promote the coordination and balance of blood and energy throughout the body, producing a series of physio-psychological reactions [86, 87]. However, the manual operation of traditional acupuncture has high requirements on the physical strength of the doctor. Therefore, after inserting the needle into the corresponding acupoint, the doctor often uses electricity to translate the acupuncture into a more obvious therapeutic effect.

3.2. EA. EA comprises stimulating the hand-inserted needles with microcurrent waves (including continuous waves and intermittent waves), which can enhance the effect of acupuncture [88]. Furthermore, neural signals generated by EA stimulation were found to cause the hypothalamus to release endogenous opioids [89], such as β-endorphins, which are thought to play an essential role in regulating the effects of stress on reproductive function [90]. Previous studies have shown that [91, 92] acupuncture can change the level of plasma β-endorphin, thereby affecting the secretion of GnRH and Gn and then regulating the HPO axis to control female reproduction. Moreover, Stener [92, 93] reported that repeated EA therapy could reduce the high pulsatility index (PI) value in women's uterine arteries to an average level, which produced beneficial effects in increasing ER. Similarly, it can also improve the phenomenon of anovulation in PCOS patients.

Kim and Bae [94] showed that EA could produce a balance that enhanced the NK cell activity and promoted Th1/Th2 cell reaction. Moreover, Li et al. [95] demonstrated that acupuncture could inhibit free radicals (FR) metabolic imbalance and thus help achieve an antioxidant stress effect, which shows that acupuncture can regulate immunity and antioxidation, which can help regulate the immune balance in the endometrial microenvironment, treat CE, and increase ER.

3.3. ACE. ACE therapy is to embed absorbable sutures into acupoints, which will continuously stimulate the embedded part, to achieve the purpose of disease treatment, health care, and body strengthening [96]. The effect of catgut embedding is like that of acupuncture, but it is more lasting than acupuncture, and its stimulation can be maintained for two

weeks or even longer [97]. A randomized controlled trial (RCT) conducted by Qin et al. [98] showed that ACE as a complementary therapy was beneficial for improving the rate of regular ovulation and clinical pregnancy.

4. Acupuncture and Infertility

4.1. Clinical Research. Female infertility results from an abnormal reproductive endocrine function [99]. Many diseases lead to female infertility, hindering the process of pregnancy from various nodes. Because acupuncture has the function of adjusting the neuroendocrine system [100], its application in the field of reproduction has attracted extensive attention. Acupuncture can act on multiple parts of the female reproductive system to improve its function through the action of neuroendocrine signaling molecules, to improve female fertility. Currently, acupuncture is mainly applied as a clinical adjuvant treatment of infertility conditions, such as IVF-ET and PCOS, as shown in Table 1.

Six RCTs [101–106] of IVF-ET patients treated with acupuncture showed a significantly increased clinical pregnancy rate than those without acupuncture. However, four other studies [107–110] reported the opposite results. Following a thorough analysis, we found that the RCTs that showed increased pregnancy rates were mainly from the last two to three years, while the RCTs that showed adverse effects were more than a decade old. Thus, it can be hypothesized that this could be due to the continuous development of acupuncture technology over time, which also improved the clinical efficacy of acupuncture.

Rashidi et al. [109] showed that acupuncture could improve the rate of high-quality embryos in IVF patients. Chen and Hau [110] showed that acupuncture could increase the estradiol (E2) and progesterone (P) levels in IVF patients and promote the endometrial artery blood flow; all of these are conducive to embryo transfer. Similarly, after follow-up, three studies showed significant differences in the rate of persistent pregnancy between the two groups [104–106]. Guven et al. [106] showed that IVF-ET patients treated with acupuncture had a higher live birth rate and a lower state-trait anxiety inventory-1 (STAI-1) score. Wang et al. [102] demonstrated that acupuncture could improve the endometrial morphology and menstruation in IVF-ET patients, and Zhou et al. [103] showed that acupuncture significantly improved blood E2 levels, the number of ova obtained, and the number of high-quality embryos in IVF patients.

Furthermore, Altutunji et al. [104] showed that acupuncture could increase blood E2 and P levels and reduce the incidence of ovarian hyperstimulation syndrome (OHSS). Therefore, it can be found that acupuncture can improve the clinical pregnancy rate of IVF-ET patients by ameliorating the level of sex hormones and promoting the endometrial condition. In addition, acupuncture also reduced the occurrence of ovarian hyperstimulation syndrome (OHSS) and alleviated patients' anxiety to a certain extent.

Three other RCTs [111–113] reported the effect of EA on IVF-ET infertility patients. Kusuma et al. [111] showed that EA could improve the number of ova obtained by IVF. Further testing found that BAX expression decreased, and BCL-2 expression increased in patients with EA treatment, which confirmed that EA could help patients get more follicles by inhibiting granulosa cell apoptosis. However, Wu et al. [112] and Xiang et al. [113] showed opposite results regarding the number of obtained ova but concluded that EA could significantly improve the rate of high-quality embryos in IVF patients. However, their research results on clinical pregnancy rates were different. We speculate that this may be related to the course of EA treatment. Studies with longer EA treatment cycles have shown better efficacy in clinical pregnancy rates than short-term EA treatment [112]. The study of Xiang et al. [113] showed that EA could significantly improve the live birth rate of IVF patients, although there was no significant difference in clinical pregnancy rate. Therefore, we can find that EA can enhance ova quality to a certain extent, but further facilitating the pregnancy outcome may require a long period of repeated EA stimulation.

Three other RCTs [114–116] reported the effect of TEAS on patients with IVF-ET infertility. Qu et al. [114] and Shuai et al. [116] reported that TEAS significantly increased the clinical pregnancy rate and live birth rate in IVF patients compared with those without TEAS intervention. Similarly, Zhai et al. [115] showed that TEAS (20/30/40 mA) showed no significant difference in clinical pregnancy rate compared to TEAS (5 mA), depicting that though TEAS is beneficial for pregnancy outcomes, in IVF-ET patients, the optimal stimulation intensity of TEAS is yet to be explored.

Four RCTs [117–120] investigated the effect of acupuncture on infertility patients with PCOS. Jiang et al. [117] and Xu et al. [119] reported that the group with acupuncture intervention had a significant increase in clinical pregnancy rate compared to the group that received only western medicine treatment regimen. However, Jiang and Xu's findings contradicted the study conducted by Yu et al. [120]. Moreover, it was reported that acupuncture can improve the cycle ovulation rate, E2 and P levels, and endometrial morphology is also envisaged to benefit patients with PCOS. Xu et al. [119] also reported that acupuncture could improve the rate of regular ovulation and reduce serum T and LH levels in infertility patients with PCOS. Nevertheless, Wu et al. [118] reported that acupuncture could not improve the live birth rate. Therefore, it is hypothesized that acupuncture could help PCOS patients ovulate by improving sex hormone levels before pregnancy, thus increasing the pregnancy rates. However, the therapeutic effects of acupuncture before pregnancy may not last until delivery.

4.2. Animal Studies. The detailed information regarding acupuncture in animals is shown in Table 2. Stener-Victorin reported that repeated EA stimulation significantly promoted the release of β-endorphins in the hypothalamus of polycystic ovary (PCO) rats and reduced nerve growth factor (NGF), corticotropin-releasing factor (CRF), and endothelin-1 (ET-1) in the ovaries of PCO rats [95, 121–123]. The β-endorphins can inhibit GnRH secreted by the hypothalamus and LH released by the pituitary [124]. Some scholars

Table 1: Characteristics of clinical studies.

First author	Year	Research type	Type of infertility	Intervention		Course of treatment	Evaluating indicator	
				T	*C*		$P < 0.05$	$P > 0.05$
Andersen D	2010	RCT	IVF-ET	Acupuncture	N	1 time	——	CPR, PPR, LBR (–)
Moy I	2010	RCT	IVF-ET	Acupuncture	N	1 time	——	CPR (–)
Rashidi BH	2013	RCT	IVF-ET	Acupuncture	N	4 times	NHQE☐	NRO, CPR, PPR, BPR (–)
Villahermosa DI	2013	RCT	IVF-ET	Acupuncture	N	4 times	CPR☐	——
Chen Q	2015	RCT	IVF-ET	Acupuncture	N	12 weeks	(E_2, P)☐, EAI☐	CPR (–)
Jiang D	2015	RCT	PCOS	Acupuncture + WM	WM	12 weeks	(CPR, ET/EM, CM)☐, AR☐	——
Wang X	2016	RCT	IVF-ET	Acupuncture	N	30 times	(CPR, EM, Menstruation)☐	——
Zhou L	2016	RCT	IVF-ET	Acupuncture	N	15 ± 2 times	(E_2, NRO, NHQE, CPR)☐	——
Qu F	2017	RCT	IVF-ET	TEAS	N	2 times	(CPR, LBR, NPY)☐	NRO, FR, NHQE, TGF-α, G-CSF (–)
Wu XK	2017	RCT	PCOS	Acupuncture + WM	WM	16 weeks	——	LBR (–)
Xu J	2018	RCT	PCOS	Acupuncture + WM	WM	8 weeks	(CPR, COR)☐, (T, LH)☐	AR, ET (–)
Yu L	2018	RCT	PCOS	Acupuncture + WM	WM	24 times	(COR, ET/EM, E_2, P)☐	CPR (–)
Altutunji AZ	2019	RCT	IVF-ET	Acupuncture	N	2 weeks	(CPR, PPR, E_2, P)☐, OHSS incidence☐	AMH (–)
Kusuma AC	2019	RCT	IVF-ET	EA	False EA	6 times	(NRO, FR, BCL-2)☐, BAX☐	GDF9, BMP15 (–)
Shuai Z	2019	RCT	IVF-ET	TEAS	N	16 ± 2 times	(CPR, LBR)☐	——
Wu HC	2019	RCT	IVF-ET	EA	False EA	12 weeks	(NHQE, CPR, PI3K/Akt mRNA)☐	NRO (–)
Dehghani AS	2020	RCT	IVF-ET	Acupuncture	N	2 times	(CPR, PPR)☐, BPR☐	——
Guven PG	2020	RCT	IVF-ET	Acupuncture	N	3 times	(CPR, PPR, LBR)☐, STAI-1 score☐	——
Xiang S	2021	RCT	IVF-ET	EA	N	24 times	(NHQE, LBR, IRS-1/PI3K/GLUT4 mRNA)☐	NRO, CPR (–)
Zhai ZJ	2021	RCT	IVF-ET	TEAS (20 mA/30 mA/40 mA)	TEAS (5 mA)	10–13 times	(NRO, ET)☐	NHQE, CPR (–)

Note. T: Test group; C: Control group; RCT: Randomized controlled trial; IVF-ET: In vitro fertilization - embryo transfer; PCOS: Polycystic ovary syndrome; EA: Electroacupuncture; TEAS: Transcutaneous electrical acupoint stimulation; N: None; WM: Western medicine; CPR: Clinical pregnancy rate; PPR: Persistent pregnancy rate; LBR: live birth rate; BPR: Biochemical pregnancy rate; COR: Cycle ovulation rate; AR: Abortion rate; FR: Fertilization rate; NRO: Number of retrieved oocytes; NHQE: Number of high-quality embryos; ET: Endometrial thickness; EM: Endometrial morphology; EAI: Endometrial arterial impedance; CM: Cervical mucus; E_2: Estradiol; P: Progesterone; STAI-1: State-trait anxiety inventory-1; OHSS: Ovarian hyperstimulation syndrome; NPY: Neuropeptide Y; TGF-α: Transforming growth factor-α; G-CSF: Granulocyte colony stimulating factor; BCL-2: B-cell lymphoma-2; BAX: Bcl2-Associated X; PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; IRS-1: Insulin receptor substrate-1; GLUT4: Glucose transporter 4; GDF9: Growth differentiation factor 9; BMP15: Bone morphogenetic protein 15; ☐: Improve; ☐: Reduce; (–): Unchanged.

Table 2: Characteristics of animal studies.

First author	Year	Experimental object	Modeling drugs	Intervention	Course of treatment (times)	Evaluating indicator	
						$P < 0.05$	$P > 0.05$
Stener-victorin E	2000	PCO rats	EV	EA	12	NGF☐	Ovarian morphology (-)
Stener-victorin E	2001	PCO rats	EV	EA	12	CRF☐	——
Stener-victorin E	2003	PCO rats	EV	EA	12	NGF☐, ET-1☐	——
Stener-victorin E	2004	PCO rats	EV	EA	12	β-Endorphins☐	NK cell, CD4 + /CD8 + T cell (-)
Zhang WY	2009	PCO rats	DHEA	Acupuncture	5	(NEI, Ovarian morphology, ET, E_2)☐, T☐	TSH, LH, P (-)
Huang J	2020	PCO rats	LE	EA	14	(AMH, PI3K, AKT)☐, (T, LH, LC3 II/I)☐	Ovarian morphology (-)
Gao W	2013	ETF rats	Mifepristone	Acupuncture	10	(NEI, CCL2, CXCL8, uNK cell subsets)☐	——
Zhu S	2016	COH mice	Gn	EA	7	(NEI, IGF-1)☐	——
Wang S	2019	POF rats	CTX	Acupuncture	21	(E_2, PI3K, Akt, BCL-2)☐, (BAX, FSH)☐	Ovarian morphology (-)
Zhong H	2019	POF rats	CTX	EA	7	(NF, LS, E2, AMH)☐, (PI3K, AKT, mTOR, S6K, 4E-BP1, FSH, LH)☐	——

Note. PCO: Polycystic ovary; ETF: Embryo transfer failure; COH: Controlled ovarian hyperstimulation; POF: Premature ovarian failure; EV: Estradiol valerate; DHEA: Dehydroepiandrosterone; LE: Letrozole; Gn: Gonadotropin; CTX: Cyclophosphamide; EA: Electroacupuncture; NGF: Nerve growth factor; CRF: Corticotropin releasing factor; ET-1: Endothelin-1; FSH: Follicle stimulating hormone; E_2: Estradiol; T: Testosterone; ET: Endometrial thickness; NEI: Number of embryo implantation; LH: Luteinizing hormone; AMH: Anti-Mullerian hormone; LC3: Microtubule associated protein 1 light chain 3; PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; CCL2: CC chemokine subfamily L2; CXCL8: CXC chemokine subfamily L8; IGF-1: Insulin growth factor; BCL-2: B-cell lymphoma-2; BAX: Bcl2-Associated *X*; NK cell: Natural killer cell; mTOR: mammalian target of rapamycin; S6K: S6 kinase; 4E-BP1: 4E-Binding Protein1; NF: Number of follicles; LS: Litter size; ☐: Improve; ☐: Reduce; (-): Unchanged.

argue that endogenous opioid drugs are closely related to the surge of LH before ovulation [125, 126]. Therefore, EA helps improve the "high-LH" state and restore normal ovulation function for PCO rats. The increase in nerve growth factor (NGF) and endothelin-1 (ET-1) was found to be associated with ovarian sympathetic excitation [127, 128], which has been reported in many inflammatory and autoimmune states [129]. The NGF is one of the target neurotrophic factors involved in developing and maintaining ovarian innervation [130]; ET-1 is a vital peptide that regulates blood flow in the ovary and is an effective and durable vasoconstrictor [122]. CRF is related to stress [131]. The repeated EA treatment was also found to improve the frequency and activation of *T* and natural killer (NK) cells, but the difference was insignificant.

Similarly, 12 cycles of EA treatment did not significantly improve ovarian morphology. However, it substantially improved the ovarian neuroendocrine function in PCO rats. The authors speculated that more extended periods of EA might translate into significant effectiveness. Zhang [132] demonstrated that acupuncture could significantly downregulate serum testosterone (*T*) and upregulate E2 expression in PCO rats, improve ovarian function, promote endometrial development, and promote embryo implantation.

Huang et al. [133] reported that EA could reduce serum *T*, LH, and AMH contents, reduce the ratio of ovarian LC3-II/I, and upregulate phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB, also known as AKT) in PCO rats. The LC3 is a biomarker of autophagy. Cytoplasmic LC3 (LC3-I) will hydrolyze a small peptide and transform it into autophagosome (LC3-II), representing a direct relationship between a higher LC3-II/I ratio and a higher the level of autophagy.

A reduction in ovarian reserve function directly affects female reproduction ability. An increase in FSH and a decrease in E2 levels are typical of decreased ovarian reserve function [134]. Follicular development and maturation result from the oocytes' coregulation, mutual influence, and surrounding granule cells. It has been found that granulosa cells provide nutrients and growth signals for oocytes, and their apoptosis is the primary reason for follicular atresia [135]. A hormone is an important signal molecule in granulosa cell proliferation and apoptosis. In ovarian dysfunction, the sensitivity of follicles to hormones reduces and declines the ovaries' secretary function for estrogen and progesterone [136], both of which are not conducive to follicular development. FSH is an essential hormone for follicular development, which can bind to the FSH receptor on the granular cell membrane to activate upstream protein kinase A (PKA) and Grb2-associated Binder2 (GAB2), and downstream target factors and PI3K/AKT pathway are then activated to slow down the apoptosis of granulosa cells [137, 138]. Zhao et al. [139] showed that repeated EA stimulation significantly increased blood E2 concentration and restored HPO axis function in ovariectomy (OVX) rats. Further analysis showed that EA increased aromatase activity, mRNA, and protein expression in OVX rats, suggesting that FSH can also induce aromatase expression, stimulate ovarian E2 secretion, and promote granulosa cell maturation [136].

Wang et al. [140] showed that manual acupuncture could regulate hormone levels in POF rats (increase E2 and decrease FSH), upregulate PI3K/AKT signaling pathway, and promote follicular development, where higher expressions of PI3K, AKT, BCL-2 gene, and proteins were observed. In contrast, the expression of BAX decreased in POF rats after manual acupuncture, which could help reduce apoptosis of granulosa cells and reduce follicular atresia. In terms of morphology, though, acupuncture did not significantly reduce the apoptosis of granulosa cells in POF rats, suggesting that acupuncture was more about improving ovarian function. However, Zhang et al. [141] reported that EA reduced the phosphorylation of PI3K, AKT, mammalian target of rapamycin (mTOR), S6 kinase (S6K), and 4E-binding protein 1 (4E-BP1) and downregulated the ovarian PI3K/AKT/mTOR activation pathway in POF mouse. In terms of hormone levels, EA can upregulate serum AMH and E2 in POF mice and downregulate serum FSH and LH levels, which agrees with the reports of other researchers; hence, acupuncture is speculated to be beneficial to the level of sex hormones in POF rats.

Female POF mice treated with EA mated with male mice, and the outcome was compared with mice not subjected to EA treatment [141]. The results indicated that the EA group's litter size was significantly increased, indicating that acupuncture can improve the fertility of POF mice. Gao et al. [142] used mifepristone in pregnant rats to establish an "embryo implantation failure model." After manual acupuncture treatment, compared with the nonacupuncture group, the number of implanted embryos, mRNA, and protein levels of CCL2 (CC chemokine subfamily L2) and CXCL8 (CXC chemokine subfamily L8) in the endometrium and uterine NK (uNK) cell subpopulations were significantly higher. The CCL2, CXCL8, and uNK cells are closely related to placental development [143]. The CCL2 can promote Th2 polarization and maintain a Th2 dominant environment, thus ensuring embryo implantation by regulating immunity [144]; CXCL8, on the other hand, stimulates the invasion of trophoblast cells outside villus in early pregnancy into the decidua by increasing MMP-2 secretion [145]. However, uNK cells in rats only appear during pregnancy. They often promote angiogenesis by expressing various cytokines, such as vascular endothelial growth factor (VEGF), placental growth factor (PGF), and angiopoietin 2 (ANG2), which is conducive to trophoblast invasion and placenta formation [146, 147]. This experiment suggested that acupuncture helps regulate the endometrial microenvironment through cytokine and chemokine pathways to aid embryo implantation during pregnancy. Zhu et al. [148] also reported that repeated EA stimulation could increase the secretion of insulin growth fact-1 (IGF-1) in the endometrium, thus improving the embryo implantation rate in mice. Fu et al. [149] reported that acupuncture could reduce the higher level of E2 in superovulation rats, and further studies found that the expression of estrogen receptor -β increased in rats' pituitary. These results suggest that acupuncture can prevent and reduce the incidence of OHSS by reducing the excessive

increase in serum E2, which reflects the bidirectional adjustment ability of acupuncture and high safety.

5. Mechanism of Acupuncture Treatment of Female Infertility

As previously described, acupuncture has a positive treatment effect on female infertility. However, the causes of infertility vary, and understanding the role of acupuncture will help apply it to the appropriate type of infertility to achieve maximum efficacy. Based on the existing studies, it was found that acupuncture is beneficial in regulating female reproductive hormones and various cellular and immune signaling molecules, which are considered helpful in improving multiple aspects of the female reproductive system, such as follicular development, regular ovulation, and embryo implantation. Therefore, acupuncture has great application value in PCOS, POI, and IVF-ET. Furthermore, as a complementary and alternative therapy, acupuncture can improve female fertility and the success rate of assisted reproductive technology. As shown in Figure 1, the therapeutic effect of acupuncture on female infertility is mainly aimed at the following three aspects: reproductive endocrine, follicular development, and embryo implantation. The various mechanistic implications of acupuncture therapy in female infertility are discussed below.

5.1. Reproductive Endocrine Pathway. The reproductive hormones are at their basic level during the follicular phase, and withdrawal of estrogen and progesterone induces menstruation. Low levels of E2 and P will alternatively promote the secretion of GnRH, encouraging the secretion of Gn (FSH and LH), which is essential for follicles development and results in the secretion of E2 and P. E2 and P are the material basis of endometrial proliferation and transformation and facilitate it in accepting embryos. This serial effect ensures the synchronization of follicular development and endometrial development required for pregnancy. The basic endocrine level of infertile women is "high Gn," which inhibits follicular growth since higher levels of Gn inhibit the secretion of E2 and P, resulting in no longer regular ovulation, leading to infertility. Acupuncture can restore normal reproductive hormone levels. While reducing GnRH, FSH, LH, and increasing E2 and P, it is more important to restore the regularity of hormone interaction, make the reproductive system usually operate, and then promote pregnancy.

5.2. Improvement in Ovarian Function. There are two main ways to improve ovarian function: reducing granulosa cell apoptosis in follicles and improving the ovarian environment. During follicular development, granulosa cells are responsible for providing nutrients required maturation of the ovum. Follicular atresia and apoptosis are closely related to granulosa cell apoptosis. Acupuncture can increase the expression of BCL-2 and reduce the expression of BAX to reduce the apoptosis of granulosa cells, hence serving as an escort for follicular development. On the other hand, the internal ovarian environment is a prerequisite for follicular development. Acupuncture can reduce the expression of NGF, CRF, and ET-1 to reduce inflammation in the ovary and promote the blood supply in the ovary.

5.3. Improvement in Embryo Implantation. The interaction of an embryo with the endometrium microenvironment to form a biological connection refers to embryo implantation. Acupuncture can promote embryo implantation by three mechanisms, that is, modulating autoimmunity, where acupuncture resists the maternal immune response to embryos (foreign objects) by increasing the expression of CCL2. Similarly, by increasing the expression of CXCL8 and MMP-2, trophoblast cells can invade decidual tissue to establish a physical connection with the endometrium. The second mechanism refers to feeding, where acupuncture increases the expression of VEGF, ANG2, and PGF to promote angiogenesis, to establish the pathway of material exchange between mother and embryo. Finally, the other mechanism embarks on embryonic development, where acupuncture helps the development of embryos in the endometrium by increasing the expression of IGF-1. The classification and summary of relevant mechanisms are shown in Figure 2.

6. Summary and Outlook

The etiology of female infertility is complex and can be multifactorial, that is, pathological, physiological, psychological, and social. This review discusses the incidence of female infertility during the whole pregnancy cycle for the first time, which is envisaged to help understand the causes of female infertility and help target the clinical diagnosis and treatment. Female reproduction needs the reproductive system to provide a reasonable "material basis" on the one hand, and the neuroendocrine system to release accurate "signal" regulation on the other hand. Thus, successful pregnancy embodies the perfect cooperation between the reproductive and neuroendocrine systems. This ideal state is reflected in the interaction between signal molecules to achieve "dynamic balance." A good "material base" is necessary for follicular development and endometrial growth.

Initially, the HPO axis is in a positive activation state: the hypothalamus releases GnRH, which prompts the pituitary gland to release FSH and LH, and the ovary begins to produce E2. Simultaneously, follicles start to develop, and intima begins to proliferate. Until the follicle maturation, LH and E2 levels significantly increase, followed by a negative activation state elicited by the HPO axis, resulting in LH and E2 levels dropping precipitously, thus inducing ovulation. Following this, the level of P rises, and the endometrium begins to prepare for embryo implantation. Hormones, as nutrients, participate in the whole process of follicular and intimal development. At the same time, its interaction precisely controls the entire process. The orderly progress of this process is the "balance state" of the female reproductive endocrine. An abnormality in any of these processes results in missing the pregnancy opportunity or might translate into

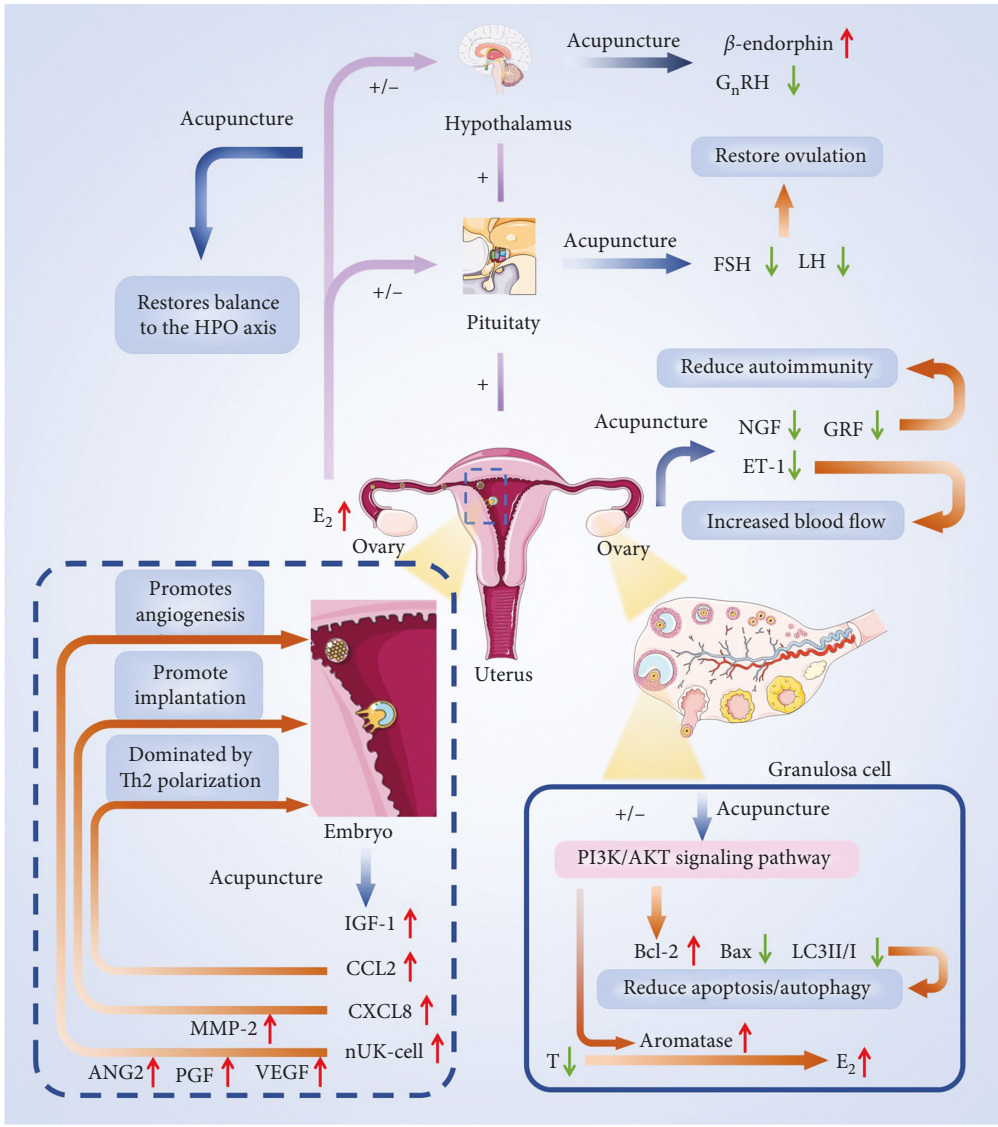

Figure 1: Mechanism of acupuncture in the treatment of infertility. The main function of acupuncture is to regulate reproductive related neuroendocrine signal molecules, in order to balance female reproductive endocrine, improve ovarian function, and promote embryo implantation. The symbol "☐" represents increased expression and activity of the molecule, and the symbol "☐" represents a decrease in the expression of this molecule. The symbol "+" represents positive feedback regulation, and the symbol "−" represents negative feedback regulation. Abbreviations are listed at the end of the article.

unacceptable conditions for pregnancy, leading to infertility. For example, high LH will inhibit the production of E2 and P by a feedback mechanism, hindering follicular maturation, resulting in failure of endometrium transformation from proliferative to secretory phase. The key to successful pregnancy lies in the regular appearance of specific signal molecules and the "steady state" of the body's internal environment.

Based on existing studies, it was observed that the mechanism of acupuncture in the treatment of infertility is mainly reflected in four ways, that is, by adjusting HPO axis balance, where acupuncture reduces the "high GnRH" and "high LH" status in PCO rats by increasing the expression of β-endorphins, which is beneficial to follicular development. The second mechanism involves improving the ovary's internal environment by increasing ovarian blood flow and reducing the ovary's immune-inflammatory response by decreasing the expression of NGF, CRF, and ET-1. The third mechanism involves increasing the expression of BCL-2 and decreasing the expression of BAX and LC3-II to LC3-I ratio to reduce granulosa cell apoptosis and promote T's transformation to E2 by activating aromatase. The final mechanism involves helping the embryo implantation by upregulating the expression of IGF-1, CCL2, and CXCL8 signaling molecules to promote placental growth and ensure embryo implantation. All these mechanisms are conducive to restoring women's normal reproductive hormone levels, improving the function of the female reproductive system, and promoting the occurrence of pregnancy. The event of infertility is often the result of an "imbalance" in the human

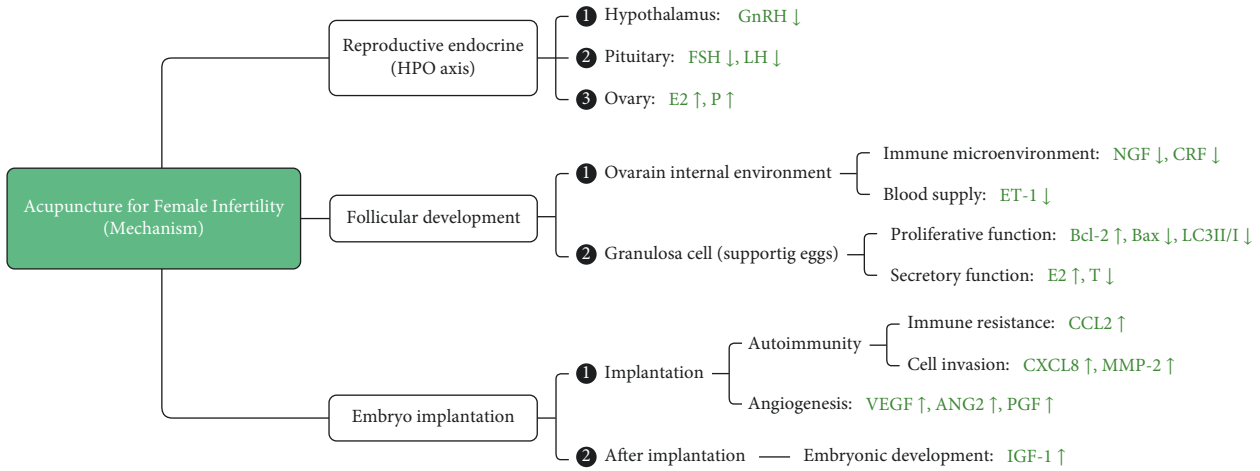

Figure 2: Classification of the mechanisms of acupuncture in treating infertility. The symbol "☐" represents increased expression and activity of the molecule, and the symbol "☐" represents a decrease in the expression of this molecule.

Table 3: Classification of clinical evaluation indexes of acupuncture in the treatment of infertility.

Evaluation category	Specific items			
Evaluation index before pregnancy				
Cervix	CM	☐	——	
Endometrium	EAI, ET, EM	☐	——	
Ovary	NRO, COR	☐	——	
Embryo	FR, NHQE	☐	——	
Menstruation	Menstrual regularity	☐	——	
Ovarian function markers	NPY, AMH, BCL-2, IRS-1, (PI3K, Akt, GLUT4) mRNA	☐	BAX	☐
Hormone level	E_2, P	☐	T, LH	☐
Security	——	OHSS incidence	☐	
Evaluation index after pregnancy				
Pregnancy outcome	CPR, PPR, LBR	☐	BPR, AR	☐

Note. CM: Cervical mucus; EAI: Endometrial arterial impedance; ET: Endometrial thickness; EM: Endometrial morphology; NRO: Number of retrieved oocytes; COR: Cycle ovulation rate; FR: Fertilization rate; NHQE: Number of high-quality embryos; NPY: Neuropeptide Y; AMH: Anti-Mullerian hormone; BCL-2: B-cell lymphoma-2; IRS-1: Insulin receptor substrate-1; PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; GLUT4: Glucose transporter 4; BAX: Bcl2-Associated *X*; E_2: Estradiol; P: Progesterone; T: Testosterone; LH: Luteinizing hormone; OHSS: Ovarian hyperstimulation syndrome; CPR: Clinical pregnancy rate; PPR: Persistent pregnancy rate; LBR: live birth rate; BPR: Biochemical pregnancy rate; AR: Abortion rate; ☐: Improve; ☐: Reduce.

internal environment, such as "high GnRH" and "high LH." Therefore, acupuncture can comprehensively regulate various signal molecules in the human body and improve reproductive function from multiple angles. Based on the above studies, it is believed that acupuncture as a complementary and alternative therapy in assisted reproduction could be meaningful.

Acupuncture has also been widely used in clinics, where in addition to its curative effect, its simple operation and fewer adverse reactions are additional advantages. Different from the endogenous reaction caused by drugs, as a surgical operation, acupuncture treatment does not involve the use of any chemicals; hence, it is considered relatively safe. At present, the current research results are not entirely consistent. We speculate that it is mainly due to clinical heterogeneity, including different acupuncture schemes and doctors' levels. Although acupuncture is easy to operate, its efficacy is determined by the angle, depth, single duration, optimal acupuncture time, treatment course of acupuncture, different acupuncture manipulations at different acupoints, and differences in individual responsiveness of patients. Differences in these control variables lead to heterogeneity between clinical studies and are the main reason for the inconsistent research results, yet the effectiveness of acupuncture cannot be ignored. Based on the existing clinical reports, we can find that the positive effect of acupuncture was reflected in all aspects conducive to pregnancy, as shown in Table 3. We can divide these evaluation indicators into two categories: prepregnancy indicators (detection indicators), which are the conditions to increase the probability of pregnancy, while the other is the observation index after pregnancy (i.e., to observe the pregnancy outcome intuitively). The reports on negative results only focused on the observation indicators after pregnancy, that is, pregnancy outcome. However, the positive effects of acupuncture on the physical state of infertile women were never denied. We

know that exploring the differences in pregnancy outcomes is more challenging than detecting indicators with more stringent requirements in research design and observation duration. The premise of pregnancy is to have a good physical state. It mainly refers to mature follicles, high-quality embryos, and good endometrium, necessary conditions for a successful pregnancy. Therefore, in the process of observation and research, the effectiveness of acupuncture may first be reflected in the above aspects, followed by pregnancy outcomes. Thus, acupuncture is believed to be equally effective in resolving infertility issues through comprehensive analysis. It also necessitates studying the effectiveness of acupuncture in treating females' infertility by comparing results from different treatment cycles among other patient groups. It is believed that acupuncture effectiveness can be significantly reflected in longer treatment cycles, also reflected in the current research results, which are envisaged to help a more comprehensive understanding of acupuncture, improving the overall physiological function of infertile women.

Therefore, optimizing the acupuncture treatment plan in an all-around way will improve the clinical curative effect and help us explore acupuncture deeply. Similarly, it is suggested that more rigorous and more targeted studies in the future may provide strong evidence and help determine the application suitability of acupuncture in the field of reproduction, making it worthy of further investigation.

7. Conclusion

Acupuncture and related therapy can restore the average balance and regularity of sex hormones in infertile women by decreasing serum FSH and LH levels and increasing E2 and P levels, which are beneficial for follicle development and maturation, regular ovulation, and the normal physiological function of the inner membrane, which is conducive to conception. As a complementary and alternative therapy, acupuncture can play a positive role in PCOS-infertility, POI- infertility, and IVF-ET, with clinical application value.

Abbreviations

POI: Premature ovarian insufficiency
PCOS: Polycystic ovary syndrome
CE: Chronic endometritis
AI: Artificial insemination
IVF: In vitro fertilization
IVF-ET: In vitro fertilization-embryo transfer
ICSI: Intracytoplasmic sperm injection
CAM: Complementary and alternative medicine
EA: Electroacupuncture
ACE: Acupoint catgut embedding
TEAS: Transcutaneous electrical acupoint stimulation
TFF: Trefoil factor family
GFM: Gelatin forming mucin
POF: Premature ovarian failure
INH-B: Inhibin B
AMH: Anti-Mullerian hormone
ROS: Reactive oxygen species
HPO: Hypothalamic-pituitary-ovary
LH: Luteinizing hormone
HPRL: Hyperprolactinemia
TSH: Thyroid stimulating hormone
GnRH: Gonadotropin-releasing hormone
HPF: Hypothalamic-pituitary failure
Gn: Gonadotropin
IGH: Idiopathic gonadotropic hypogonadism
HPA: Hypothalamus-pituitary-adrenal
CRH: Corticotropin releasing hormone
CBT: Cognitive behavioral therapy
ER: Endometrial receptivity
BCL-2: B-cell lymphoma-2
BAX: BCL-2-associated X
FR: Free radicals
RCT: Randomized controlled trial
E2: Estradiol
P: Progesterone
STAI-1: State-trait anxiety inventory-1
OHSS: Ovarian hyperstimulation syndrome
PCO: Polycystic ovary
NGF: Nerve growth factor
CRF: Corticotropin releasing factor
ET-1: Endothelin-1
LC: Light chain
T: Testosterone
PI3K: Phosphatidylinositol-3-kinase
PKB: Protein kinase B
PKA: Protein kinase A
GAB2: Grb2-associated Binder2
OVX: Ovariectomy
Mtor: Mammalian target of rapamycin
S6K: S6 kinase
4E-BP1: 4E-Binding Protein1
CCL2: CC chemokine subfamily L2
CXCL8: CXC chemokine subfamily L8
MMP-2: Matrix metalloproteinase-2
VEGF: Vascular endothelial growth factor
PGF: Placental growth factor
ANG2: Angiopoietin 2
IGF-1: Insulin growth fact-1
PI: Pulsatility index.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank the mapping help provided by Shanghai Mumuchuang Culture Communication Co., Ltd. At the same time, the authors would like to thank

FreeScience (https://www.home-for-researchers.com) for the help with the English language.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 8099853, 17 pages
https://doi.org/10.1155/2022/8099853

Research Article

Research on the Mechanism of Asperosaponin VI for Treating Recurrent Spontaneous Abortion by Bioinformatics Analysis and Experimental Validation

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Received 12 September 2021; Revised 25 November 2021; Accepted 18 April 2022; Published 23 June 2022

Academic Editor: Yue Hui Zhang

Asperosaponin VI (AS6), as the quality marker of *Dipsaci Radix*, is verified to exert therapeutic effect on alleviating recurrent spontaneous abortion (RSA). However, due to the lack of relevant research, its molecular mechanism is still unclear. We retrieved targets for AS6 and RSA, and then used their overlapped targets for PPI analysis. In addition, we used GO and KEGG enrichment analyses, and molecular docking to investigate the anti-RSA mechanisms of AS6. Furthermore, we conducted *in vitro* experiments to validate the predictions of network pharmacology. Results showed that a total of 103 AS6-associated targets and 2084 RSA-associated targets, with 49 targets overlapped. GO enrichment analysis showed 845 significant biological processes like decidualization, while KEGG pathway enrichment analysis revealed 76 significant entries including 18 signaling pathways, which were closely linked to PI3K-Akt, HIF-1, TNF, IL-17, and VEGF signaling pathways, etc. Molecular docking findings verified that AS6 had tight link with the key targets including JUN, CASP3, STAT3, SRC, and PTGS2. Notably, *in vitro* experiments revealed that AS6 treatment could exert lower expressions of JUN, pro-CASP3, CASP3, STAT3, SRC, and PTGS2 in decidual cells compared with progesterone despite the expressions of STAT3, SRC, and PTGS2 with no significant difference, and mifepristone could interfere with the effects. In general, numerous targets and multiple pathways involve during the process of AS6 treatment against RSA. Moreover, our *in vitro* research first reported that AS6 may regulate the expressions of key targets (JUN, CASP3, STAT3, SRC, and PTGS2) in decidual cells to promote decidualization, thus treating RSA.

1. Introduction

Recurrent spontaneous abortion (RSA) is a common reproductive endocrine disorder, which refers to consecutive spontaneous abortions happening twice or more before 20 weeks of gestation [1, 2]. According to statistics, over 5% of women at reproductive age suffer from RSA [3]. Multiple pathological factors lead to RSA, including genetic factors, anatomical abnormalities, endocrine disorders, infectious diseases, and thyroid dysfunction [4, 5]. Embryonic chromosomal abnormality is the main cause of spontaneous abortion in early pregnancy, which results in over 50% of first trimester miscarriages [6–8]. However, there exist approximately 50% of RSA cases with unexplained recurrent spontaneous abortion [9]. RSA seriously poses threat to the life and health of pregnant women, increasing the burden on families and society. In recent years, the therapeutic effects of traditional Chinese medicine (TCM) on RSA have gradually been proven, attracting the attention of more and more scholars [10–12].

Shoutai pill has been often used for treating unexplained recurrent spontaneous abortion in China [10]. It has been

revealed that Shoutai pill can maintain the balance of Th1/Th2 cytokines, which improves endometrial receptivity and embryo implantation thus exerting therapeutic effects on RSA [13]. To date, the hot spots of current researches focus on the active ingredients of Shoutai pill. Asperosaponin VI (AS6, Pubchem CID: 118705380) is the quality marker of *Dipsaci Radix*, which is an important drug in Shoutai pill. Pharmacological studies in recent decades have shown that *Dipsaci Radix* has a variety of biological activities, including antiuterine contraction, antiinflammatory, antiaging, antiarthritic, antiosteoporosis, fracture healing, neuroprotection, and it has been verified to benefit Chinese women from miscarriages, serving as the preferred herb in clinical treatment [14]. According to Chinese Pharmacopoeia 2020 edition, *Dipsaci Radix* exerts effects on tocolysis and uterine bleeding during pregnancy, which has been an accumulated experience for thousands of years [15]. To date, the active components isolated from *Dipsaci Radix* mainly include saponins, triterpenes, volatile oils, and alkaloids, which may have curative effects on female reproductive disorders through significantly suppressing the spontaneous contractions of the gestational uterus induced by oxidative toxins [16]. Existing evidence suggests that *Dipsaci Radix* has an important application in anti-RSA treatment [15]. Moreover, we have investigated the action mechanism of *Dipsaci Radix* extracts and AS6 in our previous research and have observed that they may exert therapeutic effects on RSA by activating decidual cells' progesterone receptor expression through Notch signaling pathway [17]. The angiogenesis disorders of the endometrium and infection play important part in RSA, and existing evidence has shown that AS6 efficiently accelerates the angiogenesis of regenerated tissue and facilitates wound healing *in vivo*, and improves vascularization of human umbilical vein endothelial cells (HUVECs) *in vitro* by the upregulation of HIF-1α/VEGF pathway [18]. Moreover, it has been revealed that AS6 also inhibits the morphological expansion of microglia cells, decreases the expression, and releases of proinflammatory cytokines, such as IL-1B, iNOS, IL-6, and TNF-a in a dose-dependent manner [19]. However, whether AS6 can treat RSA through these pathways needs further research.

In the present study, we carried out bioinformatics analysis integrated with experimental validation so as to perform systematic analysis on various targets and pathways of AS6 for treating RSA.

2. Methods

We have referred to the methods of Ren et al. [20]. Figure 1 described the study flowchart.

2.1. Data Retrieval of Network Pharmacology

2.1.1. Retrieval of AS6-Associated Structure and Targets. First, we retrieved the information of AS6-associated structure and targets by searching TCMSP platform (https://tcmsp-e.com/) [21]. Second, Pubchem website (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the AS6 structure stored as "SDF" file that we subsequently uploaded into SwissTargetPrediction platform (https://new.swisstargetprediction.ch/) [22] to get the targets associated with AS6. Third, we used the UniProt platform (https://www.uniprot.org/uniprot/) to standardize the AS6-associated target information restricted to humans.

2.1.2. Retrieval of RSA-Associated Genes and Their Corresponding Proteins. Taking "Recurrent Spontaneous Abortion" as the key word, we searched GeneCards (https://www.genecards.org/) [23] and Online Mendelian Inheritance in Man (OMIM, https://omim.org/) [24], respectively. Then we carried out data standardization through Uniprot database to obtain corresponding proteins of *RSA-associated* genes.

2.1.3. Overlapped Target Proteins (OTPs). We utilized *R* (v3.6.1) software to take the intersection of AS6- and RSA-associated targets to obtain OTPs.

2.2. Data Analysis of Network Pharmacology

2.2.1. OTPs-Associated Protein Interaction Analysis. We obtained OTPs-associated Protein–Protein Interaction (PPI) data via retrieving the STRING platform (https://string-db.org/) [25]. Next, we plotted the PPI network with the PPI information of OTPs imported into Cytoscape software (v3.7.2; https://www.cytoscape.org/) [26] and carried out network topology analysis for the calculation of the target degrees. We screened the core targets with degrees above average. Afterwards, we constructed an AS6-OTPs-RSA network via Cytoscape.

2.2.2. GO Enrichment Analysis and KEGG Pathway Analysis. We performed Gene Ontology (GO) enrichment analysis concerning biological process (BP) via clusterProfiler package (R3.6.1) and selected the enrichment results with $p < 0.05$. Then the top 20 items and 20 representative items closely related to the pathological process of RSA were presented. Additionally, we input OTPs into Cytoscape for GO.BP enrichment analysis with p-value set to 0.001, and performed network visualization to establish linkages between biological processes and targets. Next, we carried out Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of OTPs using clusterProfiler package (R3.6.1), extracted the significant enrichment results ($p < 0.05$), and plotted pathway-target network using Cytoscape.

2.3. Molecular Docking between Key Targets and AS6. We selected the top five proteins in terms of degree for molecular docking, which were recognized as the key targets in the treatment of AS6 for RSA. We adopted AutoDock Vina software (v1.1.2) [27] to perform molecular docking simulations to investigate interaction activities between AS6 and key targets. The 3D structure of AS6 was obtained by retrieving the Pubchem platform (https://pubchem.ncbi.nlm.nih.gov/). AutoDock Tools (v1.5.6) was utilized to distribute charge and combine nonpolar hydrogen for AS6 and convert the results into a PDBQT file. We searched RCSB PDB

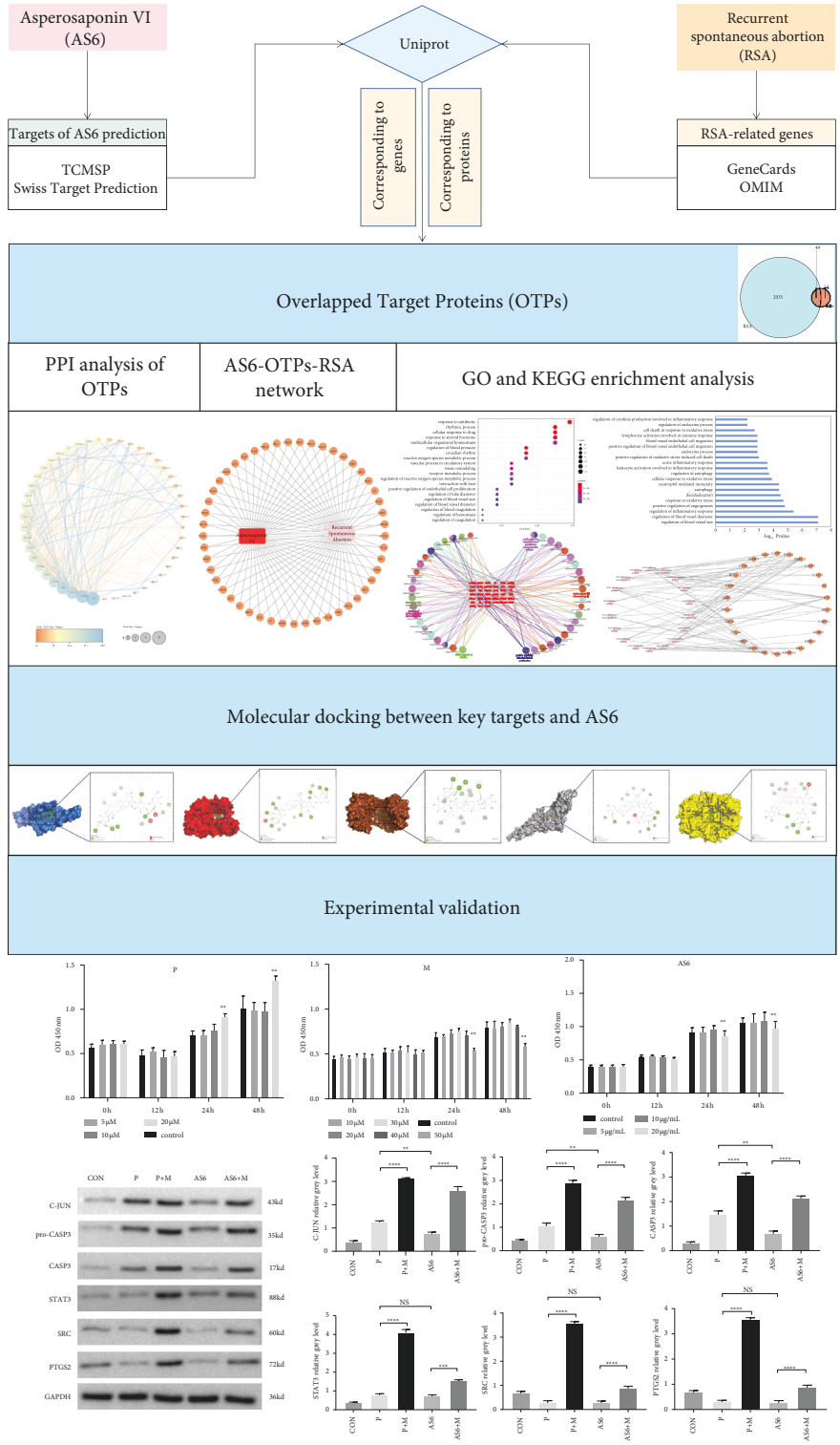


Figure 1: The flow chart of this study.

website (https://www.rcsb.org/) for the crystal structures of key targets. Then the target protein was separated from its ligand, distributed charge, and added polar hydrogen via AutoDock Tools, which would be subsequently stored as a PDBQT file. We used AutoDock Tools to determine the size and center of the docking box. Afterwards, we performed molecular docking simulations among AS6 and the target proteins with every affinity calculated. Then we analyzed and plotted the docking results of AS6 via PyMol and Discovery Studio.

Table 1: Potential target genes of AS6 in the treatment of RSA.

Number	Gene	Number	Gene
1	BCL2L1	26	PTPRC
2	F2	27	F3
3	RORC	28	F7
4	STAT3	29	LNPEP
5	GLRA2	30	CTSB
6	TYMS	31	CASP3
7	ADORA1	32	REN
8	TOP1	33	CASP7
9	PTGS2	34	CASP1
10	F2RL1	35	CAPN1
11	GBA	36	GRB2
12	JUN	37	PRKCA
13	VDR	38	ACE
14	ADRA2B	39	ITGAV
15	DRD2	40	HLA-A
16	CYP2D6	41	MMP3
17	HTR1B	42	MMP10
18	RRM1	43	SIRT1
19	ADRB1	44	MMP8
20	NR3C1	45	APEX1
21	SRC	46	HDAC6
22	XIAP	47	IGF1R
23	PLG	48	ITGA4
24	ITGB1	49	ITGB3
25	NOS2		

2.4. Validation of AS6 by in Vitro Assays

2.4.1. Cells, Reagents, and Antibodies. We obtained primary decidua cells from decidua tissues. The source of Asperosaponin VI was purchased from Guangdong Food and Drug Administration, China. We purchased progesterone and mifepristone from Sigma Aldrich. We bought 0.25% trypsin, DMEM/F12, FBS, Charcoal dextran-treated FBS, and Lipofectamine™ 2000 from Gibco. The antibodies used in this research, such as C-JUN (AF1612), CASP3 (AC030), STAT3 (AF1492), SRC (AF1831), PTGS2 (AF1924), and GAPDH (AF1186) antibodies were purchased from Beyotime (Shanghai, China). We diluted the primary antibody at a ratio of 1 : 1000 with QuickBlock™ Primary Antibody Dilution Buffer for Western Blotting (Beyotime) and the secondary antibody at a ratio of 1 : 10000 with QuickBlock™ Secondary Antibody Dilution Buffer for Western Blotting (Beyotime).

2.4.2. Isolation and Culture of Primary Decidual Cells. We obtained decidua samples at 6–9 week of gestation from singleton pregnant women who requested normal pregnancy termination or who underwent excretion of retained pregnancy products after a failed spontaneous pregnancy. All patients signed written informed consent in accordance with Declaration of Helsinki, and permission was obtained from Ethics Committee of the 1st Affiliated Hospital of Guangzhou University of TCM. The ethics code is No.K [2019] 098.

Fresh decidua tissues were taken aseptically, washed in PBS to remove blood, cut into pieces, digested with trypsin-EDTA (0.25%) for 5–10 min, and digestion was stopped by adding DMEM/F12 medium containing 10% FBS. The cell clusters in the final digestion were extracted with a 23-gauge needle, filtered through a 200-mesh sieve, centrifuged at 2000 rpm for 5 min, resuspended in DMEM/F12 medium containing 10% FBS, and incubated in flask at 5×10^5 cells/ml. After 30 min, we removed nonadherent cells and replaced the medium after 48 h.

2.4.3. Cell Counting Kit 8 Assay. We treated the decidual cells with a concentration gradient of progesterone (P), mifepristone (M), and AS6 for 12, 24, and 48 h. According to the manufacturer's protocol, we performed a cell counting kit 8 (CCK-8) assay to detect cell proliferation abilities using an optical density (OD) setting of 450 nm in the microplate reader (Varioskan Flash; Thermo Fisher Scientific, Waltham, MA, USA).

2.4.4. Western Blotting. We cultured decidual cells in 6-well plates and treated them with the specific concentration of progesterone, Asperosaponin VI with or without mifepristone according to CCK-8 screening results. After the treatment, the protein was extracted by adding 200 μL RIPA lysis buffer prepared with phosphatase inhibitor and protease inhibitor per dish (Beyotime). The protein bands were transferred to polyvinylidene fluoride membranes (Shanghai, microtiter wells) by electrophoresis and wet transfer steps, closed with QuickBlock™ Western closing solution (Beyotime) at room temperature for 30 min, added primary antibody and incubated overnight at 4°C in a shaker, then

added the corresponding secondary antibody and incubated in shaker at 24°C for 1.5 h. The antibody reactivity level was subsequently detected by gel imaging system (Bio-Rad). Finally, the grayscale values were quantitated using ImageJ software.

2.4.5. Statistical Analysis. All results were expressed as mean ± standard deviation. Student's *t*-tests were used to compare two separate samples. One-way ANOVA was used for comparison of univariate samples between multiple groups. p-value <0.05 indicates statistical significance.

3. Results

3.1. AS6-Associated Structure and Target Proteins. We obtained a total of 103 AS6-associated targets. After data standardization by the UniProt database, we obtained AS6-related target proteins called as Gene symbols. AS6-associated structure and target results were shown in supplementary Tables S1–S2.

3.2. RSA-Associated Target Information and Overlapped Target Proteins (OTPs). A total of 2084 RSA-associated target proteins were retrieved. We took the overlap of AS6- and RSA-associated targets as OTPs, which included 49 overlapped targets, as shown in Table 1 and Figure 2(a).

3.3. Construction of PPI Network and Screening Core Target Proteins. OTPs were imported into the STRING platform with the targets having no link to others hidden. We imported the PPI data into Cytoscape to draw PPI network in Figure 2(b). There were 21 target proteins predicted to be the core target proteins (Table 2), whose degrees were above average degree (9.83).

3.4. AS6-OTPs-RSA Network Plotting. Figure 2(c) shows AS6-OTPs-RSA network with 51 nodes and 98 edges included. In Figure 2(c), the orange circular nodes stand for the overlapped target proteins (OTPs). The red rectangle node stands for "Asperosaponin VI." The pink rectangle node stands for "Recurrent Spontaneous Abortion." The edges stand for the interactive relationships between Asperosaponin VI, recurrent spontaneous abortion, and the overlapped targets.

3.5. GO Enrichment Analysis. We got 845 items of biological process (BP). The top 20 items were shown in Figure 3(a). Noteworthily, we have filtrated 20 items mainly linked to autophagy, blood vessel endothelial cell migration, angiogenesis, inflammatory response, oxidative stress, decidualization, endocrine process, and immune response, which were demonstrated in Figure 3(b). Additionally, we input 49 OTPs into Cytoscape for GO.BP enrichment analysis with p-value set to 0.001. Figure 3(c) illustrated the enrichment results mainly involved in four aspects as follows: (i) inflammation-related activities, such as regulation of neuroinflammatory response and extracellular matrix disassembly which is closely associated with oxidative stress; (ii) cell cycle, such as positive regulation of endothelial cell proliferation and migration; (iii) tissue repair, such as positive regulation of response to wounding and wound healing and regulation of tissue remodeling; and (iv) endocrine metabolism process, such as regulation of cofactor metabolic process, adrenergic receptor activity, and negative regulation of synaptic transmission.

3.6. KEGG Pathway Analysis. We finally got totally 76 items including 18 key signaling pathways listed in Table 3. These signaling pathways such as PI3K-Akt, HIF-1, TNF, IL-17, and VEGF may exert regulatory functions on the process of AS6 against RSA. We conducted network visualization via Cytoscape as plotted in Figure 3(d), which established the relationship between signaling pathways and targets. Specifically, several OTPs were involved in PI3K-Akt signaling pathway (e.g., BCL2L1, ITGB1, GRB2, PRKCA, ITGAV, IGF1R, ITGA4, ITGB3), HIF-1 signaling pathway (e.g., STAT3, NOS2, PRKCA, and IGF1R), TNF signaling pathway (e.g., PTGS2, JUN, CASP3, CASP7, and MMP3), IL-17 signaling pathway (e.g., PTGS2, JUN, CASP3, and MMP3), and VEGF signaling pathway (e.g., PTGS2, SRC, PRKCA).

3.7. Molecular Docking Analysis. Among 21 core targets, the top five target proteins in terms of degree were chosen for molecular docking, including JUN, CASP3, STAT3, SRC, and PTGS2, respectively, which were considered as the key targets in the process of AS6 treating RSA. To verify how AS6 binds to the key targets, we adopted molecular docking using Autodock Vina to predict their docking interactions. Table 4 showed the docking results including affinity and interaction information.

Based on Figure 4(a), AS6 combined with JUN by forming six hydrogen bonds with the residues including Gln-30, Arg-5, Arg-21, Asp-26, and Lys-22 (binding affinity: −7.2 kcal/mol). Besides, there were three van der Waals interactions between AS6 and Tyr-18, Lys-9, and Leu-13.

Based on Figure 4(b), the docking affinity of AS6 on SRC was −9.3 kcal/mol. The residues containing Glu-339, His-319, Gln-253, Lys-152, Phe-150, Tyr-90, Thr-247, and Ser-248 linked to AS6 by forming nine hydrogen bonds, which provided a powerful electrostatic force for the combination of AS6 and SRC. Moreover, there were five van der Waals interactions between AS6 and Gln-251, Leu-322, Lys-401, Pro-250, and Ile-153.

Based on Figure 4(c), the docking affinity of AS6 on CASP3 was −9.7 kcal/mol. There existed six hydrogen bonds provided by the Arg-164, Cys-264, and Glu-124 residues in the link to AS6. Moreover, AS6 binded with the Gly-125, Thr-140, Gly-202, Tyr-197, and Glu-124 residues by six van der Waals.

Based on Figure 4(d), the docking affinity of AS6 on STAT3 was −7.6 kcal/mol. There were five hydrogen bonds provided by the Glu-324, Ser-513, Gln-247, and Cys-251 residues in the interaction with AS6. What is more, there were five van der Waals interactions between AS6 and Trp-510, Pro-336, Lys-348, Gln-326, and Trp-243.

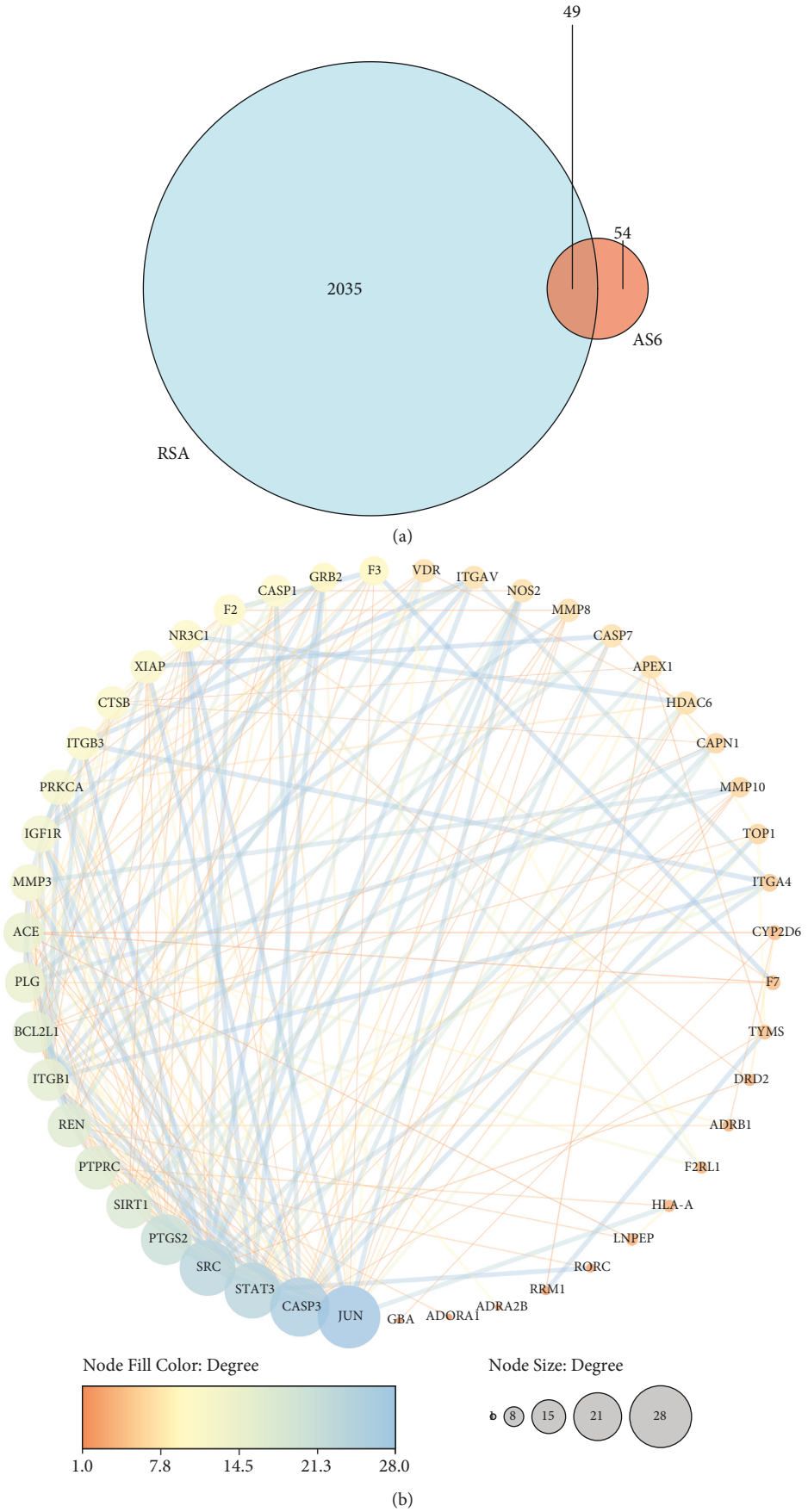
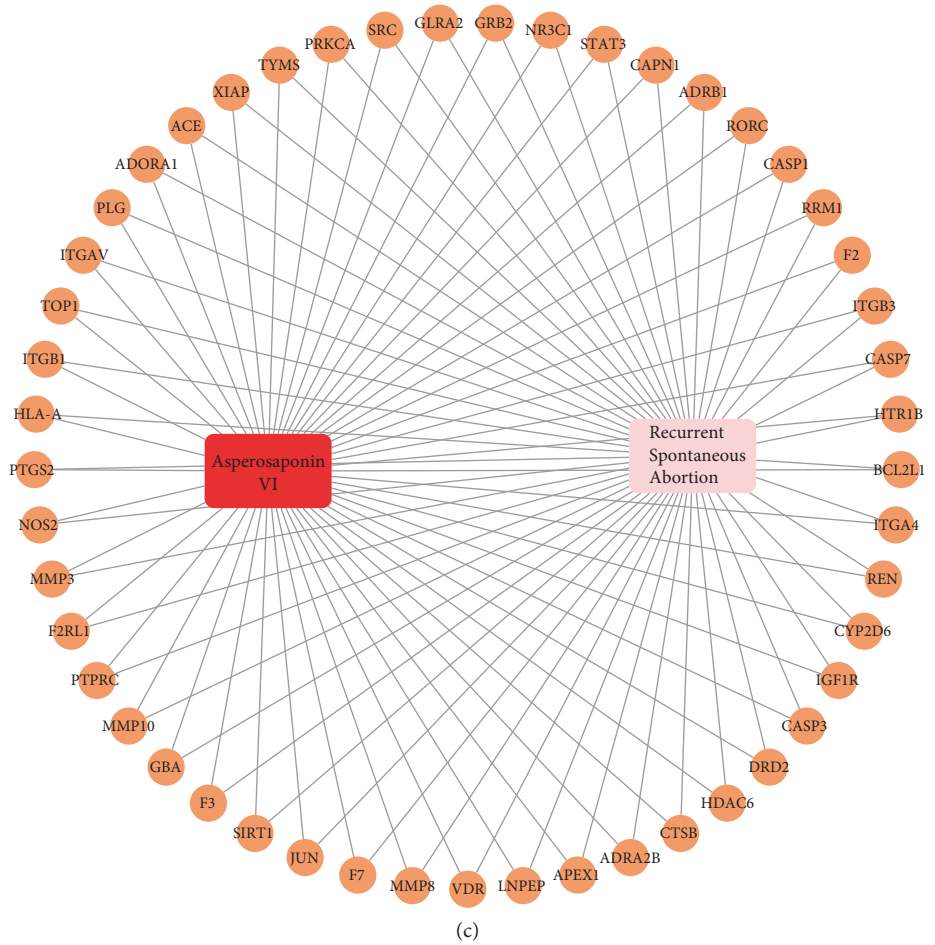


Figure 2: Continued.



(c)

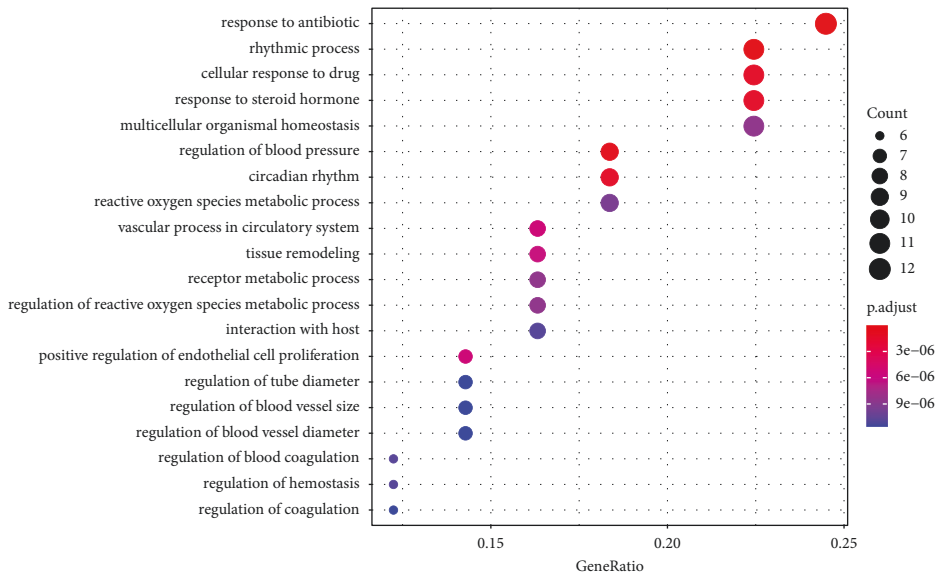
Figure 2: Venn diagram of OTPs (a), PPI network of OTPs (b), and AS6-OTPs-RSA network (c).

Table 2: Core targets of AS6 in the treatment of RSA.

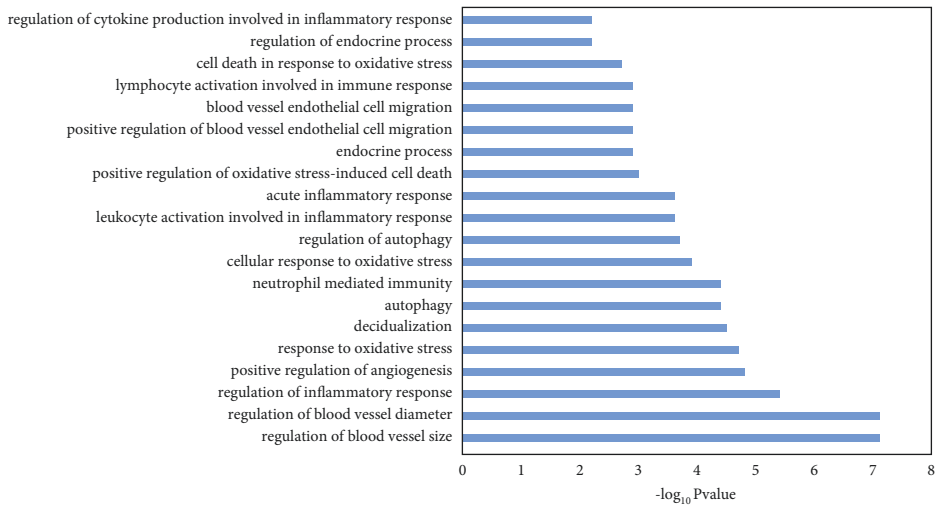
Number	Core Targets	Degree
1	JUN	28
2	CASP3	26
3	STAT3	24
4	SRC	24
5	PTGS2	21
6	SIRT1	18
7	REN	17
8	PTPRC	17
9	ITGB1	16
10	BCL2L1	16
11	ACE	15
12	PLG	15
13	MMP3	13
14	IGF1R	13
15	PRKCA	12
16	CTSB	11
17	XIAP	11
18	ITGB3	11
19	F2	10
20	NR3C1	10
21	CASP1	10

Based on Figure 4(e), the docking affinity of AS6 on PTGS2 was −10.5 kcal/mol. The Glu-236, Ser-143, and Glu-140 residues formed three hydrogen bonds in the interaction with AS6. Additionally, there were five van der Waals interactions between AS6 and Ser-143, Arg-333, Asn-144, Ser-146, and Gly-225.

3.8. CCK-8 Assay. We performed CCK-8 assays before the *in vitro* research. The concentrations of progesterone used in the study were 0 (control group), 5, 10, and 20 μmol/L. The results revealed that the progesterone concentration at 20 μmol/L exerted proliferative effect on the proliferation of decidual cells, which was selected for subsequent experiments (Figure 5(a)). The concentrations of mifepristone used in the study were 0 (control group), 10, 20, 30, 40, and 50 μmol/L. The results revealed that the mifepristone concentration at 50 μmol/L exerted suppressive effect on the proliferation of decidual cells, which was selected for subsequent experiments (Figure 5(b)). The concentrations of AS6 used in the study were 0 (control group), 5, 10, and 20 μg/mL. The results revealed that there was no cytotoxicity

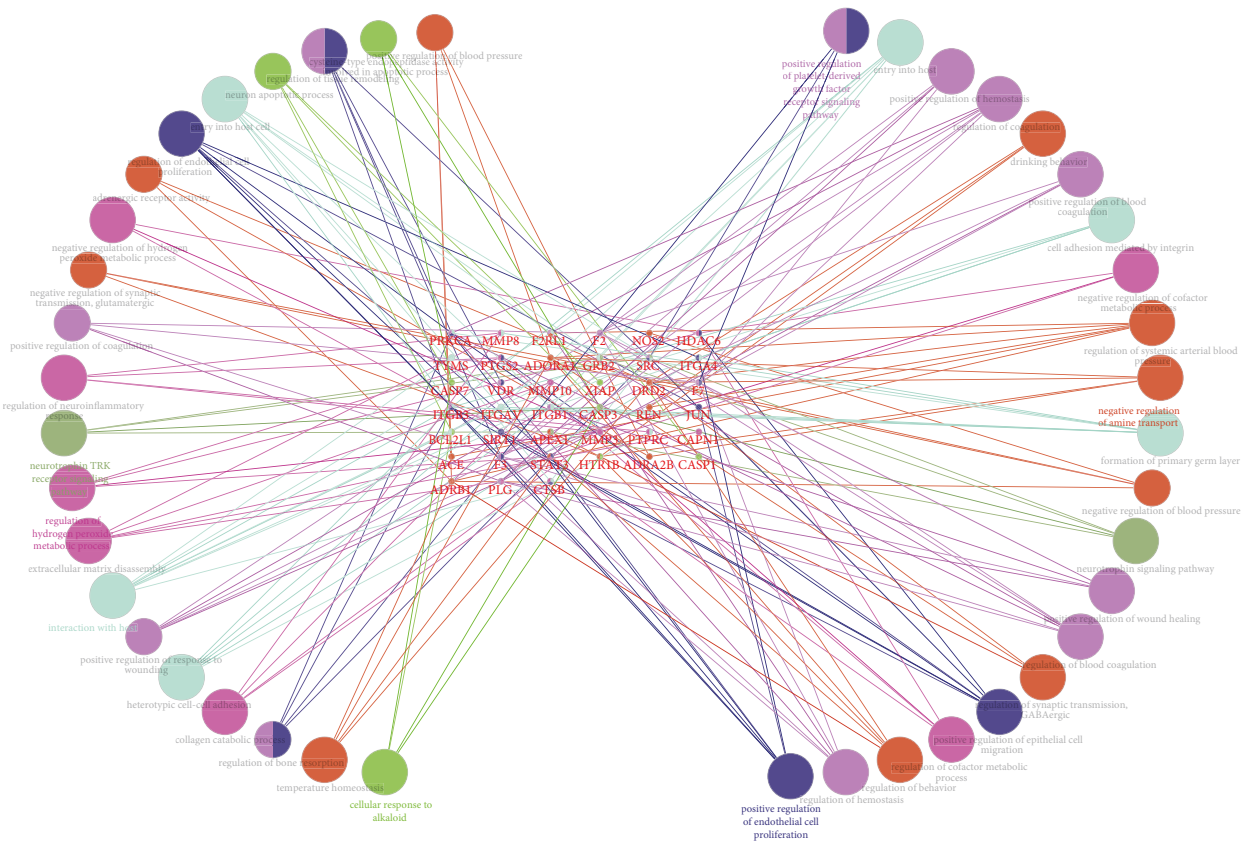


(a)

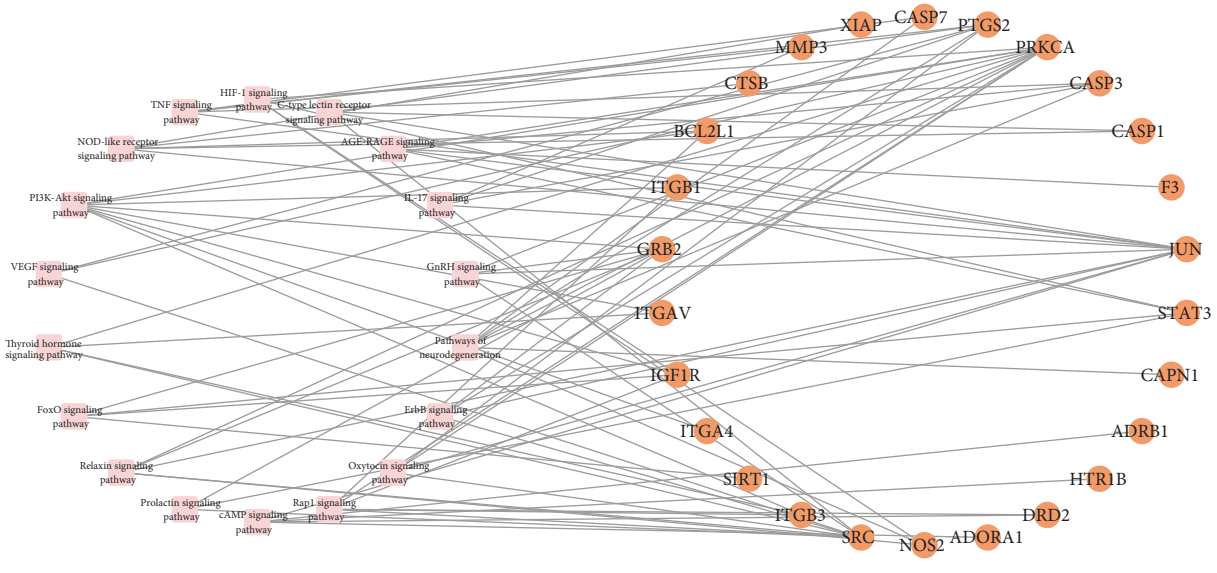


(b)

Figure 3: Continued.



(c)



(d)

Figure 3: GO.BP enrichment analysis (a–c), and pathway-target network (d). (a,b) The top and screened 20 items of biological processes in terms of p-value. (c) Different colors represent different biological process groups and node size stands for term p-value, while the edges represent the connections between biological processes and targets. (d) A pink square node represents a signaling pathway, an orange circular node represents a gene, and an edge represents a relationship between a pathway and a gene.

to decidual cells when the AS6 concentration was no higher than 10 μg/mL with the proliferation of decidual cells neither promoted nor inhibited, which was selected for subsequent experiments (Figure 5(c)).

3.9. Western Blotting Analysis. To investigate the function of Asperosaponin VI in decidual cells, we tested the expression levels of specific proteins including JUN, CASP3, pro-CASP3, STAT3, SRC, and PTGS2 to examine the influence

Table 3: KEGG pathway enrichment analysis.

ID	Signaling Pathway	Enriched Gene Number	p-value
hsa04933	AGE-RAGE signaling pathway	5	0.000238979
hsa04668	TNF signaling pathway	5	0.000404789
hsa04151	PI3K-akt signaling pathway	8	0.000751101
hsa04926	Relaxin signaling pathway	5	0.000772967
hsa04015	Rap1 signaling pathway	6	0.001119119
hsa04012	ErbB signaling pathway	4	0.001321844
hsa04912	GnRH signaling pathway	4	0.001844088
hsa04657	IL-17 signaling pathway	4	0.001918105
hsa04625	C-type lectin receptor signaling pathway	4	0.002775155
hsa04066	HIF-1 signaling pathway	4	0.003289067
hsa04621	NOD-like receptor signaling pathway	5	0.003705682
hsa04370	VEGF signaling pathway	3	0.004472814
hsa04919	Thyroid hormone signaling pathway	4	0.0047801
hsa04068	FoxO signaling pathway	4	0.006326533
hsa04917	Prolactin signaling pathway	3	0.007211213
hsa04024	cAMP signaling pathway	5	0.007706803
hsa04921	Oxytocin signaling pathway	4	0.011068984
hsa05022	Pathways of neurodegeneration	7	0.016957114

Table 4: Molecular interactions of key targets with AS6.

Compound	Target	PDB ID	Affinity (kcal/mol)	Number of hydrogen bonds	Hydrogen bonds interacting residues
Asperosaponin VI	JUN	5FV8	−7.2	6	Gln-30 (2), Arg-5, Arg-21, Asp-26, Lys-22
Asperosaponin VI	CASP3	3DEI	−9.7	6	Arg-164 (4), Cys-264, Glu-124
Asperosaponin VI	STAT3	6NUQ	−7.6	5	Glu-324 (2), Ser-513, Gln-247, Cys-251
Asperosaponin VI	SRC	2SRC	−9.3	9	Glu-339 (2), His-319, Gln-253, Lys-152, Phe-150, Tyr-90, Thr-247, Ser-248
Asperosaponin VI	PTGS2	5F19	−10.5	3	Glu-236, Ser-143, Glu-140

of Asperosaponin VI treatment via Western blotting. The treatment concentrations of progesterone, mifepristone, and Asperosaponin VI were 20 μmol/L, 50 μmol/L, and 10 μg/mL respectively, according to CCK-8 assay. As shown in Figures 5(d)–5(e), Asperosaponin VI treatment could exert lower expressions of JUN, pro-CASP3, CASP3, STAT3, SRC, and PTGS2 in decidual cells compared with progesterone, while the expressions of STAT3, SRC, and PTGS2 showed no significant difference between Asperosaponin VI-treated and progesterone-treated groups, and mifepristone could interfere the effects.

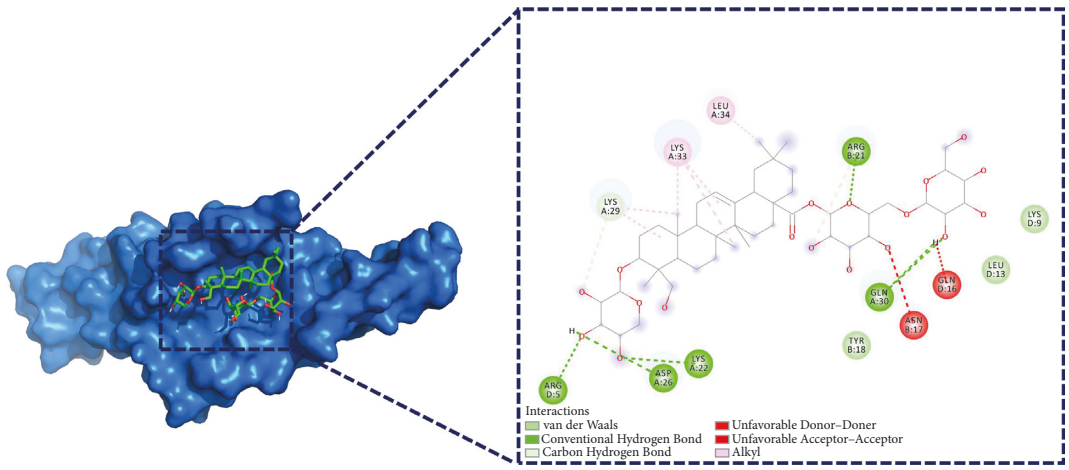
4. Discussion

Chinese traditional medicine *Dipsaci Radix*, a drug in Shoutai pills, has been widely applied in treating gynecological diseases like RSA clinically for many years. Our present study explored the mechanisms of Asperosaponin VI in treating RSA, which is an important component of *Dipsaci Radix*.

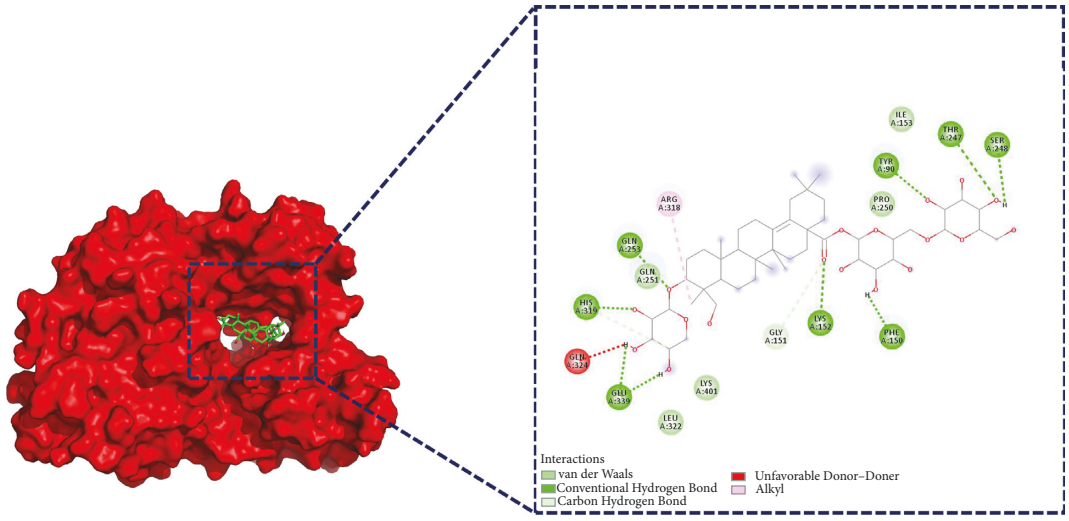
Progesterone (P) exerts essential effects on the maintenance of pregnancy, the declining level of which in blood in early pregnancy leads to necrosis of the decidua, thereby causing miscarriage [28]. Mifepristone (M) is the first-known progesterone antagonist, which eventually results in conception abortion when used postimplantation [29]. In this study, we reported the strong progesterone-like effects of Asperosaponin VI and its actions in the treatment of RSA.

According to PPI network topology analysis of OTPs, we noticed that these targets were characteristics of decidualization, autophagy, angiogenesis, oxidative stress, inflammation, and endocrine-related proteins. We identified five key targets including JUN, CASP3, STAT3, SRC, and PTGS2, which are in close conjunction with AS6 according to molecular docking findings, indicating that they may be the key targets of AS6 in treating RSA.

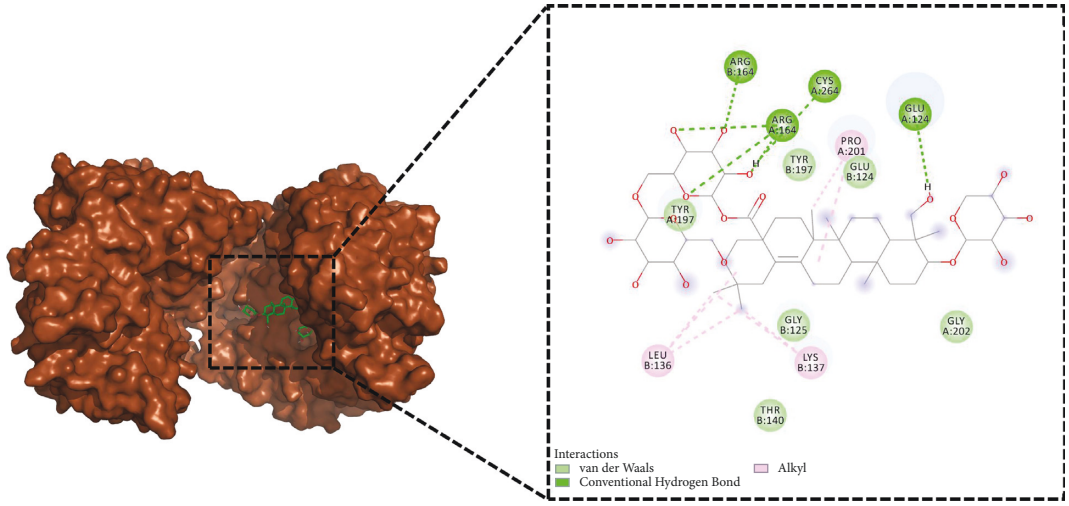
JUN (Transcription factor AP-1 subunit Jun), which is the mediator of trophoblast invasion, plays a critical role in decidualization [30, 31]. It has been revealed that down-regulation of JUN production could alter epithelial mesenchymal transition (EMT)-related molecule expression, which would impede trophoblast migration and invasion [32]. Existing research has confirmed that activation of JUN expression involves the chemokine recruitment of human first trimester decidual cells (FTDCs), triggering response to proinflammatory stimuli, which serves as an essential factor for RSA [33]. Further study has clarified that the accumulation of CX3CL1 chemokine results from the induction of



(a)



(b)



(c)

Figure 4: Continued.

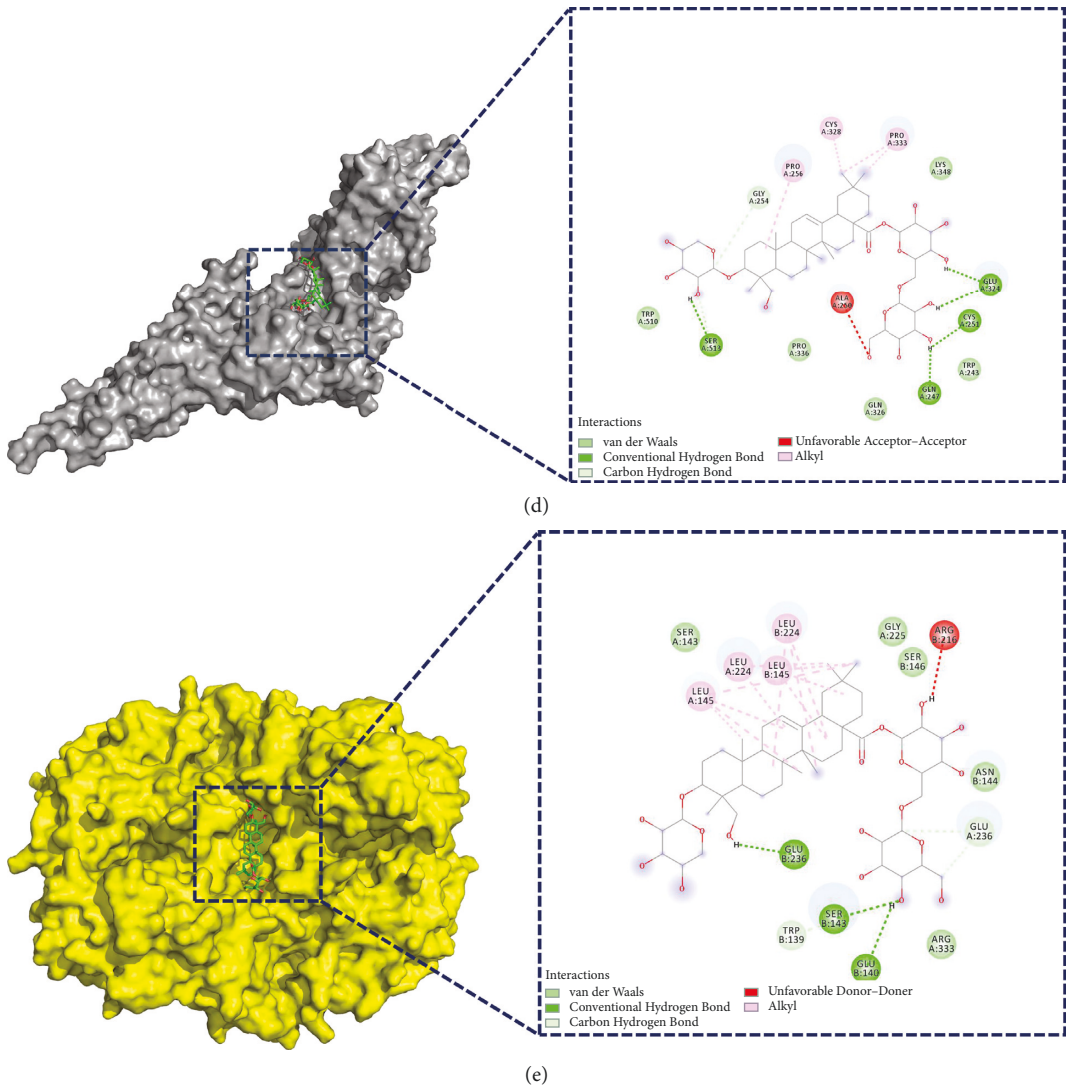


Figure 4: Simulated molecular docking of Asperosaponin VI on JUN (a), SRC (b), CASP3 (c), STAT3 (d), and PTGS2 (e).

IL-1β, TNF-α, and IFN-γ in FTDCs, which can be mediated by the activation of JUN-related signaling [34]. Notably, our experiments displayed lower expression level of JUN in decidual cells in AS6-treated group compared with progesterone-treated group, suggesting that AS6 could suppress the expression of JUN in decidual cells to promote decidualization so as to anti RSA.

Caspase-3 (CASP3) is an apoptosis-related gene, whose expression has close correlation with placental separation [35]. Some studies have identified myometrial CASP3 as a potential regulator of uterine quiescence, and uterine endoplasmic reticulum stress-unfolded protein response regulation of gestational length is CASP3-dependent [36]. Decidual cell apoptosis could be mediated by TNF-related apoptosis-induced ligand (TRAIL) via CASP3-dependent pathway, whose expression is upregulated in decidua from women suffering from RSA [37]. Moreover, the inhibition of CASP3 activity could prevent the apoptosis of uterine stromal cells, which could proliferate and then differentiate into decidual cells during the process of decidualization [38]. CASP3 exerts an essential role during the process of decidualization, while the increased expression of CASP3 in endometrium decidua indicates poor endometrial receptivity, which could lead to RSA [39]. Notably, our experiments revealed that AS6 treatment could exert lower expressions of pro-CASP3 and CASP3 in decidual cells compared with progesterone, suggesting that AS6 could downregulate CASP3 expression in decidual cells to promote decidualization so as to anti RSA.

Signal transducer and activator of transcription 3 (STAT3) phosphorylation has a close relationship with embryo implantation and decidualization [40]. RSA results from impaired trophoblast function, and further study has shown that STAT3 expression could affect trophoblast cell proliferation and migration [41]. Existing studies have confirmed that the reduction of plasmacytoid dendritic cells in RSA could be mediated by the regulation of STAT3 expression [42]. STAT3 signaling has been verified to exert anti-inflammatory IL-10 expression in decidua cells to protect pregnancy [43]. SRC (Proto-oncogene tyrosine-

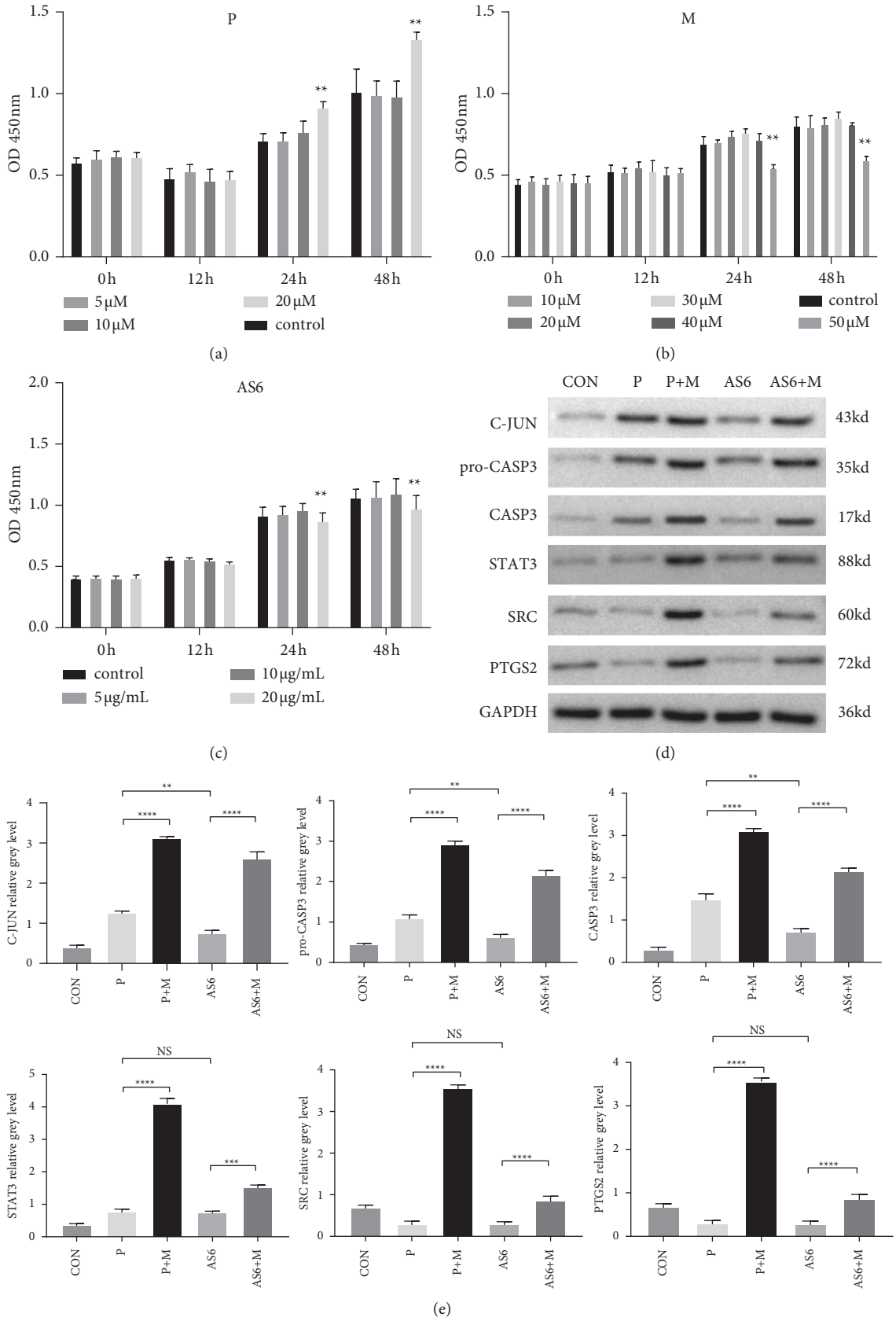


Figure 5: Asperosaponin VI influenced key targets' expression in decidual cells. Decidual cells were treated with progesterone (P), or Asperosaponin VI (AS6) respectively, with or without mifepristone (M) for 24 h in Western blotting. (a, b, c) CCK-8 assays of (P) M and AS6 (d) The protein expressions of key targets including JUN, CASP3, STAT3, SRC and PTGS2 were detected by Western blotting. (e) Representation of the relative grey level in (D) Data are displayed as mean ± standard deviation. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

protein kinase Src) is endometrial nuclear receptor cofactor, which plays an important part in regulating human endometrium remodeling [44]. It has been shown that SRC could regulate endometrial function and progesterone-related gene expression [45]. Transcriptomics has confirmed that SRC gets involved in the process of decidualization [46]. Further study has shown that the expression of SRC is necessary for invasion and migration of human decidual stromal cells, which exerts vital functions in embryo implantation and human pregnancy [47]. Prostaglandin G/H synthase 2 (PTGS2) is related to the regulation of inflammatory response, the regulation of which influences the decidualization response of endometrial stromal cells [48]. Numerous studies have shown that PTGS2 is identified as important regulators of early pregnancy events and plays a vital role in human decidualization and vascularization of the endometrial stroma [49, 50]. In our present study, we observed lower expressions of STAT3, SRC, and PTGS2 in decidual cells after AS6 treatment compared with progesterone, but the difference was not statistically significant, suggesting that AS6 may exert progesterone-like effect in the treatment of RSA.

Similar to PPI analysis, GO enrichment results show consistent results as demonstrated in Figure 3(b). Decidualization plays an indispensable role in normal pregnancy, while suppressed decidualization contributes to increased prevalence of RSA [51]. Numerous studies have confirmed that the expressions of key targets including JUN [52], CASP3 [38], STAT3 [53], SRC [46], and PTGS2 [54] play an essential role in decidualization. In the present study, we observed that AS6 displayed strong effects on the expressions of JUN, CASP3, STAT3, SRC, and PTGS2, even better than the positive control progesterone, indicating that AS6 may play a strong progesterone-like function to promote decidualization against RSA. It has been verified that autophagy makes key functions in RSA-related pathogenesis, which affects trophoblast invasion and adhesion [55]. Some evidences have illustrated that oxidative stress is one of the important factors that trigger RSA [56]. According to our present study, AS6 may be an antioxidant with a good prospect that helps reduce oxidative stress and improve RSA. Endometrial angiogenesis disorders and infection exert key functions in RSA, and it has been shown that AS6 can effectively accelerate the angiogenesis of regenerated tissues and promote wound healing, and promote the vascularization of HUVECs [18]. AS6 can also inhibit the morphological expansion of microglia, reduce the expression of pro-inflammatory cytokines such as IL-1B, iNOS, TNF-α, IL-6, IL-1B, and TNF-α in a dose-dependent manner [19].

KEGG enrichment results revealed that PI3K-Akt, HIF-1, TNF, IL-17, and VEGF signaling pathways may exert regulatory functions on AS6 against RSA. Some studies have verified that the inhibition of PI3K-Akt signaling pathway can reduce trophoblast cell proliferation and migration [57]. Moreover, studies have shown that activation of PI3K-Akt signaling pathway could promote endometrial decidualization [58]. However, whether AS6 could regulate PI3K-Akt signaling pathway to treat RSA is still unclear, which needs further identification in the future research. Our present study has shown that AS6 may treat RSA through the regulation of angiogenesis and tissue repair as described in Figure 3(b). And some studies have verified that AS6 can promote the angiogenesis of HUVECs *in vitro* by upregulating HIF-1α/VEGF pathway and can effectively promote the angiogenesis of regenerative tissues and promote wound healing *in vivo* [18]. So HIF-1 signaling pathway and VEGF signaling pathway have close connection with AS6 treatment in RSA. In addition, inflammatory response-related pathways including TNF and IL-17 signaling pathways play vital role in the pathological process of RSA. Existing study has shown that the balance between pro-inflammatory cytokines on TNF signaling pathway exerts important influence on the success or failure of the implanted embryos [59]. Abnormal expression of IL-17 in the feto-maternal interface may lead to RSA [60].

In summary, our results predict some potential therapeutic targets and pathways, providing reference for future studies on AS6 treatment against RSA. However, one limitation of this study is that further *in vivo* and *in vitro* experiments are needed to confirm our findings.

5. Conclusion

Collectively, our results revealed that AS6 may treat RSA possibly by regulating numerous signaling pathways and targets related with decidualization, autophagy, blood vessel endothelial cell migration, angiogenesis, inflammatory response, oxidative stress, and immune response, etc. Moreover, our *in vitro* study first reported that AS6 may regulate the expressions of key targets in decidual cells including JUN, CASP3, STAT3, SRC, and PTGS2 to promote decidualization, thus treating RSA.

Data Availability

The data to support the study's results came from the first author.

Ethical Approval

This study was approved by Ethics Committee of the 1st Affiliated Hospital of Guangzhou University of TCM. The ethics code is No.K [2019] 098.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All the authors have actively participated in the planning of the work, data gathering, analyzing, and writing the manuscript. All the authors have read and confirmed their participation in the manuscript. The authors Bo Xia and Peng Zhang contributed equally to this work.

Acknowledgments

The project was generously supported by the grants from National Natural Science Foundation of China (81774358), Special Projects in Key Fields of Guangdong Universities (Biomedicine and Health) (2021), Guangzhou Key Laboratory of TCM Prevention and Treatment of Female Reproductive Disorders (2022), Guangdong Provincial Key Research and Development Program (2020B1111100003), and Double First-rate and High-level University Discipline Collaborative Innovation Team Project of Guangzhou University of Chinese Medicine (2021XK04).

Supplementary Materials

Supplementary Table S1. The Structure of Asperosaponin VI. Supplementary Table S2. Targets of Asperosaponin VI. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 2574438, 18 pages
https://doi.org/10.1155/2022/2574438

Review Article

Current Research on Complementary and Alternative Medicine in the Treatment of Premature Ovarian Failure: An Update Review

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Received 19 April 2022; Accepted 24 May 2022; Published 23 June 2022

Academic Editor: Tian-Bo Liu

Complementary and alternative medicine (CAM) encompasses a wide range of different non-mainstream therapies that have been increasingly used for the treatment or adjunct treatment of various ailments, with premature ovarian failure (POF) being one of the most common conditions treated with CAM. This review updates the progress of CAM in the treatment of POF, and we focus specifically on reviewing the evidence for the efficacy and mechanisms of a range of CAM treatments in POF, including single herbal medicines and their active ingredients, compound Chinese medicines, acupuncture and moxibustion, psychotherapy, exercise, vitamins, massage, and dietary supplements. According to the literature, CAM is very helpful for improving POF symptoms, and we hope to provide some instructive suggestions for clinical treatment and experimental research in the future. However, more clinical trials are needed to prove the safety of CAM.

1. Introduction

Premature ovarian failure (POF) is a common endocrine disease leading to female amenorrhea and infertility. It is characterized by high expression of gonadotropin (follicle-stimulating hormone (FSH) ≥ 40 mIU/ml), low expression of estradiol (E2), and poor follicular development in women under 40 years old. Clinical symptoms are also accompanied by perimenopausal symptoms such as hot flashes, sweating, mood swings, sleep disorders, and low sexual desire [1–3]. At present, it is considered that the occurrence and development of POF are closely related to gonadotropin-related disorders [4], immune factors [5, 6], infectious factors [7, 8], iatrogenic factors [9], lack of metabolism-related enzymes [10], environmental factors [11], socio-psychological factors [12], and genetic factors [13] (Figure 1). The incidence of POF is about 1.0% globally, while in China, it is 1%–3.8%. Primary amenorrhea accounts for 20%–28% of POF cases, secondary amenorrhea accounts for about 4%–20% of cases, and about 74%–90% of POF cases have unknown etiology. The incidence of POF has been increasing in recent years with the development of the economy, changes in social relationships, and unhealthy lifestyles [14–16].

At present, the most common clinical treatment for POF is still hormone replacement therapy (HRT), but other therapies include in vitro activation, stem cell therapy, tissue engineering, and regenerative medicine. HRT is considered to reduce the risk of infection, osteoporosis, cardiovascular disease, and urogenital atrophy and improve the quality of life of women with POF. However, HRT does not fully restore ovarian function, and some studies have reported that HRT is a predisposing factor for breast cancer [17]. Therefore, clinicians are still looking for a new method to treat POF through complementary and alternative medicine (CAM). CAM is usually defined as "non-traditional" medicine according to the recommendations of the National Center for Comprehensive and Integrative Health (NCCIH). If unconventional methods or products are used together with conventional drugs, it is called complementary

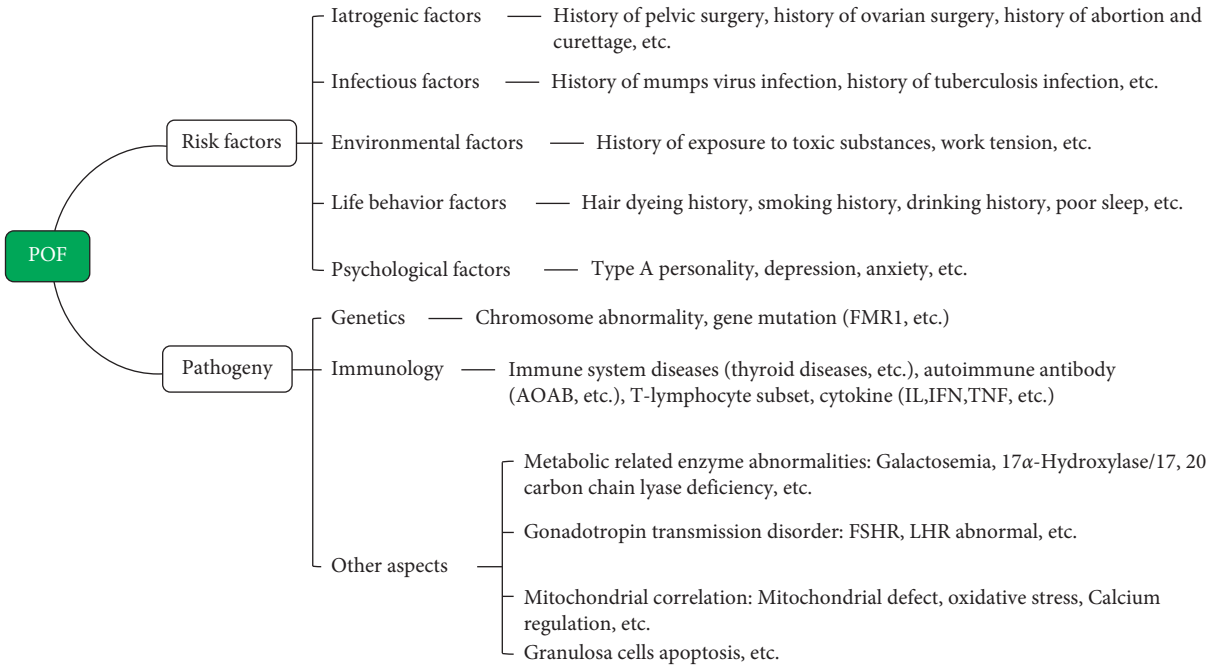

Figure 1: Risk factors and etiology of POF.

medicine, and if an unconventional method is used instead of standard treatment, it is considered an alternative method [18]. CAM includes many products and medical practices that are not conventional therapies in western countries, including not only traditional medicines and folk therapies, but also many new therapies that cannot be covered by medical insurance, such as traditional Chinese medicine (TCM; including Chinese herbal medicine, acupuncture, qigong, etc.), Indian medicine, medicinal food, health food, aromatherapy, vitamin therapy, dietary therapy, psychotherapy, spa therapy, and oxygen therapy [19, 20]. CAM is favored by many countries and nationalities because of its natural ingredients and methods, its convenience, and its low costs. It is also an effective strategy to alleviate the pain related to many diseases. Reports have suggested that the rate of CAM application has reached 9.8%–76.0% globally and that roughly 40% of adults use at least one type of CAM to treat a wide range of conditions in the USA [21, 22]. This article updates the progress in the research and application of CAM in the treatment of POF.

2. Single Herbal Medicines and Their Active Ingredients

Chinese herbal medicines have excellent efficacy in the treatment of POF, and many compound Chinese medicines have been widely used in the clinical treatment of POF due to their many advantages. Single herbal medicines are the basis for the research into compound Chinese medicines, and some of their active ingredients provide indications for the use of different Chinese herbal medicines in the treatment of POF. Current studies have found that the potential mechanisms of single herbal medicines and their active ingredients include estrogen-like effects, promoting DNA damage repair, enhancing anti-inflammatory and antioxidant capacity, regulating the expression of related pathways and proteins, and affecting prostaglandin biosynthesis, apoptosis, aging-associated, autophagy, etc., to delay POF.

2.1. Epimedium and Icariin. Epimedium, a representative of Chinese herbal medicines, has the ability to tonify the kidney and is commonly used to treat female infertility, irregular menstruation, amenorrhea, POF, and other diseases. One of the main active ingredients of Epimedium is icariin. Studies have shown that icariin has an obvious estrogen-like effect that can significantly increase the uterine coefficient, endometrial epithelial thickness, and serum E2 levels in ovariectomized mice [23]. In a rat model of POF, icariin could significantly increase the serum level of anti-Müllerian hormone (AMH), possibly by promoting the secretion of AMH in ovarian granulosa cells (GCs) and restoring normal follicular recruitment and maturation to maintain the ovarian reserve [24]. Li et al. found that icariin was effective in reducing ovarian damage by promoting DNA damage repair, suggesting that icariin might be used as a protective agent against POF [25].

2.2. Semen Cuscutae and TFSC/Cuscuta Flavonoids. Semen cuscutae is an important tonic for kidney yang and is commonly used in the treatment of gynecological diseases. Its main active chemical ingredient is Cuscuta flavonoids, which have significant effects on improving and enhancing the function of the human reproductive endocrine system. In a rat model of POF, total flavonoids from Semen cuscutae (TFSC) had a significant restorative effect on the estrous cycle and ovarian endocrine function and promoted follicular development and GCs proliferation in the ovary, indicating that they had estrogen-like effects [26]. Huang Changsheng believed that quercetin might be one of the

main effective ingredients in Cuscuta flavonoids for the treatment of POF, and in a rat model of POF, both Cuscuta flavonoids and quercetin alone could inhibit the process of POF through activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway [27, 28].

2.3. Pueraria Lobata and Puerarin. The main active ingredients in Pueraria lobata are isoflavones and compound isoflavones. Puerarin is a 4,7-dihydroxy-8-β-D-glucosyl isoflavone and is the most important active ingredient of Pueraria lobata, and it has different degrees of estrogen-like effects [29]. Bcl-2-associated X protein (Bax) and B-cell lymphoma 2 (Bcl-2) play important roles in the anti-apoptotic effect in the Bcl-2 family, and Zhang et al. [30] found that puerarin could regulate the activity of Bax and Bcl-2 and inhibit apoptosis in ovarian tissue in a mouse model of POF and thus restore ovarian function. The expression of mouse vasa homolog (Mvh) and octamer-binding transcription factor 4 (OCT4) maintain the activity of female germline stem cells, and Chen et al. [31] found that puerarin not only activated the Wnt/β-catenin signaling pathway but also increased the expression of the antioxidant factors superoxide dismutase 2 (SOD2) and nuclear factor erythroid 2-related factor (Nrf2) to reduce oxidative stress.

2.4. Ginseng and Rg1. Ginseng is a traditional anti-aging drug, and its active ingredients are mainly composed of ginsenosides. The ginsenosides can be divided into three categories, namely ginseng diols (e.g., Rb1, Rb2, Rc, Rd, F2, etc.), ginseng triols (e.g., Re, Rg1, Rg2, Rf, Rh1, etc.), and pentacyclic triterpenoid saponins such as Ro [32]. The p16 and p53–p21–p19 pathways play an important role in the aging process, and Rg1 not only upregulates the expression of follicle-stimulating hormone receptor (FSHR) in the GCs of POF mice but also downregulates aging-associated proteins in the p19–p53–p21–p16 pathway and enhances the anti-aging capacity and fertility of POF mice by improving the anti-inflammatory and antioxidant capacity of the ovary [33]. Another experiment has shown that Rg1 effectively regulates the physiological status of the ovary, induces the expression of light-chain 3 (LC3)-II to increase autophagy levels, and may delay POF by activating the PI3K/Akt/mammalian target of rapamycin (mTOR) autophagy pathway [34].

2.5. Other Single Herbal Medicines. Hematopoietic prostaglandin D synthase (HPGDS), phospholipase A2-IVA (PLA2G4A), and prostaglandin-endoperoxide synthase 1 (PTGS1) are involved in prostaglandin biosynthesis in the body, and American ginseng affects the transformation of prostaglandin H2 (PGH2) into prostaglandin D2 (PGD2) by downregulating the expression of HPGDS and PTGS1 in the ovary and thus participates in the regulation of the reproductive system [35]. Animal models have shown that American ginseng plays a role in regulating prostaglandin biosynthesis, promoting ovulation, and preventing ovarian aging, which expands our understanding of the pharmacological activity of American ginseng as an anti-POF drug [36]. The expression of PLA2G4A is positively correlated with POF, while the expression levels of pregnancy-associated plasma protein-A, stanniocalcin-2, C-C motif chemokine ligand 2, and NEL-like protein 1 are negatively correlated with POF, and Ge et al. [37] confirmed in animal models that American ginseng can prevent and alleviate POF in rats by altering the expression levels of POF-related genes in ovarian tissues. These studies indicate that American ginseng is a potential clinical treatment for the prevention of POF.

The preparation made from Cistanche formulas can improve the clinical symptoms of menopausal syndrome, and follicle-promoting and luteinizing effects of Cistanche can regulate the endocrine function of female mice with kidney deficiency. Experiments have shown that Cistanche polysaccharide significantly improves the endocrine regulatory mechanisms of the body and has an inhibitory effect on POF by affecting the expression of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and the apoptosis-related protein Bcl-2/Bax [38, 39].

Angelica is an important gynecological medicine commonly used in Chinese herbal preparations, and Angelica polysaccharide is the main active ingredient. Animal experiments have found that Angelica polysaccharide can increase the activity of SOD and inhibit the level of malondialdehyde in the blood of mice with POF and that it promotes both oxidative stress and antioxidant capacity through the Akt/Forkhead box subgroup O3 (FOXO3) pathway, thus providing a novel approach for the treatment of immune-related POF [40].

Lycium barbarum polysaccharides are one of the main ingredients isolated from Lycium barbarum, a characteristic plant in Ningxia. These polysaccharides regulate endocrine production and maintain ovarian function in mice by slowing down ovarian damage caused by anti-zona pellucida antibody, possibly through both direct actions on immunoreactive cells and indirect actions on the hypothalamic pathway [41].

A study showed that high-dose maca powder could restore ovarian function and the abnormal estrous cycle, and improve the ovarian index and serum reproductive hormone levels of POF rats with kidney yang deficiency syndrome and that it has certain conservation effects on the ovary [42]. Oyster polypeptide plays an anti-oxidative role through the SIRT1-FOXO3a-SOD2 axis and protects against POF by reducing apoptosis [43]. The effectiveness of single herbal medicines and their active ingredients in the treatment of POF is summarized in Table 1.

3. Compound Chinese Medicines

Compound Chinese medicines are composed of two or more kinds of herbs that are boiled in water, and the residue is removed to obtain a clear liquid for Chinese medicine decoctions or is further processed into other dosage forms such as Chinese patent medicines, pills, powders, or creams [19]. Compound Chinese medicines have been used to treat diseases for thousands of years and have formed their own materia medica that is separate from traditional western medical practice. More and more studies have shown that

Table 1: Summary of the curative effects of single herbal medicines in POF.

Single herbal medicine	Active ingredients	Outcomes	References
Epimedium	Icariin	FSH, LH↓; E2, AMH↑.	[24, 25]
Semen cuscutae	TFSC/cuscuta flavonoids	TFSC processing: E2↑. Cuscuta flavonoids processing: FSH↓; E2, AMH↑.	[26, 27]
Pueraria lobata	Puerarin	FSH↓; E2, AMH↑; Bcl-2↑; Bax, cleaved caspase-3↓; β-catenin, SOD2, Nrf2↑.	[30, 31]
Ginseng	Rg1	FSH↓; E2, AMH↑; IL-1b, IL-6, TNF-α↓; aging-associated proteins in the p19-p53-p21-p16 pathway↓, LC3-II↑.	[33, 34]
American ginseng	/	FSH↓; E2↑; HPGDS, PLA2G4A, PTGS1↓; PAPPA, STC2, CCL2, NELL1↑.	[34–37]
Cistanche	/	FSH↓; E2, AMH↑; IFN-γ and TNF-α↓; Bcl-2↑; Bax↓; Bcl-2/Bax ratio↑.	[39]
Angelica	AP	SOD↑; MDA↓; IL-1β, IL-6↓.	[40]
Lycium barbarum	LBP	FSH↓; E2↑; serum anti-zona pellucida antibody↓.	[41]
Maca	/	FSH, LH↓; E2↑.	[42]
Oyster	Oyster polypeptide	FSH↓; P↑; P53, Bad↓.	[43]

TFSC: total flavonoids from Semen cuscutae; AP: Angelica polysaccharide; LBP: Lycium barbarum polysaccharides. FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol; AMH: anti-Mullerian hormone; Bcl-2: B-cell lymphoma 2; Bax: Bcl-2-associated X protein; SOD2: superoxide dismutase 2; Nrf2: nuclear factor erythroid 2-related factor; IL-1b: interleukin-1b; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha; LC3-II: light-chain 3 (LC3)-II; HPGDS: hematopoietic prostaglandin D synthase; PLA2G4A: phospholipase A2-IVA; PTGS1: prostaglandin-endoperoxide synthase 1; PAPPA: pregnancy-associated plasma protein A; STC2: stanniocalcin-2; CCL2 : C-C motif chemokine ligand 2; NELL1: NEL-like protein 1; IFN-γ: interferon-gamma; SOD: superoxide dismutase; MDA: malondialdehyde; IL-1β: interleukin-1beta.

compound Chinese medicines have overall advantages in the treatments of diseases due to their ability to simultaneously affect multiple targets and pathways, and this has attracted more and more attention in the treatment of POF compared with the efficacy and side effects of pharmaceutical drugs. Several studies have shown that compound Chinese medicines have a positive effect on POF, and the side effects are significantly less than for pharmaceutical drugs. Compounds extracted from Chinese herbal medicines can significantly reduce oxidative stress in the ovary and reduce apoptosis in ovarian GCs and oocytes. Thus, they play an important role in the treatment of POF by delaying apoptosis and regulating the secretory function of ovarian GCs and have significant advantages in increasing serum E2, reducing serum FSH levels, and improving clinical symptoms [44–47].

3.1. Chinese Medicine Decoctions. Chinese medicine decoctions, known in ancient times as "Tang Ye," are important traditional preparations in TCM due to their ease of consumption, fast absorption, large drug load, and remarkable curative effect. The decoctions can be fully adapted to the needs of syndrome differentiation and treatment in TCM because of their flexibility, simplicity, low cost, quick effect, and the use of water as the solvent [48]. Chinese medicine decoctions that are commonly used in the clinical treatment of POF include Zuogui pill (ZGP), Bushen Huoxue decoction (BHD), Erxian decoction (EXD), and Yulin decoction.

3.1.1. ZGP. ZGP, from Jingyue Quanshu, is a classic prescription for nourishing yin and tonifying the kidney and is widely used in the treatment of POF. According to TCM, women are kidney-based and POF is responsible for kidney deficiency. By nourishing yin and tonifying the kidney, filling the lean marrow, improving perimenopausal symptoms of POF, correcting sexual hormone disorders, and promoting the recovery of ovarian function, ZGP can not only improve the curative effects of western medicine but can also reduce side effects caused by hormone drugs without any serious adverse events [49–51]. Zeng et al. [52] showed that after ZGP treatment female rats showed an increase in ovarian volume, the ratio of total ovarian weight to body weight, and serum AMH and E2 levels and a decrease in serum FSH levels. Peng et al. [53] established a control group, model group, three ZGP groups (high-dose, medium-dose, and low-dose), and a triptorelin group to study ZGP's inhibition of mitochondrial-dependent apoptosis of follicles in POF rats. They found that ZGP inhibited follicle loss after exposure to chemotherapeutic drugs and improved the estrous cycle in rats. Further mechanistic studies showed that the protective effect of ZGP is related to the inhibition of the activation of the mitochondrial-dependent apoptosis cascade at the gene and protein levels. By inhibiting the expression of the pro-apoptotic molecule Bax and increasing the expression of the anti-apoptotic molecule Bcl-2, ZGP can reduce the level of serum FSH and increase the level of serum E2. It has also been shown that ZGP upregulates the protein expression of growth differentiation factor-9 and its signal transduction protein Smad2 in POF mouse ovaries, and thus ZGP can promote the recruitment of primordial follicles, promote follicular development, and inhibit follicular atresia [54].

3.1.2. BHD. BHD [55–57] is a prescription developed by Professor Zhu Nansun, a master of Chinese medicine, and it exerts its therapeutic effects by tonifying the kidney and invigorating the blood, which benefits the kidney and fills the essence, fills the qi and blood, promotes blood circulation, and unblocks the collaterals to promote normal menstruation. Song et al. [58] verified the clinical efficacy of BHD in the treatment of POF patients by comparing the oral BHD group and the oral estradiol or estradiol dydrogesterone tablets group. Their results showed that BHD in the

treatment of POF could significantly improve the TCM symptoms of patients—including hot flashes, night sweats, and other clinical manifestations—reduce the levels of serum FSH, mir-23a, and ovarian artery resistance index, and increase the level of AMH. Liu et al. [59] studied the effect of BHD on immune-related POF mice and found that compared with the model group, there was a significant increase in the number of mature ovarian follicles and a decrease in the number of atretic follicles in the high-dose group, medium-dose group, low-dose group, and estradiol valerate group of BHD, demonstrating that BHD can improve ovarian function by upregulating the protein expression of transforming growth factor-beta 1 (TGF-β1), transforming growth factor-beta receptor II (TGF-β RII), and Smad2/3 in GCs. BHD can promote normal levels of ovarian hormone secretion and promote the increase in mature follicles and the formation of primordial follicles and a large number of primary follicles through the upregulation of PI3K, Akt, and Bcl-2 protein expression in GCs [60].

3.1.3. EXD. EXD is derived from the clinical manual of TCM formulations, and it warms the kidney, fills the essence, and reconciles the Chong Ren. In recent years, clinical observations and research on EXD in the treatment of POF have shown that the decoction has a clear effect on POF [61, 62]. Studies have shown that EXD has an effect on serum inflammatory factors and related growth factors in a rat model of POF. Compared with the low EXD dose group and the medium EXD dose group, the high-dose group showed significantly decreased serum levels of TNF-α, IFN-γ, FSH, and LH, significantly increased protein expression of vascular endothelial growth factor and basic fibroblastic growth factor in ovarian tissue, and increased levels of E2, showing that EXD could improve the level of sex hormone in a rat model of POF and promote the recovery of ovarian function [63]. In another study [64], treating POF mice with EXD restored the ovarian cycle to near or above the normal ovarian index, brought serum E2, FSH, and LH levels close to normal, the increased the numbers of CD3+ T-cells, CD4+ T-cells, and the CD4+ T-cell/CD8+ T-cell ratio, decreased the number of CD8+ T-cells, and increased the expression of BMP15 and Akt.

3.1.4. Other TCM Decoctions. Based on the "liver and kidney theory" [65], Longjiang Han's Department of Gynecology applies Jiawei Yuyin decoction for tonifying the kidney and soothing the liver to provide symptomatic treatment for liver depression and kidney deficiency related to POF, and the patients' perimenopausal symptoms are significantly relieved and the serum levels of sex hormones return to normal. Modified mulberry decoction (MMD) has the effects of tonifying the liver and kidney, nourishing the blood and essence, and harmonizing Chong Ren. Wang et al. used estradiol valerate and cyproterone tablets as their control group and MMD as their treatment group. The results showed that the effective rate of the control group was 66.67% while that of the treatment group was 86.67%, and after treatment, the TCM syndrome scores and FSH and LH levels in the treatment group were lower than those in the control group and the E2 level was higher than in the control group. Their study showed that MMD could effectively improve the clinical symptoms of patients with POF, regulate the level of serum hormones, and promote menstrual regularity [66]. Modified Bazhen Decoction (MBD) [67] is effective in the treatment of POF. Professor Li [68] used the empirical formula "Yangshu Bazhen Decoction," which was modified from the ancient formula "Bazhen decoction," to treat POF by emphasizing the harmony of liver and kidney, paying attention to emotional treatment, balancing yin and yang, and regulating Chong Ren, which achieved good curative effects. In addition, Danggui Buxue decoction [69], Siwu decoction [70], Wenjing decoction [71], Bushen Jianpi formula [72], and other TCM decoctions have good effects in the treatment of POF, and these emphasize the flexible composition and good curative effect of TCM decoctions and fully demonstrate the theory of disease differentiation and treatment using TCM. The effect of TCM decoctions on POF is summarized in Table 2.

3.2. Chinese Patent Medicines. Chinese patent medicines use Chinese herbs as raw materials, and these are processed into a certain dosage form according to the specified prescription and preparation process. They have the characteristics of stable nature, definite curative effects, relatively weak side effects, and convenience of consumption, transport, and storage [73–75].

3.2.1. Kuntai Capsule (KTC). KTC is made according to the ancient prescription found in the Treatise on Febrile Diseases. It can effectively regulate women's endocrine levels, improve ovarian function, and prevent the decline of ovarian function. It can be used either alone or in combination with sex hormones to treat POF [76, 77]. Zhang et al. [78] found that KTC can regulate the estrous cycle, increase hormone secretion, improve fertility, and significantly reduce follicular atresia in the case of impaired ovarian function. Transmission electron microscopy showed that KTC could effectively maintain the ultrastructure of the mouse ovary, terminal deoxynucleotidyl transferase dUTP Nick end labeling (TUNEL) staining confirmed that KTC decreased apoptosis, and Western blot showed that KTC could reduce the expression of AMH, SOD2, Bcl-2, and Bax.

3.2.2. Kunbao Pill (KBP). KBP [79, 80] has the effects of restoring deficiency of qi and blood, weakness of kidney qi, and imbalance of yin and yang, nourishing the liver, kidney, and blood, calming the nerves, and dredging the collaterals, and it is widely used in the treatment of POF in the clinic. Li et al. explored the effects of KBP on the prevention and treatment of a rat model of POF and showed that the serum FSH, LH, and ovarian TGF-β1 levels were significantly decreased in the low-, medium-, and high-dose groups of KBP and combined estrogen group. They also found that serum E2 levels significantly increased and that stem cell factor expression levels significantly decreased in

Table 2: Summary of the curative effects of TCM decoctions on POF.

Chinese medicine decoction	Ingredients	Sample size	Interventions	Model used	Outcomes	References
Zuogui pill (ZGP)	Rehmanniae radix praeparata, cornel, yam, semen cuscutae, Chinese wolfberry fruit, radix achyranthis bidentatae, colla carapacis et plastri testudinis, and antler gelatin	$N = 114$	Control group, model group, low-ZGP group, high-ZGP group, low-dose estradiol valerate, high-dose estradiol valerate, DAPT group, and DAPT + low-ZGP group	SD rats	FSH↓; E2, AMH↑; ovarian volume↑; ratio of total ovary weight to body weight↑.	[52]
Bushen huoxue decoction (BHD)	Panax notoginseng powder, rhizoma cyperi, licorice, spatholobus suberectus stem, psoralea corylifolia, rhizoma curculiginis, semen cuscutae, cornel, angelica, epimedium, salvia miltiorrhiza, ligusticum wallichii, codonopsis pilosula, rehmanniae radix praeparata, and radix paeoniae alba.	$N = 73$	BHD group and western medicine group (estradiol or estradiol didroxyprogesterone tablets)	Human study	FSH↓; AMH↑; TCM syndrome score↓; mir-23a, ovarian artery RI↓.	[58]
Erxian decoction (EXD)	Rhizoma curculiginis, epimedium, morinda officinalis, anemarrhena, phellodendron amurense, and angelica.	$N = 60$	Normal group, model group, bujiale group, low-EXD group, medium-EXD group, and high-EXD group	SD rats	FSH, LH↓; E2↑; TNF-α, IFN-γ↓; VEGF, bFGF↑.	[63]
Modified mulberry decoction (MMD)	Fructus mori, lilium brownii, angelica, rehmanniae radix praeparata, radix paeoniae alba, epimedium, cistanche, and licorice.	$N = 60$	Control group: climen tablets treatment group: MMD	Human study	FSH, LH↓; E2↑; TCM syndrome score↓.	[66]
Modified bazhen decoction (MBD)	Bupleurum, angelica, ligusticum wallichii, radix paeoniae alba, glutinous rehmannia, codonopsis pilosula, atractylodes, poria cocos, licorice, antlers, radix achyranthis bidentatae, and rhizoma cyperi	$N = 24$	Control group, POF group, MBD group, and FES group	SD rats	XIAP↑; mir-23a, mir-27a↓.	[67]
Danggui buxue decoction (DBD)	Angelica and astragalus mongholicus	$N = 50$	Control group, model group, DBD group, quercetin group, and kaempferol group	SD rats	FSH, LH↓; E2, AMH↑; cytochrome c, Bax, p53 and IL6↓; ,ESR1, AR, Bcl-2↑.	[69]
Si-Wu-Tang (SWT)	Rehmanniae radix praeparata, angelica, paeonia lactiflora pall, and ligusticum wallichii	$N = 36$	Control group, model group, low-SWT group, medium-SWT group, high-SWT group, and DHEA group	Six C57BLmice	FSH, LH↓; E2, AMH↑; TCM syndrome score↓.	[70]
Wenjing decoction (WJD)	Evodia rutaecarpa, cassia twig, radix paeoniae alba, angelica, ligusticum wallichii, donkey-hide gelatin, ophiopogon japonicus, ginger, rhizoma pinelliae, moutan bark, ginseng, and licorice	$N = 119$	Control group: HRT (progynat + progesterone capsules) observation group: HRT (progynat + progesterone capsules), WJD, and acupuncture	Human study	FSH, LH↓; E2, AMH↑; TCM syndrome score↓.	[71]

DAPT: dual antiplatelet therapy; FES: Fufang Ejiao Syrup; DHEA: dehydroepiandrosterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estrogen; AMH: anti-Müllerian hormone; RI: resistance index; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma; VEGF: vascular endothelial growth factor; bFGF: basic fibroblastic growth factor; XIAP: X-linked inhibitor of apoptosis protein; IL6: interleukin-6; ESR1: estrogen receptor 1; AR: androgen receptor.

the medium- and high-dose KBP groups and combined estrogen group, and the connective tissue growth factor expression in ovarian tissue significantly decreased in the high-dose KBP group and combined estrogen group. Together, these results indicate that KBP has the effect of regulating the hormone levels of POF, which can reduce the expression of connective tissue growth factor, TGF-β1, and stem cell factor in ovarian tissues to relieve ovarian fibrosis damage and to promote the growth and development of early follicles and thus prevent the occurrence of POF [81].

3.2.3. Other Chinese Patent Medicines. Chinese patent medicines can improve the clinical symptoms of POF and regulate the serum levels of hormones. Zishen Yutai pill (ZYP) has the effects of tonifying the kidney and spleen, nourishing blood, calming the fetus, supplementing qi, and nourishing yuan. It can effectively promote the blood supply to the female sexual organs and promote follicular development and progesterone secretion. The prescription has a good effect on promoting ovarian function. In one study, 78 patients with POF were randomly divided into a control group who took estradiol valerate tablets orally and an observation group who took estradiol valerate tablets combined with ZYP orally. The improvement in FSH, LH, and E2 contents in the observation group was significantly better than in the control group [82, 83]. Fuke Yangrong capsule is a compound preparation of TCM, and it has the effects of promoting blood circulation and menstruation, supplementing qi, nourishing the blood, tonifying the kidney, and soothing the liver. It can promote the development of the corpus luteum, accelerate the excretion of follicles, and improve ovarian function. Xiong treated patients using a sequential artificial cycle of estrogen and progesterone in the control group and a sequential artificial cycle of estrogen and progesterone combined with Fuke Yangrong capsule in the study group. The results showed that the levels of E2, FSH, and LH in the study group were significantly better than those in the control group, and this led to a significant increase in the number of sinus follicles and endometrial thickness and improved the level of sex hormones and the quality of sexual life, and thus is worthy of clinical promotion [84, 85]. Qilin pill has the functions of tonifying the kidney and essence, supplementing qi, and nourishing blood, and studies have shown that Qilin pill combined with estradiol valerate tablets can quickly and significantly reduce the serum levels of FSH and LH, increase the level of E2, and restore menstruation [86, 87].

3.3. Other Forms of Compound Chinese Medicine. Chinese medicine cream formulas are good at tonifying deficiencies. They are a commonly used TCM preparation in the gynecological clinic and are widely used because of their characteristics of slow onset of action and safety for long-term use, which is suitable for the diagnosis and treatment of POF. Moreover, the taste of Chinese medicine creams is better than Chinese medicine decoctions, which greatly improves the medication compliance of patients and thus is favored by doctors and patients [88]. To verify the efficacy of Bushen Tiaojing cream formula (BTCF) in the treatment of POF, Lu et al. [89] randomly divided 120 patients into a cream formula group, a Chinese medicine group, and a Western medicine group. In the cream formula group, the BTCF was prepared and decocted for oral administration. In the Chinese medicine group, self-prepared herbal medicine was used, and in the western medicine group, HRT was applied. After three courses of treatment, the results showed that the improvement of clinical symptoms in the cream formula group was better than that in the Western medicine group and Chinese medicine group. Compared with the Western medicine group, the cream formula group had clear advantages in treatment effect, the serum levels of E2 in the cream formula group and Chinese medicine group were higher than in the western medicine group, and the levels of FSH and LH were lower than in the western medicine group. BTCF has a significant clinical effect in the treatment of POF, and it improves clinical symptoms such as irregular menstruation, and it effectively regulates sex hormone levels and immune function [90, 91]. Yunkang oral solution (YKos) [92] has the effects of strengthening the spleen, nourishing the blood, and calming the fetus. A rat model of POF was prepared by giving cyclophosphamide. Compared with the model group, the serum E2 level of rats in the YKos group increased significantly, the FSH content decreased significantly, the ovarian and uterine coefficients increased significantly, the pathological changes of ovarian and uterine tissues improved significantly, and the expression of gonadotropin-releasing hormone receptor (GnRHR) and FSHR protein in the ovary increased significantly, indicating that YKos can effectively repair the damage to the reproductive system and can restore ovarian function through the regulation of the GnRHR-FSHR signaling pathway. The efficacy of Chinese patent medicines and other forms of compound Chinese medicines in the treatment of POF is summarized in Table 3. In addition, Ren et al. searched the Chinese science and technology journal database (VIP), the Wanfang Digital Journal Full-text Database, the China National Knowledge Infrastructure (CNKI), and other TCM literature on POF over the past 10 years and identified a total of 180 articles that met the inclusion criteria to summarize the Chinese herbal medicines with a high frequency of use in the treatment of POF as shown in Table 4 [93].

4. Acupuncture and Moxibustion

Acupuncture and moxibustion are widely used and have a variety of treatment forms. In addition to manual acupuncture and electroacupuncture stimulation, acupoint embedding and auricular acupoint pressure have also attracted much attention because of their simple operation and prolonged acupoint stimulation. Moxibustion combined with Chinese medicine decoctions can also achieve a remarkable therapeutic effect. Acupuncture and moxibustion therapy can adjust the balance of qi, blood, yin, and yang by stimulating acupoints. This has the characteristics of simple operation, safety, efficiency, and good efficacy and has been widely used in the treatment of POF. In addition, the combination of acupuncture and moxibustion with

Table 3: Summary of the efficacy of Chinese patent medicines on POF.

Chinese patent medicine	Ingredients	Sample size	Interventions	Model used	Outcomes	References
Kuntai capsule (KTC)	Rehmanniae radix praeparata, rhizoma coptidis, donkey-hide gelatin, radix paeonia alba, poria cocos, and radix scutellariae.	/	/	/	FSH, LH↓; E2, AMH↑; Kupperman score↓.	[77]
Kunbao pills (KBP)	Fructus ligustri lucidi, raspberry, semen cuscutae, Chinese wolfberry fruit, fleeceflower root, tortoise shell, Chinese wolfberry root bark, adenophora tetraphylla, ophiopogon japonicus, wild jujube kernel, glutinous rehmannia, radix paeoniae alba, radix paeoniae rubra, angelica, spatholobus suberectus stem, nacre mother of pearl, dendrobium nobile, chrysanthemum, eclipta, folium mori, radix cynanchi atrati, anemarrhena, and radix scutellariae.	$N = 60$	Blank group, model group, low-KBP group, medium-KBP group, high-KBP group, and estrogen combination group	SD rats	FSH, LH↓; E2↑; TGF-β1↓; SCF↓.	[81]
Zishen yutai pill (ZYP)	Semen cuscutae, fructus amomi, rehmanniae radix praeparata, ginseng, parasitic loranthus, donkey-hide gelatin, fleeceflower root, folium artemisiae argyi, morinda officinalis, bighead atractylodes rhizome, codonopsis pilosula, cornu cervi degelatinatum, Chinese wolfberry fruit, teasel root, eucommia ulmoides	$N = 80$	The control group (estradiol valerate tablets), the observation group (estradiol valerate tablets combined with ZYP)	Human study	FSH, LH↓; E2↑; Kupperman score↓.	[82]
Fuke yangrong capsule (FYC)	Angelica, bighead atractylodes rhizome, rehmanniae radix praeparata, ligusticum wallichii, radix paeoniae alba, rhizome cyperi, motherwort, astragalus mongholicus, eucommia ulmoides, folium artemisiae argyi, ophiopogon japonicus, donkey-hide gelatin, licorice, dried tangerine peel, poria cocos, fructus amomi	$N = 80$	The control group (sequential artificial cycle of estrogen and progesterone) and the observation group (sequential artificial cycle of estrogen and progesterone combined with FYC)	Human study	FSH, LH↓; E2↑; TCM syndrome score↓.	[85]
Qilin pill (QLP)	Prepared fleeceflower root, eclipta, epimedium, semen cuscutae, cynomorium songaricum, codonopsis pilosula, radix curcumae, raspberry, Chinese wolfberry fruit, yam, red-rooted salvia root, astragalus mongholicus, radix paeoniae alba, pericarpium citri reticulatae viride, and fructus mori	$N = 72$	Climen tablet group and climen tablets combined with QLP group	Human study	FSH, LH↓; E2↑.	[87]
Bushen Tiaojing cream formula (BTCF)	Rehmanniae radix praeparata, yam, cornel, chinese wolfberry fruit, semen cuscutae, angelica, radix cyathulae, donkey-hide gelatin, motherwort, poria cocos, and moutan bark	$N = 110$	The control group (HRT) and the observation group (HRT combined with BTCF)	Human study	FSH, LH↓; E2↑; GDF-9, BMP-15↑.	[90]

Table 3: Continued.

Chinese patent medicine	Ingredients	Sample size	Interventions	Model used	Outcomes	References
Yunkang oral solution (YKos)	Yam, teasel root, astragalus mongholicus, angelica, rhizoma cibotii, semen cuscutae, parasitic loranthus, eucommia ulmoides, psoralea corylifolia, codonopsis pilosula, poria cocos, bighead atractylodes rhizome, donkey-hide gelatin, glutinous rehmannia, cornel, Chinese wolfberry fruit, smoked plum, radix paeoniae alba, fructus amomi, alpinia oxyphylla, radix boehmeriae, radix scutellariae, and folium artemisiae argyi	$N = 108$	Control group, CTX model group, positive control group and high-YKos, medium-YKos and low-YKos groups	SD rats	FSH↓; E2↑; ovarian coefficient, uterine coefficient↑; GnRHR, FSHR↑.	[92]

CTX: cyclophosphamide; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estrogen; AMH: anti-Müllerian hormone; TGF-β1: transforming growth factor beta1; SCF: stem cell factor; GDF-9: growth differentiation factor 9; BMP15: bone morphogenetic protein 15; GnRHR: gonadotropin-releasing hormone receptor; FSHR: follicle-stimulating hormone receptor.

Table 4: The frequency of use of Chinese herbal medicines for the treatment of POF.

Drug efficacy	Number	Percentage (%)	Chinese herbal medicines and frequency
Tonic drug	21	67.86	Rehmanniae radix praeparata 164, angelica 164, semen cuscutae 163, yam 109, Chinese wolfberry fruit 106, radix paeoniae alba 76, licorice 67, fructus ligustri lucidi 66, epimedium 87, eucommia ulmoides 54, dried human placenta 46, morinda officinalis 43, bighead atractylodes rhizome 38, antler gelatin 38, radix glehniae 13, fructus mori 13, donkey hide gelatin 11, amethyst 10, ginseng 10, radix pseudostellariae 9, ophiopogon japonicus 9
Drug for promoting blood circulation and removing stasis	11	11.77	Salvia miltiorrhiza 57, ligusticum wallichii 50, radix achyranthis bidentatae 28, radix cyathulae 24, safflower carthamus 24, spatholobus suberectus stem 23, motherwort 20, peach kernel 19, eupatorium 16, radix curcumae 10, motherwort fruit 10
Astringent drug	3	6.30	Cornus officinalis 110, raspberry 24, schisandra chinensis 17
Antipyretic drug	5	5.09	Moutan bark 39, phellodendron amurense 22, anemarrhena 21, rehmannia glutinosa 20, radix paeoniae rubra 20
Diuretic and hygroscopic drug	2	3.38	Poria cocos 67, alisma orientalis 14
Qi regulating drug	2	2.84	Rhizoma cyperi 48, dried tangerine peel 20
Drug for treating external syndromes	1	1.59	Bupleurum 38
Interior-warming drug	2	1.17	Cinnamomum cassia 17, radix aconiti carmichaeli 11

Chinese medicine and western medicine can have synergistic therapeutic effects, so such combinations are often used in clinical practice. In clinical treatment, the selection of acupoints is crucial for the therapeutic effect on the disease. Zhang et al. [94] summarized the commonly used therapeutic acupoints through a literature review of acupuncture treatments of POF, and this provides a useful reference for the clinical treatment of the disease (Table 5).

4.1. Acupuncture. In a rat model of POF, it was found that acupuncture regulated the expression levels of genes and proteins related to the PI3K/Akt/mTOR signaling pathway, promoted an increase in serum E2 level, and restored ovarian function [4, 95]. Clinical studies have shown that acupuncture can not only actively adjust ovarian status in the early stage of POF but can also improve the menstrual situation, perimenopausal symptoms, and serum sex hormone levels, increase clinical efficiency, and create better conditions for pregnancy [96, 97]. Yao et al. [98] needled the Guanyuan (RN4), Guilai (ST29), Taichong (LR3), Taixi (KI3), Xuehai (SP10), Sanyinjiao (SP6), Zigong (EX-CA1), Yinlingquan (SP9), Zusanli (ST36), Shuidao (ST28), Dahe (KI12), and Tianshu (ST25) acupoints prior to ovulation and needled the Ciliao (BL32), Shiqizhui (EX-B7), Ganshu (BL18), Shenshu (BL23), Geshu (BL17), and Pishu (BL20) acupoints after ovulation and compared the effects to HRT. The results showed that the total effective rate of the acupuncture group was 90.4% and the expression levels of IFN-γ and TNF-α decreased while the levels of LH, FSH, and E2

Table 5: Modern acupuncture selection for the treatment of POF.

Acupoint	International code	Meridians	Frequency of use	Percentage (%)
Guanyuan	RN4	Ren	34	73.91
Shenshu	BL23	Bladder	29	63.04
Sanyinjiao	SP6	Spleen	28	60.87
Zhongji	RN3	Ren	21	45.65
Zusanli	ST36	Stomach	19	41.30
Zigong	EX-CA1	Extra points	18	39.13
Pishu	BL20	Bladder	18	39.13
Taixi	KI3	Kidney	17	36.96
Taichong	LR3	Liver	16	34.78
Ganshu	BL18	Bladder	16	34.78
Xuehai	SP10	Spleen	15	32.61
Qihai	RN6	Ren	14	30.43
Guilai	ST29	Stomach	9	19.57
Dahe	KI12	Kidney	7	15.22
Ciliao	BL32	Bladder	7	15.22

improved. The umbilical cord is an important orifice in the human body and is the hub of qi movement flow in the whole body. Professor Zhang Yongchen [99] developed the clinical treatment of acupuncture combined with umbilical cord therapy in the treatment of POF and used blood prick therapy to dredge the local qi and blood to treat both the symptoms and the root cause, and this had a significant curative effect and reduced patients' pain and side effects and thus is worthy of clinical promotion and application.

4.2. Moxibustion. Moxibustion therapy regulates the body's disordered physical and chemical functions by regulating the flow of qi and blood to prevent and treat disease [100]. Experiments in animal models showed that moxibustion of RN4 and SP6 can restore ovarian function and hormone levels, reduce the content of inflammatory factors IL-6 and IL-1β, and downregulate the expression levels of phosphorylated P-PI3K, P-Akt, and P-MTOR in ovarian tissues [101]. The toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) signaling pathway is the initial signal of inflammatory response. Moxibustion not only inhibits the TLR4/myeloid differentiation factor 88 (MyD88)/NF-κB signaling pathway but also indirectly decreases nod-like receptor protein-3 (NLRP3), the precursor of IL1β (Pro-IL1β), and the precursor of IL18 (Pro-IL18). It can also directly inhibit pyroptosis induced by the thioredoxin-interacting protein (TXNIP)/NLRP3/Caspase1 signaling pathway and can regulate cell death through pyroptosis to alleviate ovarian failure in POF [102]. In addition, Liu [103] used Shenque moxibustion, Guanyuan moxibustion, and fire-dragon moxibustion to treat patients with POF and found that moxibustion improved patients' menstrual activity, clinical symptoms, and serum sex hormone levels and that it was easy to perform and accept and is a safe and effective method for the clinical treatment of POF.

4.3. Electroacupuncture Stimulation. Electroacupuncture stimulation of acupoints can effectively activate the functions of relevant organs. Through different intensities of electrical stimulation, it can not only increase the blood supply and nutrition of the treatment site and improve microcirculation but can also regulate neuroendocrine function [100, 104]. In a rat model of POF, it was found that electroacupuncture could improve the local morphology of the ovary, inhibit follicular atresia, promote follicular development, and improve ovarian function [105]. It protects the ovary by upregulating the expression of the apoptosis suppressor Bcl-2 protein and downregulating the expression of the pro-apoptotic factor Bax protein in ovarian GCs, and it improves POF by mediating the PI3K/AKT/mTOR pathway [106, 107]. Other animal model experiments have shown that electroacupuncture has a good therapeutic effect on POF, which is achieved by inhibiting ferroptosis and apoptosis that are induced by glutathione peroxidase 4 (GPX4) targeting the xC-system [108]. In addition, Zhang et al. found that warm acupuncture at "RN4" combined with electroacupuncture at "SP6" could regulate the serum sex hormone levels, improve the reproductive endocrine environment, and reduce the inflammatory response of the ovary in a rat model of POF [109].

4.4. Clinical Application of Combined Acupuncture and Moxibustion. Acupuncture and moxibustion therapy are often used in combination in clinical application. Wang et al. [110] used abdominal acupuncture combined with moxibustion to treat POF, and Yu et al. [111] used umbilical needle therapy combined with mixed moxibustion to treat POF. In addition, Wu et al. [112] randomly divided 50 patients with POF into an acupuncture group and a western medicine group. The acupuncture group used ST36 and RN4 warm acupuncture combined with Baliao (BL31, BL32, BL33, and BL34) ginger moxibustion, and the western medicine group was given oral climen. The results showed that in the acupuncture group, FSH and FSH/LH decreased, E2 increased, the peak systolic velocity of ovarian artery blood flow signal increased, the artery resistance index and pulsatility index decreased, the ovarian volume increased, and the AFC increased. The total effective rate was 92.0%, which was higher than that of the western medicine group (88%). In addition, Tian et al. [113] applied electroacupuncture

combined with heat-sensitive moxibustion to treat 60 patients with POF, and its total effective rate reached 93.3% with the improved menstrual cycle and symptom scores.

4.5. Acupuncture and Moxibustion Combined with Drugs. Acupuncture is often used as an auxiliary treatment method in TCM and western medicine to adjust endocrine levels and improve the clinical symptoms and the pregnancy rate of infertility patients with POF [114–116]. Lai et al. [117] proposed the clinical use of "Tongyuan acupuncture" combined with TCM in the treatment of POF. Studies have found that moxibustion combined with artificial cycle therapy in the treatment of POF can adjust the levels of sex hormones and improve clinical indicators compared with the effect of artificial cycle therapy alone [118, 119]. In addition, Chi [120] used Yougui pills, acupuncture, and moxibustion combined with sequential therapy to treat POF, and the results showed that the effective rate was up to 90.48%, the TCM syndrome score and Kupperman score significantly reduced, and the ovarian function index, hemodynamic index, and sex hormone levels significantly improved. Acupuncture and moxibustion combined with Chinese and western medicine can not only reduce the incidence of adverse reactions and improve the safety of treatment but can also increase the clinical efficacy and is thus worth promoting [121–123]. Some studies have shown that electroacupuncture combined with compound Chinese medicines has good efficacy in the treatment of POF, but the sample sizes in the studies were small, and the mechanism of action and long-term efficacy need to be further observed and studied [124, 125].

4.6. Acupoint Catgut-Embedding. Acupoint catgut-embedding therapy is an improved acupuncture method in which biodegradable medical sutures are embedded into specific acupoints and regions of the body. This can stimulate the meridians and harmonize qi and blood and has the advantages of simple operation and long stimulation effect, and thus it has become important in the treatment of gynecological diseases and can be used for the treatment of POF with good efficacy [126, 127]. Chen et al. [128] used Chinese medicine for tonifying the kidney and nourishing the liver combined with acupoint catgut-embedding to treat POF, and the total effective rate of clinical efficacy reached 86.67% and significantly improved clinical symptoms such as menstrual cycle, vaginal dryness, and loss of sexual desire, and it increased serum sex hormone levels, endometrial thickness, and ovarian volume. In addition, studies on acupoint catgut-embedding combined with Chinese patent medicines, Chinese medicine decoctions, and western medicine have shown that acupoint catgut-embedding combination therapy can improve and restore ovarian function of patients, thus providing strong evidence for its clinical promotion [129–131].

4.7. Auricular Acupoint Pressure. Ear acupoints are closely related to the Zang-Fu organs and meridians, and stimulating specific acupoints of ear acupoints can regulate autonomic nerve function and relieve the uncomfortable symptoms caused by autonomic nerve dysfunction in patients with POF [132]. Shen [133] compared Modified Guishen Pill combined with auricular acupoint therapy (the combined treatment group) with sequential estrogen-progesterone therapy (the artificial cycle group) and observed the efficacy after 3 months of treatment. The results showed that the total effective rate of the combined treatment group was 93.3% and the TCM symptom score, FSH, and LH levels were significantly lower than those of the artificial cycle group, while the E2 level was significantly increased in the combined treatment group. Ye et al. [134] treated POF using Qingxin Jianpi decoction combined with auricular acupoint pressure, with a total clinical effective rate of 82.9% and proposed that regulating the heart and spleen is an important link that cannot be ignored in the treatment of POF. In conclusion, auricular acupoint pressure therapy is often used as an auxiliary therapy for the treatment of POF. Selecting auricular acupoints according to the results of TCM syndrome differentiation can not only enhance the pertinence of clinical treatment but can also reduce the adverse reactions of drug treatment and achieve better therapeutic effects.

5. Other Treatments

In clinical studies, psychotherapy, exercise therapy, oral vitamins, massage, and other methods have been shown to have good therapeutic effects in the treatment of gynecological diseases. These methods are often used in combination with hormones and Chinese herbal medicines, which can improve the therapeutic effect and the quality of life of patients.

5.1. Psychotherapy. POF causes a great deal of physical and psychological stress in women, so psychological nursing is essential in the treatment of this disease. Psychological suggestion, targeted care, strengthening communication with patients about the disease, and enhancing communication with family members [135, 136] are commonly used in the treatment of POF and can improve anxiety, depression, and quality of life and can increase treatment compliance and treatment effect in patients with POF [137]. Cui et al. [138] proposed a psychosomatic treatment with TCM for POF that is based on regulating the movement of qi. Clinical studies have shown that combination therapy such as the psychosomatic treatment of TCM and estrogen-progesterone combined with psychological counseling has clinical promotion and application value by improving the happiness index of patients with good clinical efficacy [139–143].

5.2. Exercise. There is a close causal relationship between exercise and human physical and mental health. Physical exercise can improve body functions, and regular exercise can reduce stress, improve negative moods such as anxiety and depression, support sleep, and reduce chronic stress-related diseases [144]. Animal experiments have also shown

that exercise can improve depression-like behavior in ovariectomized rats [145]. In a clinical study, Wu et al. [146] found that Baduan Jin exercises improved ovarian function, increased ovulation rates, and improved the quality of life of patients with low ovarian reserve function. Exercise therapy is simple, easy to implement, and does not increase the patient's financial burden, and it has a high acceptance among patients and strong operability, which make it highly applicable to patients with POF [147].

5.3. Vitamins. Vitamin E is a fat-soluble vitamin with strong antioxidant effects. Reasonable supplementation of vitamin E can enhance the antioxidant protection of the ovary and can increase ovarian reserve. Vitamin E combined with HRT is often used in the clinical treatment of POF [148, 149]. Zou et al. [150] further suggested that coenzyme Q10 combined with vitamin E can enhance the protective antioxidant effect on the ovaries in elderly women, improve ovarian reserve, and increase ovarian responsiveness. It has been shown that POF is a high-risk factor for osteoporosis, and the combination of calcium tablets, calcitriol, and vitamin D has definite efficacy in the treatment of POF with osteoporosis [151].

5.4. Massage. Massage, which can relax tendons, invigorate blood, improve fitness, and prevent disease, is often used clinically in combination with Chinese herbal medicine in patients with POF. Such combined therapy can improve the levels of various serum sex hormones and has good efficacy in the treatment of POF [152, 153]. In addition, Feng et al. [154] adopted electroacupuncture combined with pelvic floor muscle massage, which can not only improve the local blood flow of the ovary but can also adjust the clinical symptoms and endocrine levels of the patients as a whole to treat POF.

5.5. Diet. TCM has always held to the concept of the homology between medicine and food. Studies have shown that a high-calorie diet may contribute to metabolic disorders including diabetes, obesity, and hyperlipidemia and may also affect female fertility by directly impairing oocyte health and differentiation or by indirectly interfering with the pituitary-hypothalamic axis [155]. Chen et al. [156] explored the relationship between dietary nutrition and POF by comparing different daily intakes of protein, carbohydrate, and dietary fiber in two groups of POF patients, and they proposed the view that a deficiency of carbohydrate and dietary fiber is associated with POF. However, there is still a lack of studies examining the relationship between dietary nutrition and POF, which needs to be explored further.

6. Conclusions

In recent years, clinical and scientific researchers have paid more attention to POF and its associated symptoms such as amenorrhea and infertility. As the research has progressed, more and more CAM therapies have become widely accepted and well used, including herbal medicine, acupuncture, psychology, exercise, vitamins, massage, diet, etc. These therapies can significantly improve the patient's symptoms and hormone levels and can be used in combination with other treatments to enhance their efficacy. However, to increase the benefits of these alternative medical interventions for POF patients worldwide, the standardization of effective CAM treatments, larger sample sizes, and more randomized controlled trials are still needed to confirm the effectiveness and safety of CAM for POF.

Data Availability

No data were used to support the findings of this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Yue Li conducted the research, wrote the article, collected, and organized the data; Feng-juan Han conceived and designed the study; Meng-yu Yan, Qiao-chu Chen, Ya-ya Xie, and Chen-yu Li amended the article.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (82074484).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1269530, 11 pages
https://doi.org/10.1155/2022/1269530

Research Article

Therapeutic Effects of Xianlu Oral Solution on Rats with Oligoasthenozoospermia through Alleviating Apoptosis and Oxidative Stress

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Received 6 April 2022; Accepted 2 June 2022; Published 18 June 2022

Academic Editor: Tian-Bo Liu

Idiopathic oligoasthenozoospermia (iOAZS) is one of the major causes of male infertility, and the ideal therapies for iOAZS have not been established yet. Traditional Chinese medicine (TCM), including Xianlu oral solution (XL), has been widely used as an adjunct treatment for male infertility in the clinic. However, the underlying mechanisms of XL treatment on iOAZS are still not known. Here, we found that XL treatment has therapeutic effects on ornidazole (ORN)-induced OAZS model rats through the amelioration of testis tissues spermatogenesis and the improvement of sperm concentration and motility. Moreover, XL treatment ameliorated the serum hormone levels, mitochondrial membrane potential, apoptosis status, and oxidative stress status in the testis tissues of iOAZS model rats. These findings identify a potential mechanism underlying the therapeutic effects of Xianlu oral solution on iOAZS, and Xianlu oral solution may be used as a traditional Chinese medicine (TCM) therapy for male infertility caused by iOAZS in clinical practice.

1. Introduction

Male infertility is an emerging global public health issue. Approximately 7% of the male population is diagnosed with some type of infertility, such as asthenozoospermia, oligozoospermia, teratozoospermia, some combination of them, or azoospermia [1, 2]. Approximately 19% and 63% of these men with infertility were categorized as men having asthenozoospermia (AZS), combined with oligozoospermia (OZS), and/or teratozoospermia, respectively [3]. A multitude of causes can lead to asthenozoospermia (AZS) or oligozoospermia (OZS) including gene abnormality [4–9], unhealthy lifestyle, prolonged duration of sexual abstinence, infection, abnormal immunity, and urogenital diseases [10–12]. However, no clear causes have been diagnosed in some cases using routine clinical examinations, and these cases have been categorized as idiopathic AZS or OZS (iAZS or iOZS). Some therapies for AZS or OZS have been established such as treating infection or varicocele by antibiotics or surgery, changing lifestyle, avoiding toxic environmental exposures, and maintaining regular intercourse and ejaculation [13–16]. Additionally, some severe cases of AZS or OZS caused by genetic factors benefit from the application of intracytoplasmic sperm injection (ICSI) [17]. However, ideal therapies for iAZS or iOZS have not been established.

Traditional Chinese medicine (TCM) has been widely used as an adjunctive treatment for many kinds of infertility. It has been reported that upregulation of CatSper1 (cation channel of sperm) by Sheng-Jing-San, a TCM recipe treatment, improves the sperm motility of AZS rats and that oral administration of Wuzi Yanzong (WZYZ) formula can restore the destroyed testicular structure of oligoasthenozoospermic model rats [18, 19]. *Cistanche tubulosa* (CT), echinacoside (ECH), and phenylethanol glycosides from *C. tubulosa* (CPhGs) could also attenuate poor sperm quality and testicular toxicity through upregulation of steroidogenic enzymes via the CYP450-3β-HSD pathway in Leydig cells of

bisphenol A- or hydrocortisone-induced animal models [20, 21]. Additionally, studies have found that new Wenshen Shengjing decoction (WSSJD) treatment could repair cyclosporine-induced testicular damage in mice by increasing testosterone levels in the testes and decreasing the apoptosis of spermatogenic cells; *Cuscuta chinensis* Lam. and *Lycium barbarum* L. treatment could regulate the expression of Bcl-2, BAD, and BAX, thus reducing cell apoptosis and improving sperm counts and the viability of *Tripterygium wilfordii* Hook. (GTW) polyglycoside-treated rats [22, 23].

Xianlu oral solution (XL), consisting of several traditional Chinese medicines, has been used clinically in China for the treatment of men with infertility with decreased sperm count and motility induced by kidney-yin deficiency. However, the effect and underlying mechanisms of Xianlu oral solution for the treatment of iOAZS remain unknown. In the present study, we first investigated whether XL treatment exerts its action on iOAZS model rats. Then, we investigated the possible underlying mechanisms for its treatment of iOAZS.

2. Materials and Methods

2.1. Animals. Sexually mature male Sprague-Dawley rats, weighing 180–200 g at the beginning of the experiment, were purchased from the Department of Laboratory Animal Science, Peking University Health Science Center. All the rats were housed in separate cages under the following standard conditions: temperature (18–24°C), humidity (55–65%), and dark cycle (12 h-light/12 h-dark cycle), with ad libitum access to food and water. All experimental protocols were approved by the animal care and use committee of Peking University (approved number: LA2021371).

2.2. Animal Model of Oligoasthenozoospermia. A rat model of iOAZS was developed by intragastric administration of ornidazole (ORN), as described previously [24]. In brief, adult male rats were intragastrically administered with ORN at a dose of 400 mg/kg body weight once per day from day 1 to day 14. The control rats received a 0.2% carboxymethylcellulose sodium (CMC-Na) solution (vehicle of ORN) throughout the experiment. Development of the iOAZS rat model was determined by assessment of the epididymal sperm motility and count as follows.

2.3. Xianlu Oral Solution Administration for Animals. Xianlu oral solution (XL, a gift from Changchun Leiyunshang Pharmaceutical Company) was kept at room temperature before use. The iOAZS model rats were intragastrically administered low-dose XL (1.5 ml/kg/d), middle-dose XL (3.0 ml/kg/d), high-dose XL (6.0 ml/kg/d), or equal amounts of normal saline (NS) once per day from day 15 to day 35. Meanwhile, the XL- and NS-treated iOAZS model rats were intragastrically administered with ornidazole (ORN, 400 mg/kg/d) once per day to maintain the pathological state of idiopathic oligoasthenozoospermia. The effects of XL treatment on iOAZS rats were also determined by assessment of the epididymal sperm motility and count.

2.4. Enzyme-Linked Immunosorbent Assay (ELISA). Serum samples of rats were collected and kept at 4°C before use. ELISA kits (MEIMIAN, China) were used to quantify the levels of follicle-stimulating hormone (FSH, MM-70867R1), luteinizing hormone (LH, MM-0624R1), testosterone (T, MM-0577R1), and blood urea nitrogen (BUN, MM-20555R1), and ELISA kits (MEIBIAO, China) were used to quantify the levels of alanine aminotransferase (ALT, MB-6892B) and aspartate aminotransferase (AST, MB-6891B) according to the manufacturer's instructions.

2.5. Hematoxylin and Eosin (H&E) Staining. Under deep anesthesia, the testes tissues of rats were removed quickly, fixed in 4% neutral buffered formalin, dehydrated through an ethanol series, and cleared twice in 100% xylene. For embedding, the testes tissues were transferred to pure paraffin wax for 1 h at 60°C. For H&E staining, 5 μm testicular sections were dewaxed in xylene, rehydrated through ethanol series, and then stained with H&E. Images were acquired using a light microscope (OLYMPUS, Tokyo, Japan).

2.6. Computer-Assisted Sperm Analysis (CASA). Cauda epididymal sperm of rats were collected immediately after euthanasia and prepared as described in a previous article [25]. In brief, two caudal epididymides were placed in 2 ml preheated phosphate buffer saline (PBS), slightly cut into three pieces and incubated for 5 min at 37°C in a 5% CO_2 incubator. Ten microliters of the sperm suspension were used for the assessment of sperm motility and concentrated by using a CASA system (WLJY-9000, Beijing Weili New Century Science and Technology Development Co., Ltd, Beijing, China). The following parameters were evaluated: rapid progressive motility (grade A sperm, %), progressive motility (grade A + B sperm, %), and sperm concentration (million/ml), as well as the parameters of sperm motility such as straight-line velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s), amplitude of lateral head displacement (ALH, μm), linearity (LIN, %), and straightness (STR, %) were also evaluated.

2.7. Sperm Morphological Staining. Sperm morphological staining was performed by the Diff–Quik method using the sperm morphological fast staining kit (G2572, Solarbio, Beijing, China) according to the manufacturer's instructions. In brief, 20 μl of the sperm suspension were added to the slides, smeared, and dried in air. The slides were soaked in Diff staining buffer 1 for 20 s and then soaked in Diff staining buffer 2 for 10 s. Images were acquired using a light microscope (OLYMPUS, Tokyo, Japan).

2.8. Sperm DNA Staining. Sperm DNA staining was performed by the AO method using the sperm nucleus DNA staining kit (DA1210A, Leagene Biotechnology, Beijing, China) according to the manufacturer's instructions. In brief, 20 μl of the sperm suspension was added to the slides, smeared, and dried in air. The slides were fixed in the stationary buffer for 15 min, washed in ddH_2O, and swung to

remove the redundant water. Then, the slides were stained in AO staining working solution for 5 min. The slides were observed under a confocal microscope (Zeiss LSM710), and images were captured with ZEISS ZEN software (Carl Zeiss).

2.9. Detection of Mitochondrial Membrane Potential (MMP). For the MMP analysis of testicular tissues in rats, the mitochondrial membrane potential assay kit with JC-1 (C2006, Beyotime Biotechnology, Jiangsu, China) was used following the manufacturer's instructions.

2.10. Western Blotting. A piece of testicular tissue from the rats was immediately homogenized in ice-cold RIPA lysis buffer containing 1 mM phenylmethanesulfonyl fluoride (P0013B, Beyotime Biotechnology, Jiangsu, China). The homogenates were centrifuged at 12,000 g for 10 min at 4°C to yield the total protein extract in the supernatant. The concentration of protein was measured with a bicinchoninic acid (BCA) assay kit (Pierce/Thermo Scientific), and equal amounts of protein samples (60 μg) were denatured and then separated in 10% sodium dodecyl sulfate-polyacrylamide gels and then transferred onto PVDF membranes. The membranes were incubated with the following primary antibodies overnight at 4°C: rabbit monoclonal anti-Bcl-2 (1 : 1000, Cell Signaling Technology (CST), cat# 3498), rabbit polyclonal anti-caspase-3 (1 : 1000, CST cat# 9662), rabbit polyclonal anti-4-hydroxynonenal (4-HNE) (1 : 1000, Abcam, cat# ab48506), and mouse monoclonal anti-α-tubulin (1 : 1000, CST, cat# 3873). The membranes were washed in TBST and incubated with the indicated horseradish peroxidase-conjugated secondary antibody including goat anti-rabbit IgG antibody (1 : 2000, Biodragon Immunotechnologies, Suzhou, Jiangsu, China, cat# BF03008) and goat anti-mouse IgG antibody (1 : 2000, Biodragon Immunotechnologies, cat# BF03001) for 1 h at room temperature and then washed in TBST. Immunoreactive bands were visualized by using a Tanon 5200 chemiluminescence detection system (Tanon, Shanghai, China). The bands were quantified with a computer-assisted imaging analysis system (ImageJ, NIH).

2.11. Oxidative Stress Assessments. Total antioxidant capacity (cat# S0119, total antioxidant capacity assay kit with ABTS method), glutathione peroxidase (GPx) (cat# S0058, total glutathione peroxidase assay kit with NADPH), and superoxide dismutase (SOD) (cat# S0109, total superoxide dismutase assay kit with NBT) activities as well as the levels of hydrogen peroxide (cat# S0038, hydrogen peroxide assay kit) and malondialdehyde (MDA) (cat# S0131M, lipid peroxidation MDA assay kit) in testicular tissues of rats were measured using commercial kits purchased from Beyotime Biotechnology according to the manufacturer's instructions.

2.12. Statistical Analysis. All the statistical analyses were performed with GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA, USA). The data were presented as the mean ± standard error of the mean (mean ± SEM). One-way ANOVA followed by Sidak's post hoc test was used for multiple comparisons of three groups. The significant differences between groups are represented as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3. Results

3.1. Effects of XL Treatment on the Testis Index and Serum Hormone Levels of OAZS Rats. To evaluate the effects of XL treatment on iOAZS rats, we first examined the alterations in body and testicular tissue weights of the rats in different groups. The body and testis weights of iOAZS rats were decreased, although the testis index (ratio of testis/body weight) was not altered. Additionally, a high-dose XL (XL-H) treatment improved the body and testis weights of iOAZS rats (Figures 1(a)–1(c)). Then, using ELISA, we found an increase in the FSH and LH levels of high-dose XL-treated rats, but the testosterone level was not changed (Figures 1(d)–1(f)). Moreover, we found that ORN and XL treatment had no side effects on the liver and kidney since the levels of ALT, AST, and urea were not changed in ORN- and XL-treated rats (Figures 1(g)–1(i)). These results suggest that XL treatment could improve testis spermatogenesis in iOAZS rats.

3.2. XL Treatment Improved Testis Spermatogenesis in iOAZS Rats. To further determine whether XL treatment had therapeutic effects on iOAZS rats, we first evaluated testis spermatogenesis in rats. The hematoxylin and eosin (H&E) staining of the testis tissue showed that, on day 35 after exposure of ORN to rats, the spermatogenic cells and spermatids in the seminiferous tubules were decreased and the seminiferous tubules were disordered in the testis tissues in iOAZS rats compared with vehicle-treated rats (Figure 2(a)). Moreover, we found that XL treatment improved testis spermatogenesis in iOAZS rats in a dose-dependent manner, and the disruption of seminiferous tubules and decrease of spermatogenic cells were alleviated after XL treatment in iOAZS rats (Figure 2(b)). These results suggest that XL treatment ameliorated spermatogenesis in iOAZS rats.

3.3. XL Treatment Enhanced the Sperm Concentration and Motility of iOAZS Rats. To further enhance our understanding of how XL treatment improved spermatogenesis in iOAZS rats, we examined the alteration of sperm quality in ORN- and XL-treated rats. Using the CASA technique, we found a significant reduction in sperm concentration and sperm motility, including grade A and grade A + B sperm in iOAZS rats compared with vehicle controls (Figures 3(a)–3(c)). Other parameters of sperm motility, including straight-line velocity (VSL), curve-line velocity (VCL), average path velocity (VAP), amplitude of lateral head displacement (ALH), linearity (LIN), and straightness (STR), were consistently decreased in iOAZS rats (Figures 3(d)–3(i)). Additionally, a high-dose XL treatment significantly improved the sperm quality of iOAZS rats, as indicated by augmented sperm concentration and sperm motility including grade A sperm, grade A + B sperm and other

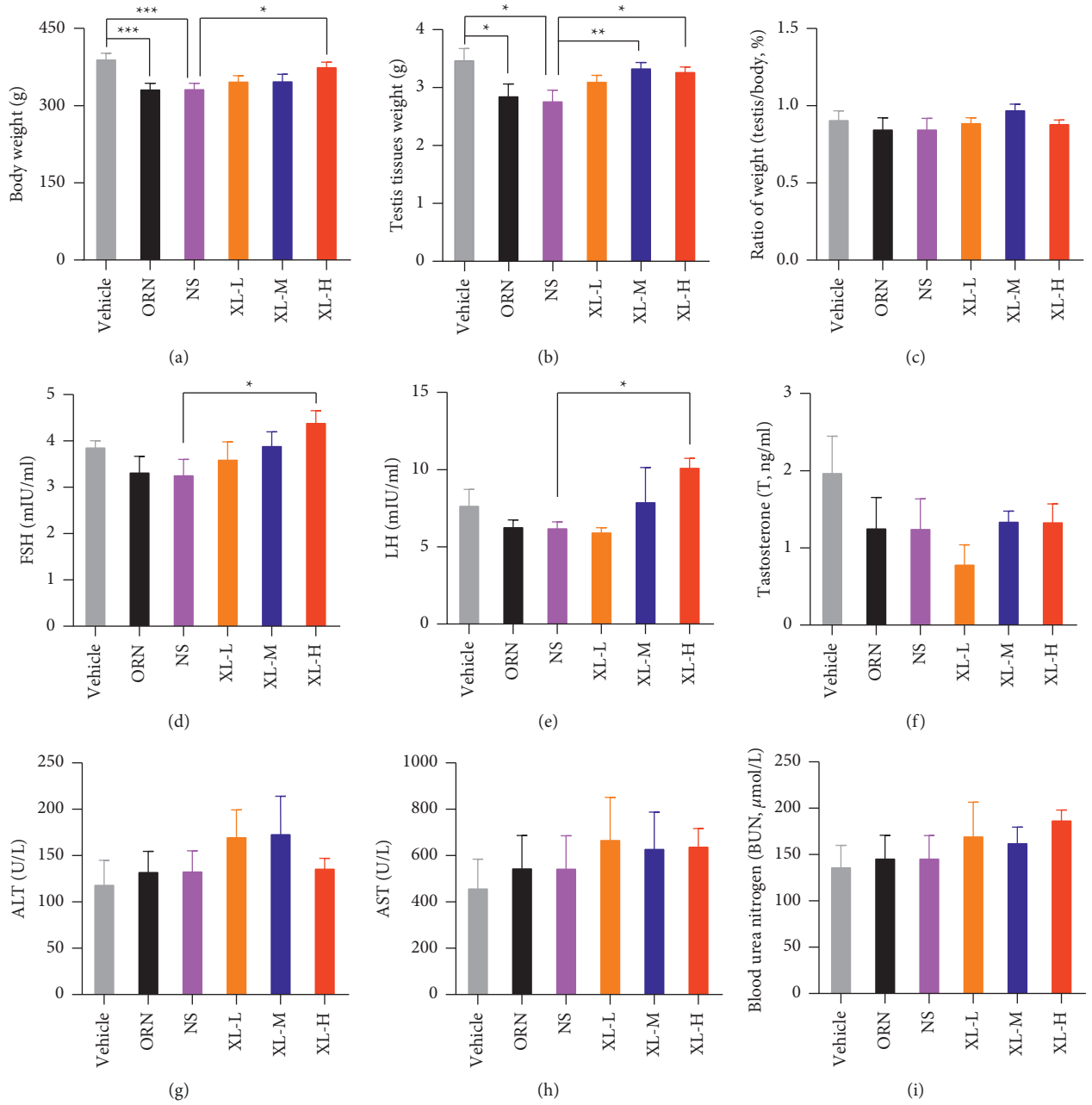

Figure 1: Improvement of FSH and LH levels in the serum of iOAZS rats with XL treatment. (a–c) Body weight, testis tissue weight, and the ratio of testis tissue weight to body weight. All data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. One-way ANOVA followed by Sidak's post hoc test, $n = 8$ to 10 rats per group. (d–f) The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T). (g–i) The levels of aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN). All data were presented as mean ± SEM. $^{*}P < 0.05$. One-way ANOVA followed by Sidak's post hoc test, $n = 5$ to 6 rats per group.

parameters of sperm motility such as VSL, VCL, VAP, ALH, LIN, and STR (Figures 3(a)–3(i)). Consistently, low- and medium-dose XL treatment enhanced sperm motility (grade A and grade A + B sperm) and some parameters of sperm motility such as VSL, VAP, and LIN (Figures 3(a)–3(i)). However, abnormal sperm morphology and DNA fragment index (single-stranded DNA/double-stranded DNA) were not altered by ORN or XL treatment in rats (Supplementary Figure 1). These data suggest that XL treatment enhanced the sperm concentration and motility of iOAZS rats.

3.4. Effects of XL Treatment on the Mitochondrial Membrane Potential (MMP) and Apoptosis Status of iOAZS Rats. To further clarify the underlying mechanism contributing to the improvement of XL treatment in iOAZS rats, we first evaluated the mitochondrial membrane potential (MMP) and apoptosis status of ORN- and XL-treated rats. Using a JC-1 assay kit, we found that MMP was reduced in the testis tissues of OAZS rats and was increased after high-dose XL treatment of iOAZS rats (Figure 4(a)). Western blotting results showed that XL treatment abrogated the reduced

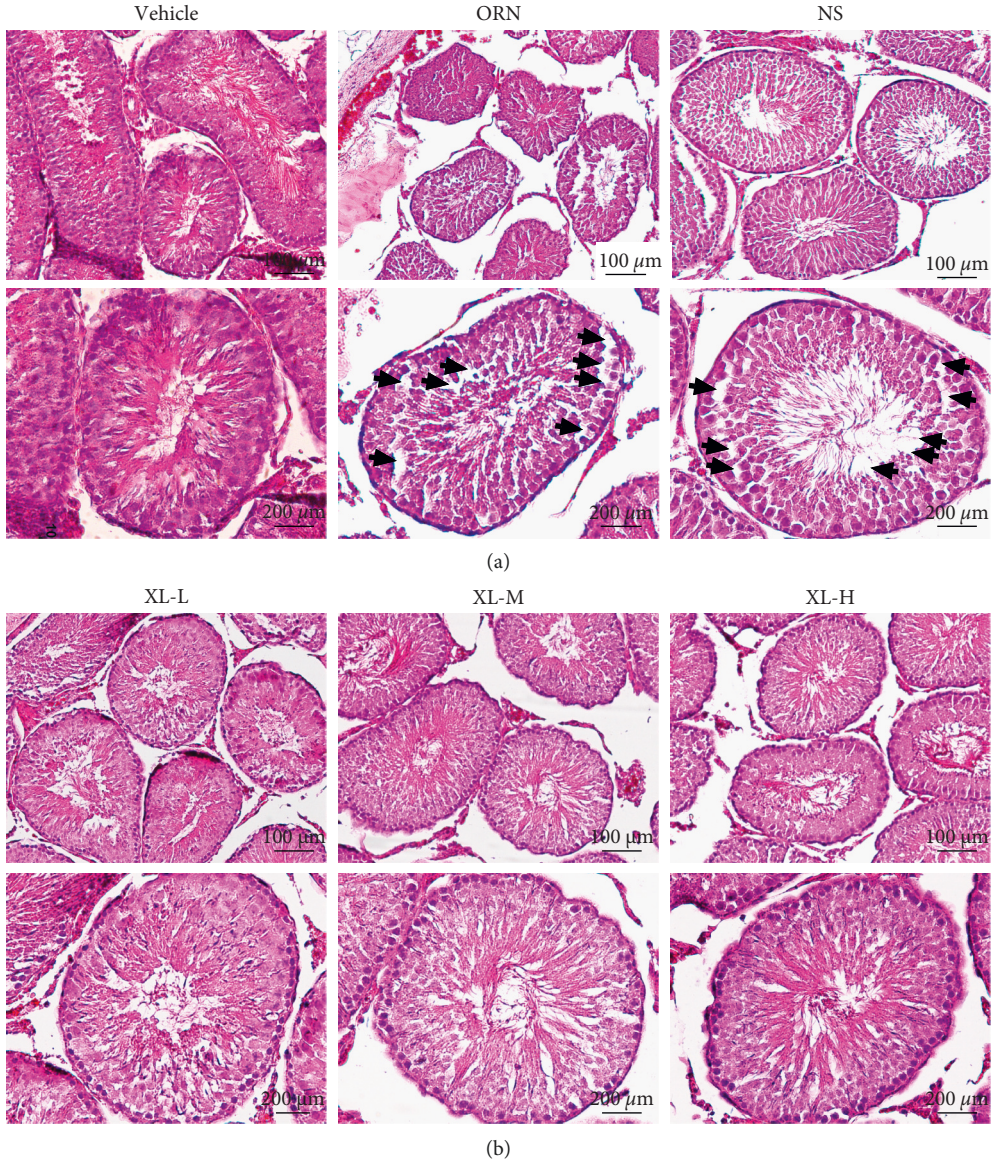

Figure 2: Ameliorated testis spermatogenesis of iOAZS rats with XL treatment. (a) H&E staining of rat testis tissues of CMC-Na (vehicle)-, ORN-, and normal saline (NS)-treated rats. (b) H&E staining of rat testis tissues of low-dose XL (XL-L), medium-dose XL (XL-M), and high-dose XL (XL-H) treated rats. Scale bar = 100 μm or 200 μm. Arrows indicate the areas without spermatogenic cells.

abundance of Bcl-2 protein in the testis tissues of OAZS rats (Figure 4(b)) and only high-dose XL treatment attenuated the increased level of caspase-3 protein in the testis tissues of iOAZS rats (Figure 4(c)). These data indicate that XL treatment ameliorated the mitochondrial membrane potential (MMP) and apoptosis status in the testis tissues of iOAZS rats.

3.5. Effects of XL Treatment on the Oxidative Stress Status of iOAZS Rats. We also found a decline in total antioxidant capacity, total GPx, and SOD activities, as well as a rise in the level of hydrogen peroxide, MDA, and protein expression of 4-hydroxynonenal (4-HNE) in the testis tissues of iOAZS rats (Figures 5(a)–5(f)). Moreover, high-dose XL treatment ameliorated the augmented oxidative stress in the testis tissues of iOAZS rats, as manifested by enhanced total antioxidant capacity, total GPx, and SOD activities and attenuation of the level of MDA and protein expression of 4-HNE (Figures 5(a)–5(f)). Taken together, we suggest that XL treatment exerts its therapeutic actions on iOAZS rats by ameliorating the mitochondrial membrane potential, apoptosis status, and oxidative stress status in testis tissues.

4. Discussion

In this study, we demonstrated that Xianlu oral solution treatment has a therapeutic effect on iOAZS by ameliorating the serum hormone levels, mitochondrial membrane potential, apoptosis status, and oxidative stress status in the testis tissues. This study provides a novel mechanism for Xianlu oral solution treatment on iOAZS, and Xianlu oral

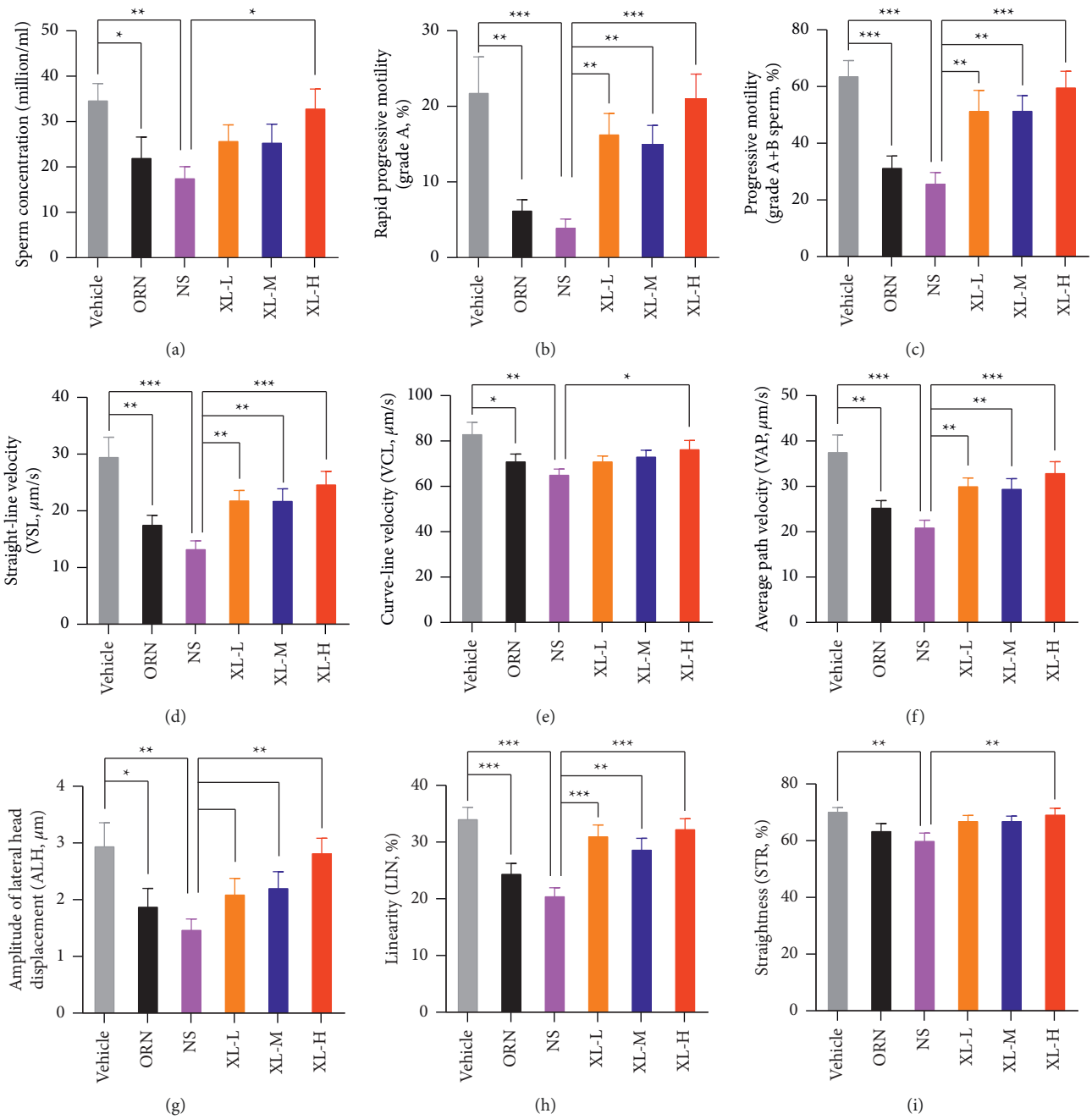

Figure 3: Enhanced sperm concentration and motility of iOAZS rats with XL treatment. (a–c) Sperm concentration, rapid progressive motility (grade A sperm), and progressive motility (grade A + B sperm). (d) Straight-line velocity (VSL), (e) curve-line velocity (VCL), (f) average path velocity (VAP), (g) amplitude of lateral head displacement (ALH), (h) linearity (LIN), and (i) straightness (STR). All data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. One-way ANOVA followed by Sidak's post hoc test, $n = 10$ to 11 rats per group.

solution may be used as a traditional Chinese medicine (TCM) therapy for male infertility caused by iOAZS in clinical practice.

Multiple causes can lead to oligoasthenozoospermia (OAZS), including infections and varicocele-induced reproductive system disease, sex chromosome abnormalities (Klinefelter's syndrome and Y chromosome microdeletions), and defects in spermatozoa flagella microstructure or function such as primary ciliary dyskinesia (PCD) or multiple morphological abnormalities of the sperm flagellum (MMAF) [26, 27]. Prostatitis or varicocele could result in augmentation of the autoimmune response and seminal inflammatory factors, increased apoptosis, increased oxidative stress, and spermatozoa DNA damage, resulting in oligoasthenoteratozoospermia [28–31]. However, there were also some cases without clear causes,

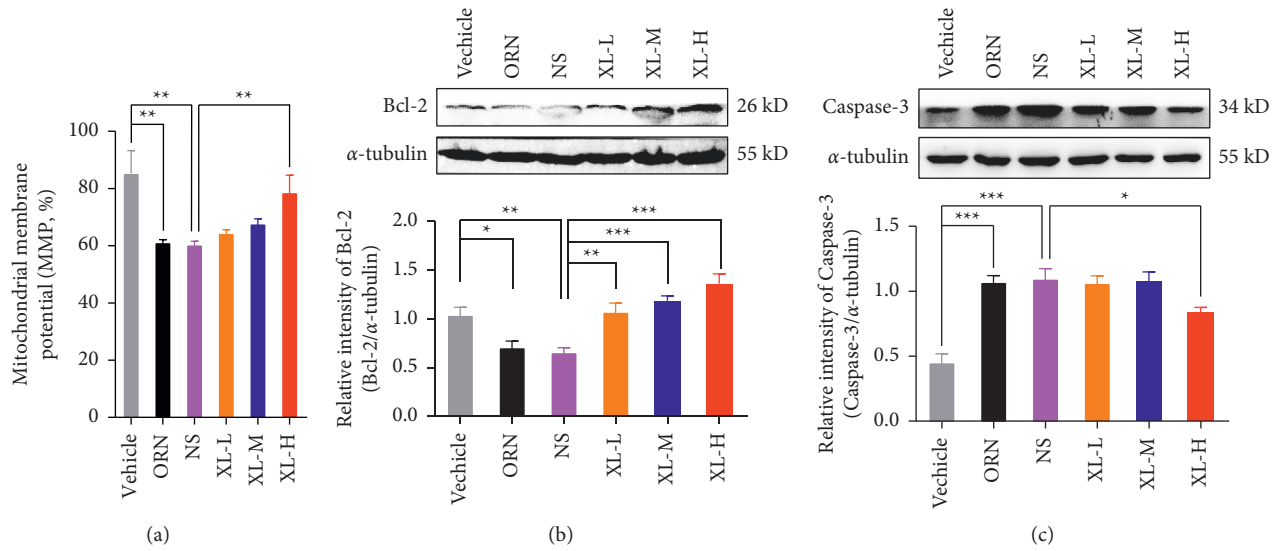

FIGURE 4: Improved mitochondrial membrane potential (MMP) and apoptosis status of iOAZS rats with XL treatment. (a) The mitochondrial membrane potential of testis tissues. (b, c) Expression of Bcl-2 and caspase-3 protein in the testis tissues. All data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. One-way ANOVA followed by Sidak's post hoc test, $n = 4$ to 5 rats per group.

categorized as idiopathic oligoasthenozoospermia (iOAZS). To date, there have been no ideal therapies for iOAZS, although methods such as treating infection or varicocele by antibiotics or surgery, changing in lifestyle, avoiding toxic environmental exposures, and maintaining regular intercourse and ejaculation have been established for OAZS, as well as the application of intracytoplasmic sperm injection (ICSI) for some severe OAZS patients caused by genetic factors [14–17].

Traditional Chinese medicine (TCM) has been widely used for the treatment of male infertility. Wenshen Shengjing decoction treatment could repair testicular damage and increase testosterone levels in the testes of cyclosporine-induced OAZS mice [20]. The components of Xianlu oral solution (XL), such as *Cuscuta chinensis* Lam., *Lycium barbarum* L., Fructus Ligustri Lucidi, and *Panax ginseng* C. A. Meyer, could ameliorate spermatogenic dysfunction and improve the sperm quality of different model animals by attenuating testis oxidative damage and apoptosis of spermatogenic cells, as well as by augmenting sex hormones [23, 32–35]. Our results showed that high-dose Xianlu oral solution (XL) treatment improved the FSH and LH but not the testosterone level in the serum, as well as without side effects on liver and kidney function of ORN-induced iOAZS rats. We think XL may influence the serum hormone levels of OAZS rats through the hypothalamic-pituitary-gonad axis (HPG). Furthermore, we found that XL treatment also ameliorated testis spermatogenesis in iOAZS rats by alleviating the disruption of seminiferous tubules and increasing the spermatogenic cells in a dose-dependent manner. Likewise, Sheng-Jing-San treatment improved the sperm motility of AZS model rats, and Wuzi Yanzong (WZYZ) formula administration restored the destroyed testicular structure of oligoasthenozoospermic model rats [18, 19]. *Cistanche tubulosa* (CT), echinacoside (ECH), and phenylethanol glycosides from *C. tubulosa* (CPhGs) treatment also attenuated the poor sperm quality and testicular toxicity of bisphenol A- or hydrocortisone-treated animals [20, 21]. In line with these findings, we found that high-dose XL treatment improved the sperm quality of ORN-induced iOAZS rats, including the augmentation of sperm concentration and sperm motility, including grade A sperm, grade A + B sperm, and other parameters of sperm motility such as VSL, VCL, VAP, ALH, LIN, and STR. Consistently, low- and medium-dose XL treatment enhanced sperm motility (grade A and grade A + B sperm) and some parameters of sperm motility, such as VSL, VAP, and LIN. We also evaluated the abnormal sperm morphology and DNA fragment index of XL-treated iOAZS rats since DNA fragmentation and epigenetic abnormalities of sperm were associated with sperm quality of men with infertility [36–39]; however, our results showed that XL treatment had no effect on abnormal sperm morphology and DNA fragment index of iOAZS rats. We speculated that these results may be due to the lack of alterations in abnormal sperm morphology and DNA fragment index after ORN treatment.

During the occurrence of spermatogenesis disorder, abnormal mitochondrial function, cell apoptosis, and oxidative stress are also involved. Excessive ROS generation of mitochondria, abnormal assembly of mitochondria, or structural defects in mitochondrial membranes are associated with asthenozoospermia or oligozoospermia [40–44]. Consistently, we demonstrated that mitochondrial membrane potential (MMP) was decreased in the testis tissues of iOAZS rats and was increased after high-dose XL treatment of iOAZS rats. We also showed that XL treatment mitigated the cell apoptosis of iOAZS rats since XL treatment abrogated the reduced abundance of Bcl-2 protein and attenuated the increased level of caspase-3 protein in the testis tissues of iOAZS rats. Likewise, the new Wenshen Shengjing decoction, *Cuscuta chinensis* Lam. and *Lycium barbarum* L. treatment reduced sperm cell apoptosis by regulating the

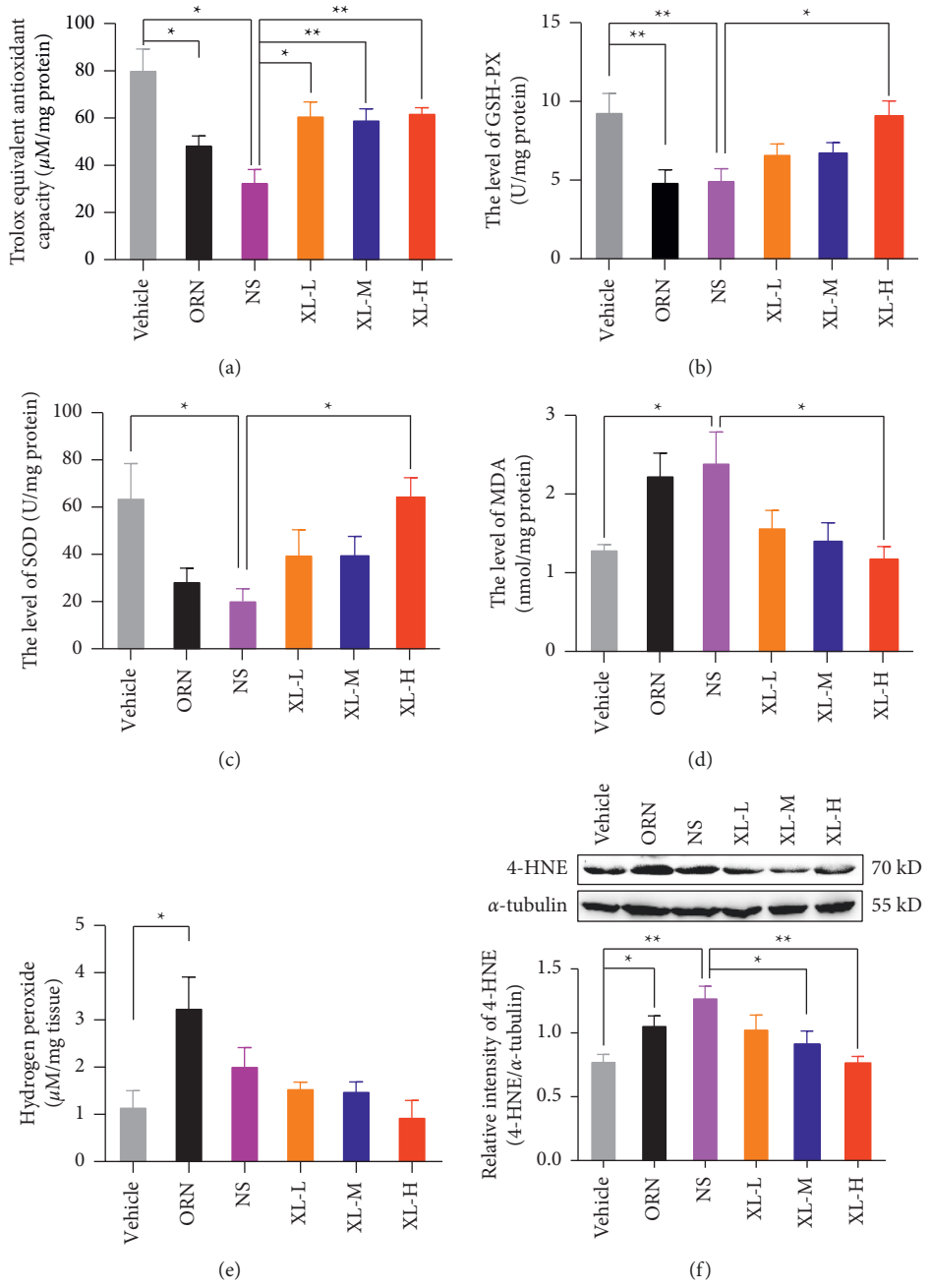

Figure 5: Alleviated oxidative stress status in the testis tissue of iOAZS rats with XL treatment. (a–c) Total antioxidant capacity represented by trolox-equivalent antioxidant capacity, total glutathione peroxidase (GPx), and total superoxide dismutase (SOD). (d–f) The level of hydrogen peroxide, lipid peroxidation represented by malondialdehyde (MDA), and protein expression of 4-hydroxynonenal (4-HNE). All data were presented as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$. One-way ANOVA followed by Sidak's post hoc test, $n = 4$ to 8 rats per group.

expression of Bcl-2, BAD, and BAX, thus improving sperm counts of *Tripterygium wilfordii* Hook. polyglycoside-treated rats [31, 43].

Oxidative stress occurs when there is an imbalance between ROS and antioxidants, and oxidative stress in testis tissues can be caused by varicocele and infection, abuse of alcohol and drugs, radiation, metabolic diseases, and mental stress [15, 45, 46]. Male infertility is associated with excessive oxidative stress and lipid peroxidation of sperm [38, 39, 47]. It is well known that oxidative stress is a major cause of male infertility since it can induce sperm nuclear and mitochondrial DNA (mtDNA) damage, telomere shortening, epigenetic alterations, and Y chromosomal microdeletions [48]. Spermatozoa are more susceptible to oxidative stress and lipid peroxidation because plasma membrane of spermatozoa contains a large amount of polyunsaturated fatty acids (PUFAs), and oxidative stress and lipid peroxidation will lead to the generation of MDA and 4-HNE, causing damage to spermatozoa [49, 50]. Y chromosomal microdeletions caused by oxidative stress during the

differentiation and maturation processes in the male reproductive tract may lead to male infertility, such as azoospermia or severe oligozoospermia [51]. Epigenetic abnormalities such as hypomethylation induced by oxidative stress were also found in oligozoospermic men [36, 37]. We also found a decline in total antioxidant capacity, total GPx, and SOD activities, as well as a rise in the level of hydrogen peroxide, MDA, and protein expression of 4-HNE in the testis tissues of iOAZS rats. Moreover, the high-dose XL treatment ameliorated the augmented oxidative stress in the testis tissues of iOAZS rats, such as the enhanced total antioxidant capacity, total GPx, and SOD activities and attenuation of the level of MDA and protein expression of 4-HNE. Likewise, *Epimedium brevicornu* Maxim (Yinyanghuo) and Fructus Ligustri Lucidi (Nvzhenzi) may have some therapeutic effects on OAZS by alleviating hydrogen peroxide-induced oxidative damage [52, 53].

5. Conclusion

In conclusion, this study demonstrates that intragastric administration of ORN to rats could produce a reduction of sperm concentration and motility, whereas Xianlu oral solution treatment increased the sperm concentration and motility by increasing the serum FSH and LH hormone levels, augmenting the mitochondrial membrane potential, mitigating apoptosis, and ameliorating oxidative stress status in the testis tissues of ORN-treated rats. Xianlu oral solution may be used as a therapy for iOAZS patients in clinical practice.

Data Availability

The data that support the findings in this study are available from the corresponding author upon reasonable request.

Ethical Approval

Approval was obtained from the IACUC of Peking University (approved number: LA2021371).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hui Jiang and Zhe Zhang conceived the overall study and supervised all the experiments. Zi-Run Jin and Ya-Lei Cao performed the experiment and drafted the manuscript. Zhi-Chao Luo collected the samples and helped to draft the manuscript. Qian-Cheng Zhao, Yu Xi, and Jia-Ming Weng performed some experiments or analyzed the data. All authors have read and approved the final manuscript. Zi-Run Jin and Ya-Lei Cao contributed equally to this work.

Acknowledgments

This work was funded by the National Key Research and Developmental Program of China (no. 2021YFC2700203), Natural Science Foundation of Beijing Municipality (no. 7222208), and National Natural Science Foundation of China (nos. 81901535, 82101676, and 82071698).

Supplementary Materials

Supplementary Figure 1. No alterations in sperm morphology or DNA integrity were observed in iOAZS rats treated with XL. (A) Representative images and summary of sperm morphology. The red arrow indicates the sperm with abnormal morphology. All data were presented as mean ± SEM. One-way ANOVA followed by Sidak's post hoc test, $n = 24$ visual fields from 6 rats per group. (B) Representative images of sperm DNA integrity. Green indicates the double-stranded DNA, and red indicates the single-stranded DNA of sperm. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 4291477, 9 pages
https://doi.org/10.1155/2022/4291477

Research Article

Tai Chi for Overweight/Obese Adolescents and Young Women with Polycystic Ovary Syndrome: A Randomized Controlled Pilot Trial

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Received 13 April 2022; Accepted 3 June 2022; Published 17 June 2022

Academic Editor: Arthur De Sá Ferreira

Background. Exercise is one of the recommended interventions for polycystic ovary syndrome (PCOS), and current evidence has shown that Tai chi may have favorable effects. The objective of this randomized controlled pilot trial was to study the feasibility and potential effects of Tai chi for overweight/obese adolescents and young women with PCOS, so a future definitive randomized controlled trial (RCT) can be well designed and implemented. *Materials and Methods*. This study recruited 50 patients who were randomly assigned to two groups (Tai chi and self-monitored exercise) at a ratio of 3 : 2. The intervention lasted for 3 months, and the feasibility and effectiveness outcomes were measured. *Results*. A total of 42 patients completed the study, including 24 in the Tai chi group and 18 in the control group. Compared with the self-monitored exercise group, there was a significantly decreased body mass index (BMI) in the Tai chi group adjusted for baseline BMI. The testosterone level and lipid profile were also decreased compared to controls; the same tendency was also observed for the homeostasis model assessment of insulin resistance (HOMA-IR), but the difference did not achieve statistical significance. Twenty-four (out of 30, 80%) patients in the Tai chi group and 18 (out of 20, 90%) patients in the self-monitored exercise group completed the data collection. A total of 36 exercise sessions were held in both groups. Patients in the Tai chi group took a mean of 34.0 ± 2.21 classes (93.06%), and those in the self-monitored exercise group engaged in 32 ± 3.06 exercise sessions (88.27%) out of the 36 required exercise sessions. *Conclusions*. The present pilot study was feasible to deliver; there was a decrease in BMI, testosterone level, and lipid profile for PCOS patients in the Tai chi group at 3 months. In a future definitive trial, lower recruitment rate and outcome measurements lead to poor patient acceptance such as the 5-time point oral glucose tolerance test need to be considered and one fixed type of aerobic exercise and supervision from the investigator for the control group are also needed. Trial registration: ClinicalTrials.gov, NCT02608554.

1. Background

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorders, and it affects 5–20% of reproductive-age women [1]. The clinical characteristics of PCOS include both reproductive features and metabolic features [2, 3], and being overweight/obese worsens all clinical features of PCOS.

Treatment aims in PCOS include achieving a healthy weight, improving any underlying hormonal disturbances, preventing future reproductive and metabolic complications, and improving the quality of life [4]. Lifestyle interventions, including dietary, exercise, behavioral, or their combination, are recommended as first-line management for PCOS patients [4]. A 5% to 10% weight loss in PCOS patients is considered clinically significant and is associated with metabolic, reproductive, and psychological health benefits [4]. A Cochrane systematic review reported that lifestyle treatment might result in a modest reduction in weight and an improvement in abdominal obesity, namely, a

mean difference in weight for lifestyle changes compared to minimal treatment of around 1.68 kg [5]. Several published systematic reviews suggested that there is limited evidence concerning exercise alone on reproductive outcomes; however, exercise may have favorable effects on body composition and insulin resistance [6–10].

Tai chi is an exercise system that follows Chinese medicine theory and has been practiced in China since the seventeenth century. The five styles which are practiced most commonly today are the Yang, Chen, Wu, Sun, and Woo styles [11]. In the past few decades, an increasing number of studies have focused on its beneficial effects in treating diseases and maintaining health, and it has received increasing attention in the West [12, 13]. The physiological and psychosocial benefits of Tai chi on chronic diseases that are very closely related to PCOS, such as obesity, cardiovascular diseases (CVDs), and type 2 diabetes [14–16], have been confirmed.

Although exercise is recommended as one of the first-line approaches for the treatment of PCOS, specific recommendations for exercise prescription are lacking, and further research into these fields is necessary. We performed this study to address whether a randomized controlled trial (RCT) of Tai chi in treating overweight/obese adolescents and young women with PCOS was an appropriate trial design and was feasible with regard to (i) recruitment and retention and (ii) treatment fidelity. In addition, we wished to assess the potential effectiveness of a 3-month Tai chi intervention and collect and synthesis data, from which the sample size of a definitive RCT could be estimated. This pilot trial may not be adequately powered for assessing effectiveness; however, together with the feasibility outcomes, the results obtained will provide data to estimate the parameters required to design a definitive RCT in the future.

2. Methods

2.1. Trial Design. The present study was a single-blind (assessors), parallel randomized clinical pilot trial. Unequal randomization of 3 : 2 was adopted since it has been suggested that it is better to have as many participants receive the intervention as is feasible [17]. The present study was registered at ClinicalTrials.gov (NCT02608554) and compliant with Consolidated Standards of Reporting Trials (CONSORT) [18] and CONSORT extension to randomized pilot and feasibility trials [17]. The study was conducted in accordance with the Declaration of Helsinki and received approval from the First Affiliated Hospital of the Heilongjiang University of Chinese Medicine Institutional Review Boards (approval number: HZYLLKT201500201). The details of the trial protocol were reported in another paper [19].

2.2. Participants. The present study was conducted in the First Affiliated Hospital, Heilongjiang University of Chinese Medicine. Patients were recruited from the Gynecology Outpatient Clinic. All study visits and Tai chi interventions took place at the abovementioned hospital. Self-monitored exercises were held in a home-based environment.

Inclusion criteria were used as follows: (i) women aged between 18 and 35 years [20]; (ii) patients who confirmed diagnosis of PCOS according to the modified Rotterdam criteria, and all subjects had anovulation plus either polycystic ovaries and/or hyperandrogenism; (iii) patients with at least two years after menarche; (iv) patients whose body mass index (BMI) is equal to or greater than 23 kg/m^2 [21]; and (v) patients with no desire to have children within 6 months. PCOS was defined by the modified Rotterdam criteria as oligomenorrhea or amenorrhea, together with the presence of ≥12 antral follicles (≤9 mm) and/or ovarian volume >10 mL on transvaginal scanning, and/or clinical/biochemical hyperandrogenism. Oligomenorrhea was defined as an intermenstrual interval >35 days and less than eight menstrual bleeds in the past year. Amenorrhea was defined as an intermenstrual interval >90 days. Clinical hyperandrogenism in mainland China is defined as Ferriman–Gallwey (FG) score ≥5 [22].

Exclusion criteria were as follows: (i) administration of other medications known to affect the reproductive function or metabolism within the past 3 months, including oral contraceptives, gonadotropin-releasing hormone (GnRH) agonists and antagonists, antiandrogens, gonadotropins, antiobesity drugs, Chinese herbal medicines, and antidiabetic drugs, such as metformin and thiazolidinediones, somatostatin, diazoxide, and calcium-channel blockers; (ii) patients with other endocrine disorders, including 21-hydroxylase deficiency, hyperprolactinemia, uncorrected thyroid (including hypothyroidism, hyperthyroidism, and/or thyroid autoimmunity) disease, and suspected Cushing's syndrome; and (iii) patients with known severe organ dysfunction or mental illness.

2.3. Interventions. The 24 forms of the simplified Tai chi program (a modified Yang style) recommended as a health-benefiting sport by the General Administration of Sport of China were applied [23]. Patients attended a 60 min exercise session three times per week for 12 weeks, with an intensity based on their original level of physical activity. Each session comprised 40 min of Tai chi training plus a 10 min warm-up and cool-down. Tai chi training was instructed by experienced Tai chi instructors who were qualified in teaching. Patients in the self-monitored exercise group were asked to add extra exercise in addition to their routine exercise. Self-monitored exercise consisted of brisk walking, cycling, jogging, or any other aerobic exercise for 60 min, three times per week for 12 weeks [24]. Adherence to the exercise was tracked via self-reported logs.

2.4. Outcome Measurements. The primary outcome was the BMI change from baseline. BMI was calculated using weight (kg)/height (m^2). The secondary outcomes included (i) homeostasis model assessment of insulin resistance (HOMA-IR), fasting plasma glucose (FPG), and fasting insulin (FINS); (ii) hormone profile including testosterone (T), androstadiendione (AND), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), follicle-stimulating hormone (FSH), luteinizing

hormone(LH), and oestradiol (E_2); (iii) fasting-lipid metabolic profile: triglycerides (TG), cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C); (iv) the weight, waist-to-hip ratio, and FG score before and after treatment; (v) feasibility outcomes including participant recruitment rates, retention rates, and treatment fidelity monitored through attendance records; and (vi) adverse events. We failed to collect data from all participants including oral glucose tolerance tests (OGTTs), blood pressure, or the presence of acne because a large proportion of the enrolled patients refused to undergo these examinations.

2.5. Randomization and Allocation Concealment. After the baseline evaluation, 50 subjects will be allocated randomly into one of the two groups in a ratio of 3 : 2. The identification code and random number, which are unique for each subject, were generated using SAS 9.2 by an independent agency (TCM Translational Medicine Research Center, First Affiliated Hospital, Heilongjiang University of Chinese Medicine). These assignments were put into sealed and opaque envelopes. The envelopes will only be opened after the subject has completed baseline clinical assessments.

2.6. Blinding. The present trial is a single-blinded trial. Outcome assessors and people responsible for statistical analysis will be blinded to the randomization status.

2.7. Statistical Analysis. The effect size of Tai chi intervention on PCOS was not available by the time when this study was designed. As this trial is an exploratory study mainly to evaluate the feasibility of a large clinical trial, sample size was determined by recommendations for a pilot trial, and $n = 30$ was recognized as a reasonable minimal sample size for pilot studies [25,26]. For such a trial designed with 90% power and two-sided 5% significance, the sample size was set to 50 patients with an allocation ratio of 3 : 2 as described in the protocol [19], and the standardized effect sizes is medium (0.5) [27].

All analysis was performed using SAS version 9.3. Data were summarized using means (±SDs) for continuous variables. An analysis of covariance (ANCOVA) model was used to compare the mean changes in outcomes from baseline to the end of the intervention, including baseline measurement covariates. Comparisons between the groups were made using the *t*-test (two-tailed) or the Mann–Whitney *U* test. A paired *t*-test or the Wilcoxon rank test was used to compare the variables from the baseline to the end of the intervention. $P < 0.05$ was considered statistically significant.

3. Results

Fifty patients were enrolled in the trial; 30 were randomized to the Tai chi group and 20 to the control group. Eight patients terminated the study prematurely; thus, 42 patients completed the end of treatment assessments and were included in analysis (Figure 1). Their anthropometric data and baseline characteristics are shown in Table 1.

3.1. Primary Outcome. The primary outcome measurement was the BMI change from baseline. After 3 months of treatment, BMI significantly decreased in the Tai chi group compared with before treatment. However, BMI did not differ before and after treatment in the self-monitored exercise group. Compared with the self-monitored exercise group, there was a significantly decreased BMI in the Tai chi group adjusted for baseline BMI (Table 2).

3.2. Secondary Outcome. After treatment, the Tai chi group showed favorable changes in body weight, FINS, and HOMA-IR with no significant differences between the groups. For the hormonal profile, *T* significantly decreased in the Tai chi group compared with the self-monitored exercise group. In addition, Tai chi showed a significant effect in alleviating lipid profile disorders, including decreased TG, TC, and LDL-C, compared with the self-monitored exercise group (Table 3).

Recruitment was conducted in the Gynecology Outpatient Clinic, First Affiliated Hospital, Heilongjiang University of Chinese Medicine. A total of 1023 subjects were screened prior to eligibility assessment, and we excluded 508 of them since these subjects lived far away from the trial site and could not participate in Tai chi exercise under supervision. A total of 515 subjects were assessed for eligibility, 443 were excluded for not meeting inclusion criteria, 15 were excluded because they did not want to participate in the trial, and 7 were excluded for taking other medications that could have affected the outcome. The primary barriers for participating in this study were patients who lived too far away from the hospital or had no time to participate due to work or household activities.

Fifty patients were enrolled in the trial. After three months of intervention, 24 (of 30, 80%) patients in the Tai chi group and 18 (of 20, 90%) patients in the self-monitored exercise group completed the data collection. Reasons for dropping out are recorded in Figure 1.

A total of 36 Tai chi classes were held during the intervention, and patients attended a mean of 34.0 ± 2.21 classes (93.06%). Patients in the self-monitored exercise group undertook 32 ± 3.06 exercise sessions (88.27%) out of 36 required exercise sessions.

3.3. Adverse Events. There were no adverse events reported during the study period.

4. Discussion

In this study, we designed a pilot RCT to assess the feasibility and potential effectiveness of Tai chi in treating overweight/obese adolescents and young women with PCOS. The main results showed that the major impediment to recruitment was a long distance from the trial site and no time to exercise, which were also the main reasons for dropping out.

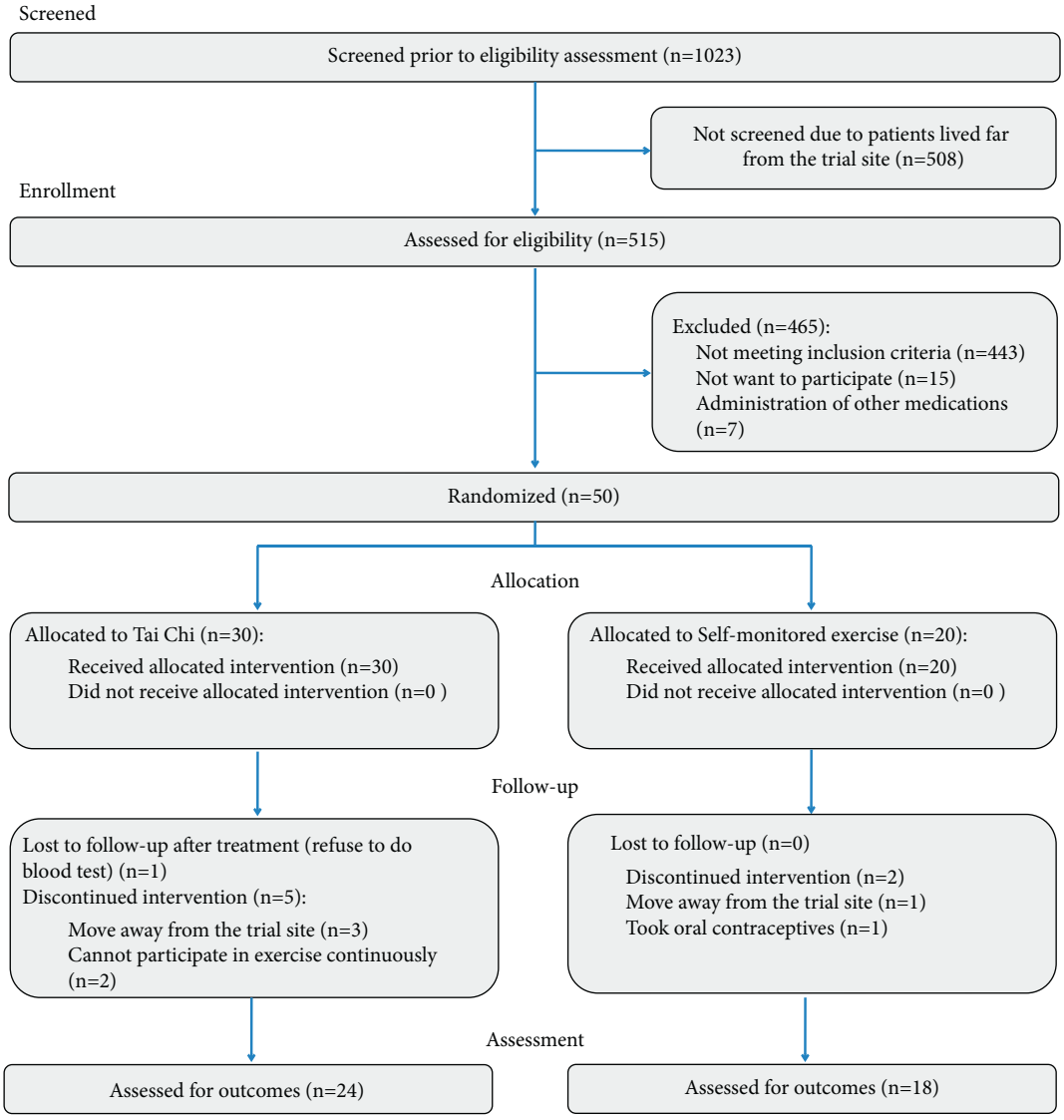

Figure 1: Flow diagram of the participants through the study.

Table 1: Characteristics of the study participants.

	Tai chi group ($n = 24$)	Self-monitored exercise group ($n = 18$)
Age (years)	23.2 ± 4.38	22.9 ± 4.64
Weight (kg)	74.99 ± 11.62	78.33 ± 13.45
Height (m)	1.62 ± 0.05	1.63 ± 0.05
BMI (m/kg^2)	28.41 ± 4.03	29.57 ± 4.47
Waist circumference (cm)	97 ± 8.73	96.4 ± 13.67
Hip circumference (cm)	107.27 ± 7.9	106.95 ± 8.05
Waist-to-hip ratio (cm/cm)	0.9 ± 0.05	0.9 ± 0.08

Data are expressed as the mean ± standard deviation. BMI: body mass index; FG score: Ferriman–Gallwey score.

However, the trial also found that Tai chi may significantly decrease the BMI, T level, and lipid profile; the same tendency was also observed for HOMA-IR, but the difference did not achieve statistical significance compared to self-monitored exercise. Since this pilot trial was intentionally designed without adequate power to test the efficacy, these effectiveness outcomes should be interpreted with caution.

The recruitment rate of this pilot study seemed lower than that in other exercise studies in PCOS [28, 29]. The barriers to recruitment could partially be because, in this pilot trial, Tai chi needed to be practiced under the guidance of an experienced instructor and only subjects who lived near the clinical site and who were available during the exercise sessions could participate. However, this situation may have little effect when Tai chi is used as a treatment because after mastering Tai chi practice, patients can exercise on their own, which improves the flexibility of time and location. And, online learning will also be a strategy to promote more patients to involve. The retention in the Tai chi group was similar to that in the self-monitored exercise group and other reports [28, 30], which suggests that Tai chi may be a feasible intervention for women with PCOS. Five-

Table 2: Primary outcome measure.

	Tai chi group ($n = 24$)		P value	Self-monitored exercise group ($n = 18$)		P value	Differences in changes between the groups at 3 months (P value)
	Baseline	3 months		Baseline	3 months		
BMI (m/kg^2)	28.41 ± 4.03	27.39 ± 3.58	0.008$^{\Delta}$	29.57 ± 4.47	29.62 ± 3.95	0.524	0.003*

Data are expressed as the mean ± standard deviation. BMI: body mass index; *indicates $P < 0.05$ vs. the self-monitored exercise group; $^{\Delta}$ indicates $P < 0.05$ vs. baseline.

Table 3: Characteristics of study participants.

	Tai chi group ($n = 24$)		P value	Self-monitored exercise group ($n = 18$)		P value	Differences between the groups at 3 months (P value)
	Baseline	3 months		Baseline	3 months		
Weight (kg)	74.99 ± 11.62	73.08 ± 11.55	0.009$^{\Delta}$	78.33 ± 13.45	77.97 ± 12.1	0.547	0.203
Waist-to-hip ratio (cm/cm)	0.9 ± 0.05	0.9 ± 0.04	0.956	0.9 ± 0.08	0.91 ± 0.08	0.481	0.833
FPG (mmol/L)	4.89 ± 0.89	4.84 ± 0.54	0.542	4.79 ± 0.53	4.83 ± 0.38	0.997	0.964
FINS (μU/ml)	20.08 ± 12.39	13.99 ± 8.2	0.035$^{\Delta}$	19.35 ± 10.26	20.36 ± 14.1	0.275	0.073
HOMA-IR	4.51 ± 3.15	3.05 ± 1.82	0.042$^{\Delta}$	4.07 ± 2.0	4.36 ± 3.12	0.396	0.094
T (ng/dL)	61.84 ± 20.96	49.89 ± 15.77	0.045$^{\Delta}$	63.92 ± 26.49	66.59 ± 28.15	0.822	0.032*
AND (ng/mL)	274.21 ± 146.29	312.71 ± 182.51	0.53	285.3 ± 146.62	296.83 ± 136.46	0.892	0.758
SHBG (nmol/L)	19.34 ± 10.78	22.74 ± 9.64	0.093	28.61 ± 37.42	23.24 ± 13.73	0.512	0.890
DHEAS (μg/dl)	7.17 ± 5.26	5.16 ± 2.45	0.017$^{\Delta}$	5.92 ± 2.66	4.98 ± 2.51	0.01$^{\Delta}$	0.818
LH (mIU/mL)	9.25 ± 5.08	4.54 ± 1.45	0.09	11.11 ± 12.01	4.42 ± 1.1	0.253	0.779
FSH (mIU/mL)	4.54 ± 1.66	6.96 ± 3.6	0.748	4.45 ± 0.99	7.7 ± 5.01	0.882	0.581
LH/FSH	2.19 ± 1.09	1.58 ± 0.74	0.024$^{\Delta}$	2.28 ± 1.65	1.74 ± 1.11	0.232	0.556
E_2 (pg/mL)	58.81 ± 20.36	47.99 ± 18.42	0.052	62.2 ± 54.92	59.85 ± 19.16	0.68	0.049*
TG (mmol/L)	1.79 ± 1.35	1.33 ± 0.61	0.308	2.29 ± 1.29	2.3 ± 1.25	0.475	0.006*
TC (mmol/L)	4.13 ± 1.09	3.81 ± 0.66	0.431	4.78 ± 1.18	4.75 ± 1.32	0.585	0.11*
HDL-C (mmol/L)	1.07 ± 0.28	1.07 ± 0.27	0.936	1.11 ± 0.21	1.12 ± 0.28	0.86	0.572
LDL-C (mmol/L)	2.63 ± 0.71	2.43 ± 0.65	0.368	3.01 ± 0.99	3.13 ± 0.99	0.893	0.009*
FG score (score)	5.0 ± 2.52	4.71 ± 2.93	0.206	4.35 ± 2.58	5.0 ± 3.27	0.327	0.817

*indicates $P < 0.05$ vs. the self-monitored exercise group; $^{\Delta}$indicates $P < 0.05$ vs. baseline; data are expressed as the mean ± standard deviation. FPG: fasting plasma glucose; FINS: fasting insulin; HOMA-IR: homeostasis model assessment of insulin resistance; T: testosterone; AND: androstadienone; SHBG: sex hormone-binding globulin; DHEAS: dehydroepiandrosterone sulfate; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E_2: oestradiol; TC: cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FG score: Ferriman–Gallwey score.

time point OGTT had low acceptance to patients, especially at the end of treatment visit. Acne was difficult to document precisely because patients were not willing to remove makeup. Blood pressure was not fully documented because these young patients thought it was irrelevant to the condition.

BMI will be used as the primary outcome measure for the future definitive trial. Data from the present trial showed that the BMI value in the Tai chi and control group was 27.39 ± 3.58 m/kg^2 and 29.62 ± 3.95 m/kg^2, respectively. Sixty-six participants per group will be required to achieve a 90% statistical power ($\alpha = 0.05$) for changes between the intervention group and the control group. With an estimated 20% attrition, we plan to recruit a total of 160 participants.

Tai chi has been reported to alleviate the lipid profile disorders in other conditions [31–34], which was also observed in this trial. A significant difference was observed between the two intervention groups in TC, TG, and LDL-C. BMI and HOMA-IR were also decreased in the Tai chi group, which seems consistent with reports that Tai chi could contribute to weight loss and improve insulin resistance [35], and the effect seemed similar to other aerobic exercises [36]. Aerobic exercises could improve insulin measures in women with PCOS, especially those involved in more vigorous activity and/or more frequent weekly exercise or sessions of longer duration [37]. Vigorous aerobic activity at least three days per week for 30 min or more is recommended. A heart rate monitor or maximal oxygen consumption (VO_{2max}) guided intensity levels (~60% VO_{2max}) are advised to obtain insulin-related benefits [37].

Little is known about the effect of Tai chi on sex hormones. In this trial, we observed a decrease in *T* levels in the Tai chi group. Current evidence has shown that improvements in androgens are more likely with resistance or strength training instead of aerobic exercise [29, 37]. Yoga, another popular mind-body exercise, has also been reported to improve *T* levels [38]. Additional studies are warranted to confirm these findings.

As a treatment for PCOS, it is suggested that a minimum of 120 minutes of vigorous intensity exercise per week is necessary to provide favorable health outcomes [6]. A systematic review compared the effects of high-intensity interval training (HIIT) and moderate-intensity steady-state

exercise (MISS) in PCOS patients [37]. The authors reported that neither exercise type improved HOMA-IR, MISS improved the relative maximal oxygen consumption and anthropometric profile, and decreased the TC (moderate-quality evidence), and MISS exercise appeared to be more effective in improving cardiorespiratory fitness and BMI in women with PCOS than HIIT [39].

The exercise prescription of Tai chi still needs future investigation, specifically in terms of exercise intensity, frequency, and duration. It has been shown that the intensity of Tai chi is approximately 52–63% of the heart rate range [40], which is comparable to low- or moderate-intensity aerobic exercises [41–43]. The intensity of Tai chi varies depending on the training style and posture [11]. The Yang style is the most popular due to its gentleness and extensive stretching; the Chen style requires more strength and involves more skipping movements, which may consume the most energy among all five branches [44]. A recently published systematic review compared different training styles of Tai chi among patients with type 2 diabetes, and the results showed that different styles could result in variable effectiveness [45]. The most suitable form of Tai chi for PCOS still needs further study. We also noticed that there are studies of modified Tai chi [46–48] or Tai chi combined with other exercises (e.g., theraband) [49, 50]. These modifications may point to a potential strategy for treating PCOS patients.

It has been confirmed that exercise programs that incorporate ~170 min of exercise/weekly improved insulin sensitivity more than a program utilizing ~115 min of exercise/weekly among PCOS patients [51]; therefore, practicing Tai chi at least 3 times per week may be suitable, which was well accepted in the present pilot trial. However, current evidence is insufficient to support whether long-term Tai Chi training is superior to short-term training [45]. Another point also needs to be elucidated, that is, what is the optimal time of day to exercise? A crossover study of HIIT for type 2 diabetes showed that afternoon HIIT was associated with better glucose control than morning HIIT [52]. Additional studies are necessary to elucidate the exercise prescription of Tai chi.

Given the preliminary nature of a feasibility study, the present study has several limitations. First, this trial only enrolled overweight/obese women with PCOS, and the effects of Tai chi need future investigation in normal-weight subjects, and reproductive outcomes, such as the live birth rate and the ovulation rate, need to be considered. Second, the present trial did not investigate the impact of Tai chi on quality of life. As a mind-body exercise, Tai chi is reported to have favorable effects in improving the quality of life of the subjects [53–55], and it is well known that PCOS is associated with a lower quality of life compared to healthy women [56–59]. Studies in this area should be carried out in the future. Last but not the least, we used multiple aerobic exercises in the control group without supervision from the investigator, this may cause bias between the groups. A good strategy of supervision from investigators or quantitative measurements of exercise intensity are needed in the future.

5. Conclusions

Tai chi is a mind-body approach that may be considered a therapeutic option in the multidisciplinary management of PCOS, and the present pilot study was feasible to deliver. There was a decrease in BMI, testosterone level, and lipid profile for PCOS patients in the Tai chi group at 3 months. In a future definitive trial, lower recruitment rate may be fixed by online teaching, a 5-time point oral glucose tolerance test which leads to poor patient acceptance need to be considered, one fixed type of aerobic exercise and supervision from the investigator for the control group are needed, and quantitative measurement of exercise intensity will also be considered. The evidence generated from the present pilot trial will inform further definitive trials that are required to evaluate the effectiveness of Tai chi on PCOS and to develop optimized exercise prescriptions.

Abbreviations

PCOS: Polycystic ovary syndrome
RCT: Randomized controlled trial
BMI: Body mass index
HOMA-IR: Homeostasis model assessment of insulin resistance
CONSORT: Consolidated Standards of Reporting Trials
FG: Ferriman–Gallwey
GnRH: Gonadotropin-releasing hormone
FPG: Fasting plasma glucose
FINS: Fasting insulin
T: Testosterone
AND: Androstadiendione
SHBG: Sex hormone-binding globulin
DHEAS: Dehydroepiandrosterone sulfate
FSH: Follicle-stimulating hormone
LH: Luteinizing hormone
E_2: Oestradiol
TG: Triglycerides
TC: Cholesterol
HDL-C: High-density lipoprotein cholesterol
LDL-C: Low-density lipoprotein cholesterol
OGTTs: Oral glucose tolerance tests
ANCOVA: Analysis of covariance
VO_{2max}: Maximal oxygen consumption
HIIT: High-intensity interval training
MISS: Moderate-intensity steady-state exercise.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The studies involving human participants were reviewed and approved by the Institutional Review Board of First Affiliated Hospital, Heilongjiang University of Chinese Medicine (approval number: HZYLLKT201500201).

Consent

The participants provided their written informed consent to participate in this study.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors' Contributions

Conceptualization was carried out by LY and H-LH. Data curation was performed by P-CL and G-JJ. Formal analysis was performed by Z-MW and X-LZ. Funding acquisition was carried out by LY. Investigation was carried out by P-CL. Methodology was performed by LY and W-YJ. Validation was carried out by P-CL, Z-MW, G-JJ, and G-YH. Original draft was prepared by LY and W-YJ. Review and editing were carried out by LY, P-CL, Z-MW, X-LZ, G-JJ, W-YJ, G-YH, and H-LH. All authors read and approved the final manuscript.

Acknowledgments

The research team thanks Prof. Guangying Cao for her great input at coaching patients in the Tai chi group and Lihui-Kou,Chunyan Li, YanqiuLi, HuijunZheng, LixueFeng, and Qian Yin for their assistance during the conduct of the trial. This research was funded by the State Administration of Traditional Chinese Medicine of People's Republic of China, grant number: JDZX2015058; National Foundation of Natural Science of China, grant number: 81804139; and the Heilongjiang Provincial Administration of Traditional Chinese Medicine, grant number: ZHY2020-130.

Supplementary Materials

CONSORT 2010 checklist of information to include when reporting a randomized trial. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 5929694, 13 pages
https://doi.org/10.1155/2022/5929694

Research Article

Proteomic Analysis of Human Follicular Fluid Reveals the Pharmacological Mechanisms of the Chinese Patent Drug Kunling Pill for Improving Diminished Ovarian Reserve

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Received 10 April 2022; Accepted 13 May 2022; Published 27 May 2022

Academic Editor: Min Hu

Objective. To explore the pharmacological mechanism of a Chinese patent drug (Kunling Pill (KLP)) on improving diminished ovarian reserve based on proteomic analysis. *Methods*. A total of 18 patients divided into three groups (the normal ovary reserve (NOR), diminished ovary reserve (DOR), and KLP groups) undergoing assisted reproductive technology by standard ovarian stimulation protocols were recruited to collect follicular fluid. Data-independent acquisition mass spectrometry was used to identify differentially expressed proteins by nano-LC-MS/MS. Bioinformatic analysis was conducted to predict the functions and pathways of the identified proteins. Clinical, hormonal, and biochemical parameters were also analyzed in the three groups. *Results*. A total of 144 differentially expressed proteins were screened out, including 56 proteins that were downregulated and 88 proteins that were upregulated in the DOR group compared with the NOR group, while 27 proteins were shared in the KLP-treated group. Among them, 10 proteins were upregulated and 17 proteins were downregulated in the KLP-treated group compared with the DOR group. The most enriched biological processes accounted for 28 GO terms, including cellular process, biological regulation, metabolic process, and regulation of biological process. Significant pathways were associated with fatty acid elongation, fatty acid degradation, fatty acid metabolism, nicotinate and nicotinamide metabolism, and valine, leucine, and isoleucine degradation. *Conclusion*. Our study provides the proteome profiles of human follicular fluid from DOR patients treated by KLP. Functional analyses of proteome datasets revealed that core proteins (SAA1, MIF, and PRDX5) and related pathways (fatty acid metabolism, nicotinate and nicotinamide metabolism, and tyrosine and purine metabolism) are possible pharmacological mechanisms through which KLP improves DOR. Therefore, these findings may help better understand the complex mechanisms through which DOR is treated by the Chinese patent drug KLP.

1. Introduction

With lifestyle changes, a trend for postponement of pregnancy due to later marriage, and later childbearing, the number of infertile couples in China has increased. According to clinical reports, more than 20% of women with infertility are diagnosed with diminished ovarian reserve (DOR) in China (ref. in Chinese, not shown) [1, 2]. The main reasons for DOR include genetics, aging, stress, endometriosis, reproductive endocrine disorders, chemotherapy and radiotherapy, excessive drinking, autoimmune diseases, chemical toxins, ovarian induction agents, certain systemic

diseases, previous mumps infection, and some unclear etiologies [3]. Although there is no generally accepted definition of DOR, it has been described as a pathophysiological ovarian state characterized by poor fertility outcome, poor ovarian response, and an abnormal ovarian reserve [4, 5].

In the clinic, many treatment approaches have been attempted to improve reduced ovarian reserves, such as estrogen replacement therapy [6, 7], dehydroepiandrosterone [8], resveratrol [9], coenzyme Q10 [10], and stem cell therapy [11]. However, the evidence to support these interventions is weak. Some challenges include unexpected adverse reactions such as breast cancer, heart disease, and thrombus and an increased incidence of estrogen or androgen-related malignancies [12]. Therefore, researchers have sought safer and more effective medicines for treating DOR.

In traditional Chinese medicine (TCM) theory, DOR is categorized as a liver depression and kidney deficiency syndrome (Gan Yu and Shen Xu Zheng), thereby reinforcing the TCM notion that the kidney and liver are important therapeutic targets for DOR. Kunling Pill (KLP, also called Kunling Wan), a standardized Chinese patent drug approved in China since 2004, has been widely used for the treatment of polycystic ovary syndrome (PCOS) [13] and DOR and for improving pregnancy outcome in the clinic (ref. in Chinese). However, little is known regarding its underlying mechanisms in treating DOR. One reason for this is that the formulation of KLP involves complex components (see supplemental data, S1), leading to variability in its ingredients. Another reason is that conventional research methods applied to TCM preparations face many difficulties due to the multiple components and multiple targets of preparation such as KLP.

Proteomics, a core technology in the current postgenomic era, plays an important role in discovering biomarkers, diagnosing disease phenotypes, and revealing the pharmacological mechanisms of various interventions [14, 15]. In recent decades, the technology and instruments for proteomics have made rapid progress, including mass spectrometry (MS) technology, protein fragmentation techniques, and bioinformatics [16, 17]. In this study, a data-independent acquisition mass spectrometry (DIA-MS) technique was used to scan and identify all peptides in the human follicular fluid (FF) in order to explore the possible pharmacological mechanisms of KLP in the treatment of DOR.

The FF is formed in the secondary follicle stage (antral follicles) and provides the microenvironment surrounding the growing oocyte. The FF contains complex materials, such as proteins and cytokines, which can provide unique insights into the processes regulating healthy follicle development [18]. Alterations in the proteomic signature of the FF might therefore reveal the molecular mechanisms involving small antral follicle-associated proteins and oocyte maturation-associated proteins and might also help to decipher the underlying pathophysiology of ovarian disorders [19–21]. Importantly, in the in vitro fertilization (IVF) process, the FF can be easily obtained during the extraction of oocytes from the follicle, which makes it a useful source for experimental research. Proteomic approaches have been used to study the pathological mechanism of PCOS [22–24], endometrial cysts [25, 26], poor ovarian response [27], ovarian hyperstimulation syndrome [28], and recurrent abortion [29], but they have not been applied to DOR. The incidence of DOR has been increasing, and it shows a trend for occurring in younger patients, but it has been difficult to determine the effect of DOR on FF function. This study aimed to identify protein expression changes in the FF of DOR patients, and our results strongly indicate that proteomic expression patterns in the FF undergo significant alterations in DOR patients.

2. Materials and Methods

2.1. Experimental Design and Workflow. The experimental design and workflow are shown in Figure 1. In brief, a total of 18 patients undergoing assisted reproductive technology (ART) by standard ovarian stimulation protocols were recruited to collect FF. Among them, 6 patients had normal ovary reserve (NOR), 6 patients had diminished ovary reserve (DOR), and 6 patients had DOR and were treated with KLP (lot # 20191022) for 3 months.

All patients were recruited from the Reproductive and Genetic Medical Center, Dalian Municipal Women and Children's Medical Center (Group), from March 2020 to March 2021. The inclusion criteria were anti-Müllerian hormone (AMH) $\leq$ 1.1 ng/ml and/or antral follicle count <5–7 and age <40 years. The exclusion criteria were patients with PCOS, abnormal liver and kidney function, endometriosis, endocrine diseases, or chromosomal abnormalities.

This work was approved by the Ethics Committee of Dalian Municipal Women and Children's Medical Center (No. 2020010), and all participants gave written informed consent.

All participants were infertile women who were scheduled for their first IVF/intracytoplasmic sperm injection cycle. Of these, 6 cases had NOR and were used as the control group, 6 cases had DOR and were used as the disease control, and 6 cases had DOR and were treated with KLP as the experimental group.

2.2. Serum Sex Hormone Levels and Embryo Quality Determination. Levels of serum follicle-stimulating hormone (FSH), estradiol (E_2), and AMH were determined by an automated multianalysis system with a chemiluminescence instrument (DX1800 Beckman Coulter, USA).

Embryos were divided into four grades based on pronuclear stage score, development speed, number of blastomeres, size, morphology, cytoplasm fragment ratio, and embryo quality score of the cleavage stage. Grade 1 embryo is as follows: blastomeres of equal size, regular morphology, bright, and no fragments. Grade 2 embryo is as follows: blastomeres of unequal size and/or fragmentation <10%. Grade 3 embryo is as follows: 10–50% fragments. Grade 4 embryo is as follows: fragmentation >50%. Embryos with more than six cells at grade 1 or 2 on day 3 after egg collection were defined as high-quality embryos. Embryos with less than 30% fragmentation were available and could be transplanted or frozen (Figure 2).

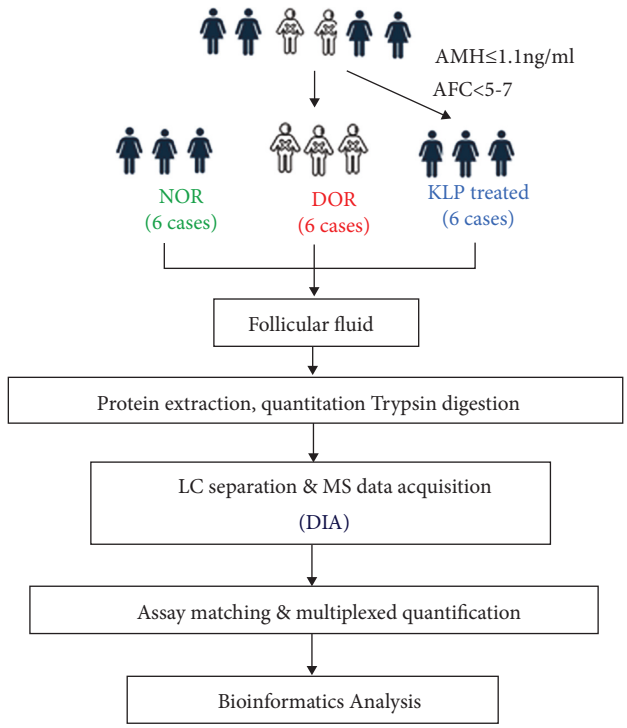

Figure 1: Experimental design and workflow.

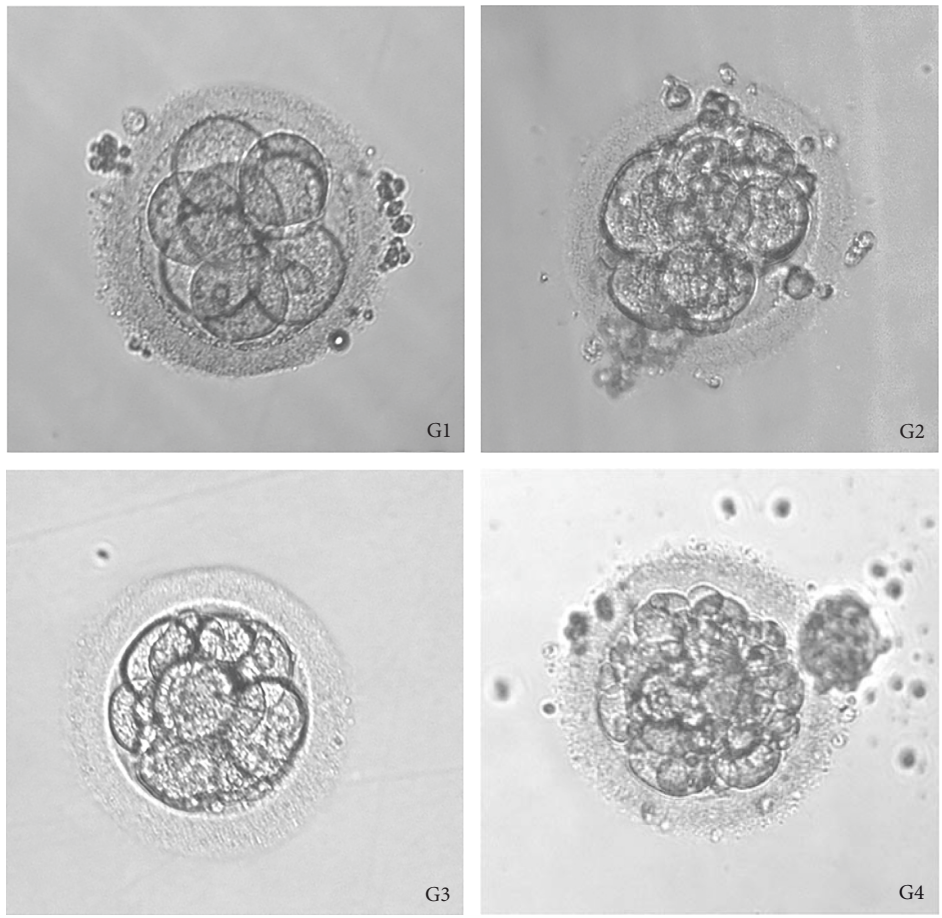

Figure 2: Representative images of embryos of different grades. *Note.* Grade 1 embryo: blastomeres of equal size, regular morphology, bright, and no fragments. Grade 2 embryo: blastomeres of unequal size and/or fragmentation <10%. Grade 3 embryo: 10–50% fragmentation. Grade 4 embryo: fragmentation >50%.

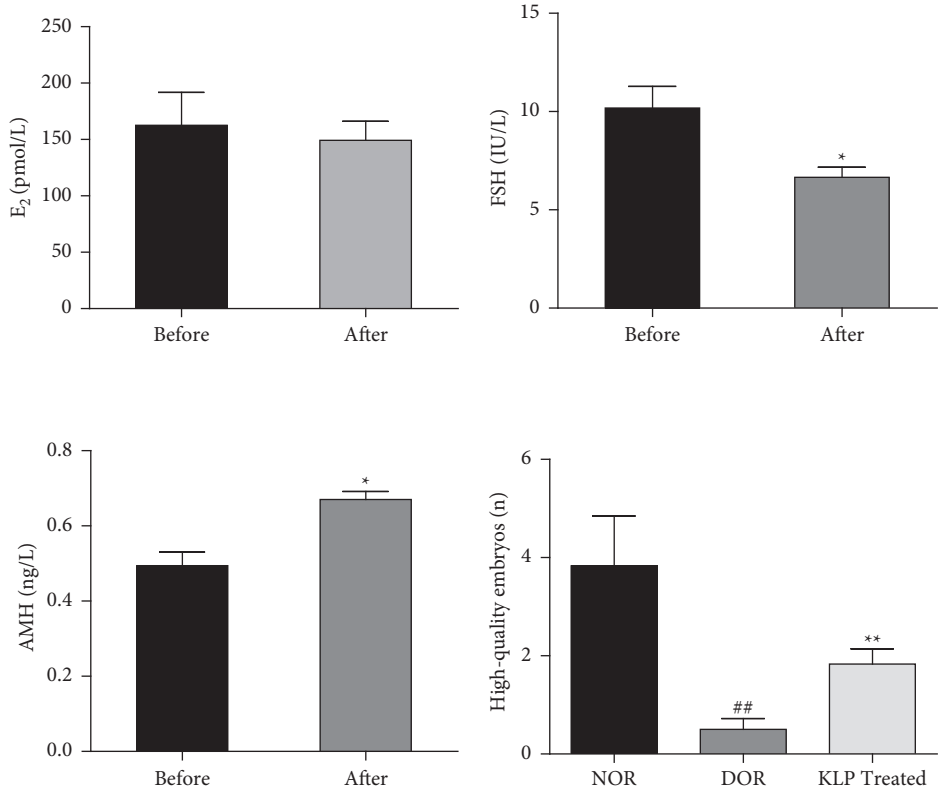

Figure 3: Serum levels of E_2, FSH, and AMH and the number of high-quality embryos. The values are shown as the mean ± SEM ($n = 6$ per group). (a) After treatment with KLP, the levels of E_2 were not significantly different compared with before treatment. (b) The level of FSH after treatment was significantly lower compared with before treatment ($^{*}P < 0.05$). (c) The level of AMH was significantly higher compared with before treatment ($^{*}P < 0.05$). (d) The number of high-quality embryos in the three groups. DOR group compared with the NOR group, ($^{\#\#}P < 0.01$); KLP treatment group compared with the DOR group ($^{**}P < 0.01$).

2.3. Collection and Preparation of FF. We used a conventional procedure to collect FF [30, 31]. For all experiments, FF was obtained from mature follicles (diameter ≥18 mm) and the protein samples were prepared as follows. FF samples were collected when the oocytes were aspirated under the guidance of transvaginal ultrasound. During oocyte retrieval, 2.5 ml of macroscopically clear FF lacking visible blood contamination was collected from all participants. After centrifugation at 4, 000 × g for 10 min at 4°C, the supernatant liquid was drawn and immediately frozen in liquid nitrogen and stored at −80°C until use.

(1) Protein extraction: the FF samples were denatured using five volumes of cold acetone, precipitated at −20°C overnight, and centrifuged at 25, 000 × g and 4°C for 15 min, and the supernatant was discarded. The precipitate was air-dried, sonicated three times in an ice bath (frequency 50 Hz), and centrifuged at 25, 000 × g and 4°C for 15 min to obtain the supernatant. DTT was added to a final concentration of 10 mM and incubated at 56°C in a water bath for 1 h. IAM was added to a final concentration of 55 mM and incubated in the dark for 45 min, and the supernatant was obtained by centrifugation at 25, 000 × g at 4°C for 15 min. The supernatant was the protein solution. (2) Protein quality control: the protein was quantified by the Bradford method [32]. (3) Protein enzymatic hydrolysis: a total of 100 μg of protein solution per sample was diluted in four volumes of 50 mM NH_4HCO_3, and 2.5 μg of trypsin enzyme was added (protein : enzyme = 40 : 1) and digested for 4 h at 37°C. The resulting peptides were desalted using a Strata X column and vacuumed to dryness. (4) Peptide fractionation: HPLC analysis was carried out on an LC-20AB liquid phase system (Shimadzu, Japan), and the separation column was a Gemini C-18 column (4.6 mm × 250 mm). The dried peptide sample was redissolved with mobile phase A (5% ACN, pH 9.8), injected, and eluted at a flow rate of 1 mL/min with mobile phase B (95% ACN, pH 9.8) for 10 min. The elution peak was monitored at a wavelength of 214 nm, and one component was collected per minute. All of the samples were combined according to the chromatographic elution peak map to obtain 10 fractions, which were then freeze-dried.

2.4. Data-Dependent Acquisition (DDA) and DIA Analysis by Nano-LC-MS/MS. The extracted peptide sample was redissolved with mobile phase A (2% ACN, 0.1% FA) and centrifuged at 20, 000 × g for 10 min at 4°C, and the supernatant was taken for injection. The sample was first concentrated and desalted in a trap column and then connected in series with a self-assembled C18 column (35 cm × 150 μm, 1.8 μm). The proteins were separated on a Thermo UltiMate 3000 UHPLC liquid chromatograph at a flow rate of 500 nL/min with the following effective gradient: 0–5 min, 5% mobile

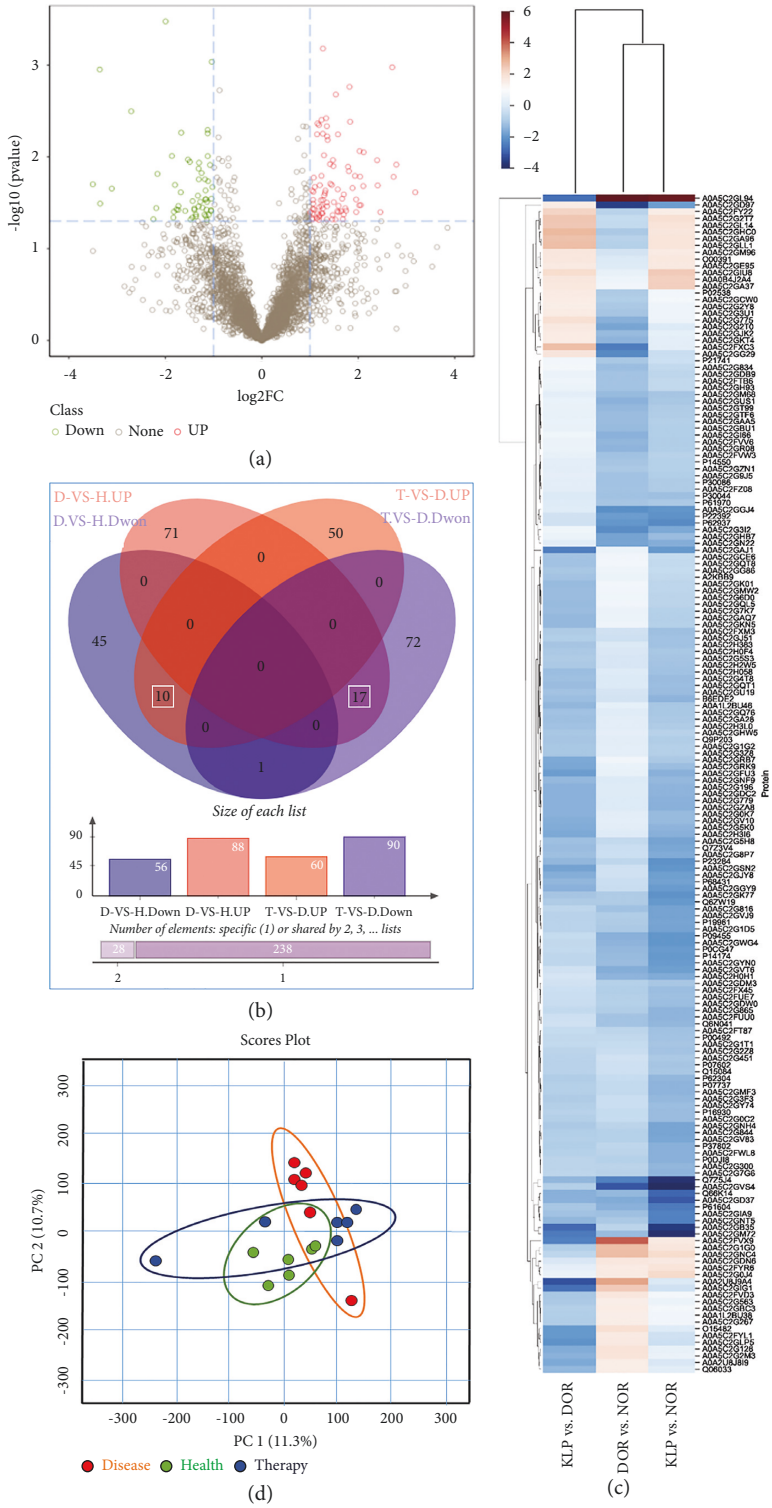

Figure 4: DDA and quantitative protein detection. (a) Identification of DEPs in DOR FF. (b) Venn diagram of the DEPs between the DOR group and the KLP-treated group. (c) Cluster analysis chart of the identified DEPs (higher red and blue intensities indicate a higher degree of upregulation and downregulation, respectively). (d) PCA chart.

phase B (98% ACN, 0.1% FA); 5–130 min, from 5% B to 25% B; 130–150 min, from 25% B to 35% B; 150–160 min, from 35% B to 80% B, 160–175 min, 80% B; and 175–180 min, from 80% B to 5% B. The peptides separated by liquid phase were ionized by a nano-ESI source and injected into a tandem mass spectrometer Fusion Lumos (Thermo Fisher Scientific, San Jose, CA) for DDA mode detection.

For DDA analysis, the main parameters were as follows: ion source voltage of 2 kV, MS scan range of 350–1,500 m/z, MS resolution of 120,000, maximum ion

Table 1: Differentially expressed proteins in the KLP-treated group compared with the DOR group.

Swiss-Prot Id	Gene name	Description	Up or down
O00391	QSOX1	Sulfhydryl oxidase 1	↑
P27169	PON1	Serum paraoxonase/arylesterase 1	↑
A0A0B4J2A4	ACAA2	3-Ketoacyl-CoA thiolase, mitochondrial	↑
P02763	A1AG1	Alpha-1-acid glycoprotein 1	↑
B7Z1F8	B7Z1F8	cDNA FLJ53025	↑
Q5SNT2	SAMP	Transmembrane protein 201	↑
P07360	CO8G	Complement component C8 gamma chain	↑
Q5T4S7	UBR4	E3 ubiquitin-protein ligase UBR4	↑
Q6LDG4	C2	Complement protein	↑
A0A5C2H1W2	N	IG c1918_light_IGLV1-51_IGLJ3	↑
P16930	FAH	Fumarylacetoacetase	↓
P0DJI8	SAA1	Serum amyloid A-1 protein	↓
P14174	MIF	Macrophage migration inhibitory factor	↓
O75446	SAP	Histone deacetylase complex subunit SAP30	↓
P30044	PRDX5	Peroxiredoxin-5	↓
P23284	PPIB	Peptidyl-prolyl cis-trans isomerase B	↓
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	↓
Q15084	PDIA6	Protein disulfide-isomerase A6	↓
P07602	PSAP	Prosaposin	↓
P62304	SNRPE	Small nuclear ribonucleoprotein E	↓
P68431	H3C1	Histone H3.1	↓
Q15084	PDIA6	Protein disulfide-isomerase A6	↓
Q7Z3V4	UBE3B	Ubiquitin-protein ligase E3B	↓
Q7Z5J4	RAI1	Retinoic acid-induced protein 1	↓
A0A0B4J2A4	ACAA2	3-Ketoacyl-CoA thiolase, mitochondrial	↓
B6EDE2	HEL180	Epididymis luminal protein 180	↓
P00492	HPRT1	Hypoxanthine-guanine phosphoribosyltransferase	↓

implantation time (MIT) of 50 ms, MS/MS collision type of HCD, collision energy NCE of 30, MS/MS resolution of 30,000, MIT of 100 ms, and dynamic exclusion of 30 s. The start m/z for MS/MS was fixed at 100. Precursors for the MS/MS scan satisfied the charge range of 2+ to 6+, and the top 20 precursors had intensities greater than 2E4. The AGC was MS 4E5, MS/MS 5E4.

For DIA analysis, LC-separated peptides were ionized by nano-ESI and injected into a Fusion Lumos tandem mass spectrometer (Thermo Fisher Scientific, San Jose, CA) in DIA mode. The main parameters were ion source voltage of 2 kV, MS scan range of 400–1500 m/z, MS resolution of 60,000, and MIT of 50 ms, and the 400–1500 m/z range was equally divided into 44 continuous window MS/MS scans. The MS/MS collision type was HCD, and MIT was 54 ms. Fragment ions were scanned in Orbitrap with an MS/MS resolution of 30,000, collision energy of 30, and AGC 5E4.

2.5. Data Analysis. Proteins in the DDA data were identified using MaxQuant (http://www.maxquant.org), and the identification results were used for spectral library construction [33]. For large-scale DIA data, the mProphet algorithm was used to perform the analytical quality control, thus obtaining a large number of reliable quantitative results. The identified proteins from spermatozoa were analyzed by gene ontology (GO) (http://david.abcc.ncifcrf.gov/home.jsp) and the Kyoto Encyclopedia of Genes and Genomes Database (KEGG) (http://www.genome.jp/kegg). Principal component analysis (PCA) of the quantified proteins was performed with the Unscrambler software (version 9.8). Based on the quantitative results, the differentially expressed proteins (DEPs) between the comparison groups were identified, and we performed functional enrichment analysis, protein-protein interaction analysis, and subcellular localization analysis of the DEPs using the Web tool STRING (http://string-db.org). Other databases for bioinformatic analysis were the UniProt protein database and the NCBI databases (including GenBank, RefSeq, Swiss-Prot, and PDB). The sex hormone data were analyzed by Student's *t*-test in SPSS 22.0, where $P < 0.05$ was considered significant.

3. Results

3.1. Efficacy Evaluation of KLP. The clinical features of the study participants are summarized in the supplementary data (Table S2). Age, body mass index, and serum levels of E_2, FSH, LH, homocysteine, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and lipoprotein were not statistically significantly different between the NOR and DOR groups ($P > 0.05$).

The levels of E_2 were not significantly different before and after treatment with KLP (Figure 3(a)). However, compared with before treatment the levels of FSH decreased significantly after treatment with KLP (Figure 3(b)), while the levels of AMH were significantly elevated (Figure 3(c)). Moreover, compared with the NOR group the number of high-quality embryos in the DOR group decreased significantly, while the number of high-quality embryos increased

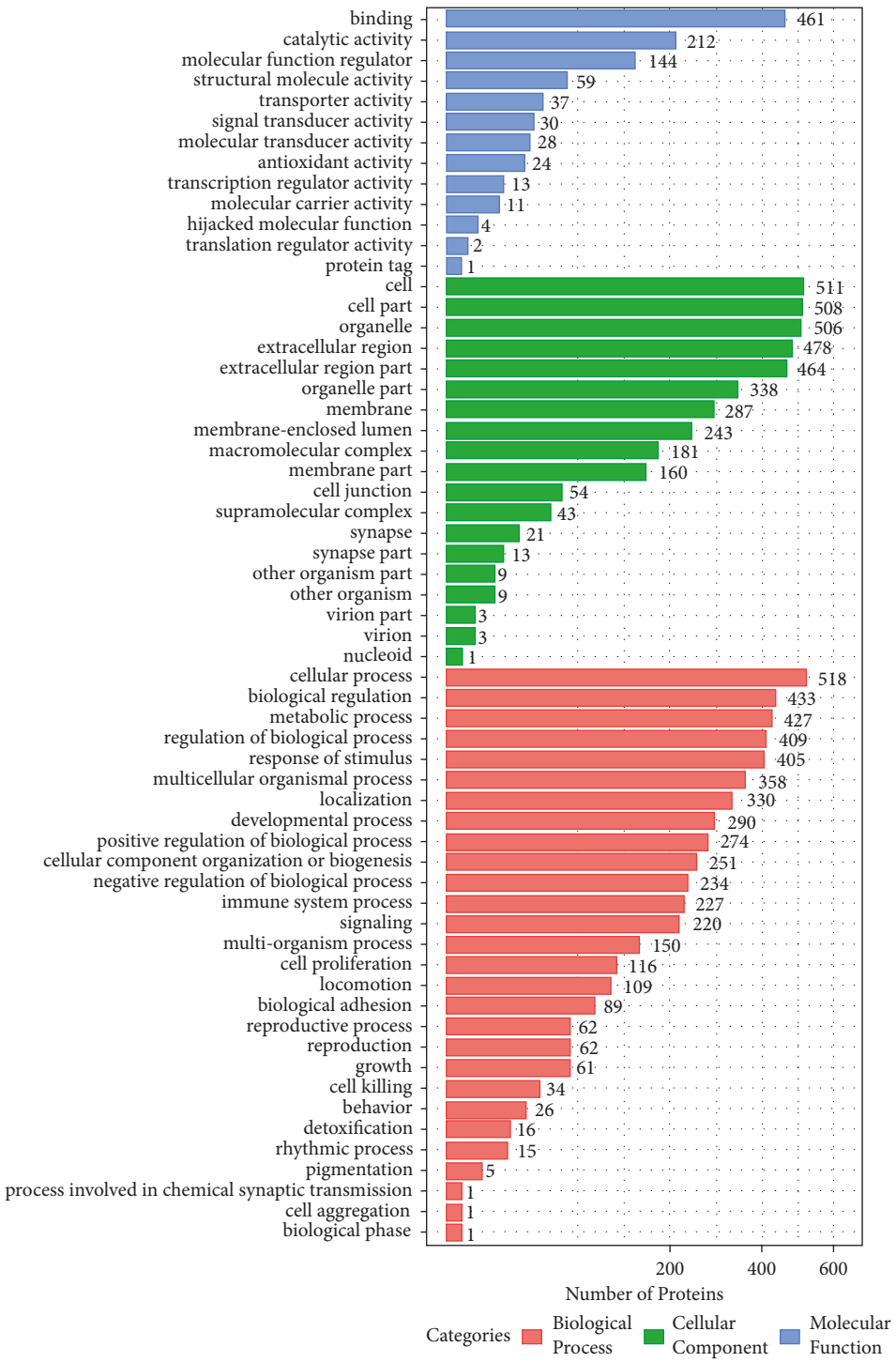

Figure 5: Bar graph of gene ontology (GO) classification of all identified FF proteins by DDA and quantitative protein detection. The length shows the number of DEPs associated with the GO term. The large numbers of proteins in the categories of metabolic process and biological process provide strong support for our hypothesis.

significantly in the KLP treatment group compared with DOR groups (Figure 3(d)).

3.2. Quantitative Protein Detection. Conventional DDA-MS was used to establish and analyze a spectral library of human FF obtained from 18 subjects, i.e., 6 NOR patients, 6 DOR patients, and 6 DOR patients treated with KLP. We identified 10,887 peptides and 3,774 proteins, and then, the DIA method was adopted for MS data collection. After calculating the fold changes and P value through the MSstats package, two filtration criteria (fold change >2 and P value <0.05) were used to identify significant DEPs. The differences between the comparison groups are presented in Figures 4(a) and 4(b).

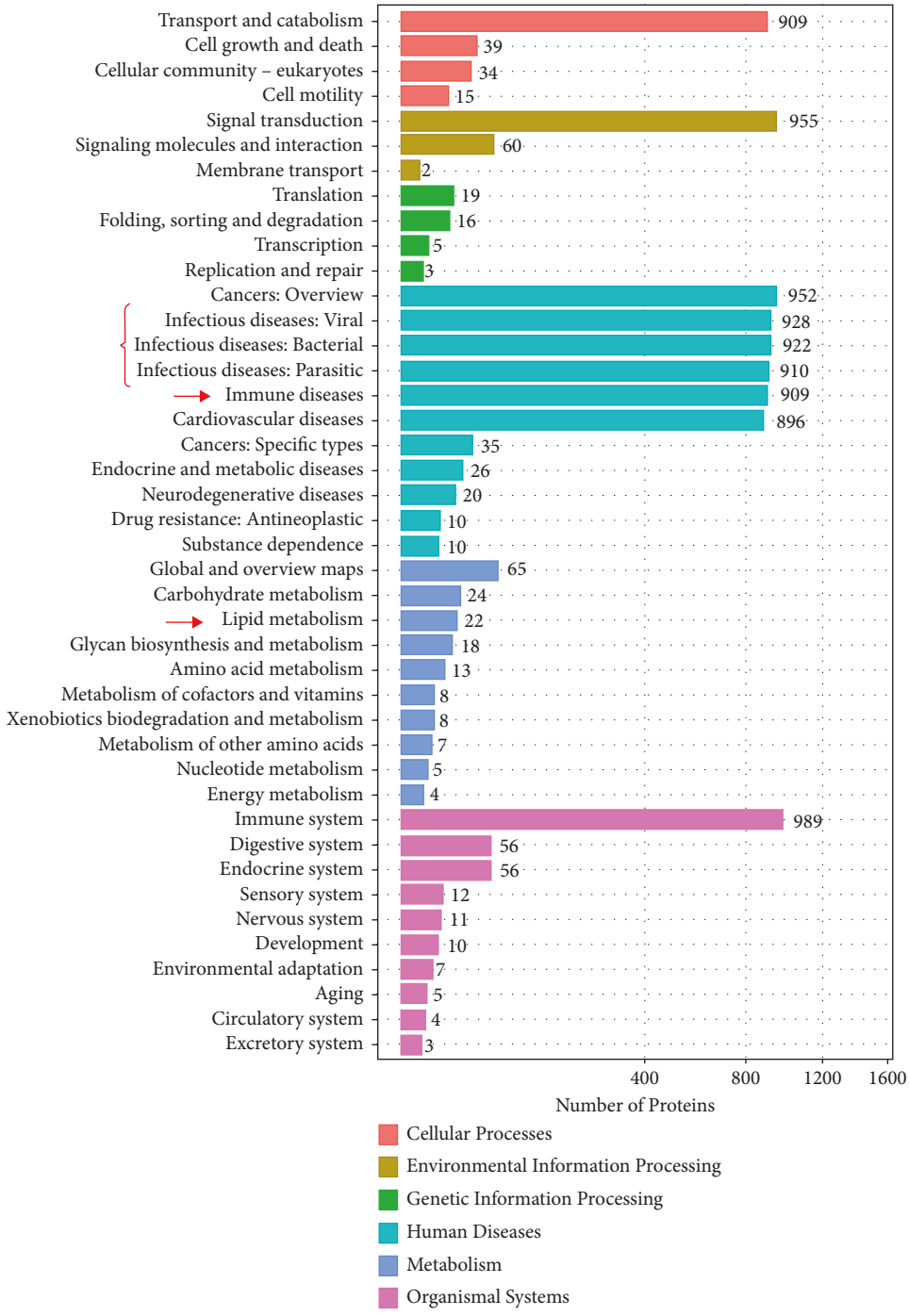

Figure 6: Bar graph of the KEGG pathway analysis of DEPs in the KLP-treated group vs. the DOR group. The bar length shows the number of all DEPs associated with the GO and KEGG terms.

According to the Venn diagram, 56 proteins were downregulated and 88 proteins were upregulated in the DOR group compared with the NOR group, and 27 of the proteins were shared in the KLP-treated group. Among these, 10 proteins were upregulated and 17 proteins were downregulated in the KLP group compared with the DOR group. The identified proteins are listed and described in Table 1. In addition, all DEPs were used to draw a cluster analysis chart (Figure 4(c)), which intuitively illustrates the expression differences among the three groups. PCA of the qualified FF proteins showed that samples from NOR (health), DOR (disease), and KLP-treated (therapy) were in separate clusters (Figure 4(d)). The first two PCs explained 22% of the total variance and could distinguish the three species.

3.3. Bioinformatic Analyses. To evaluate the functional significance of all of the identified proteins, the Blast2GO software was used to perform gene ontology (GO) annotation analysis. As shown in Figure 5, the most enriched

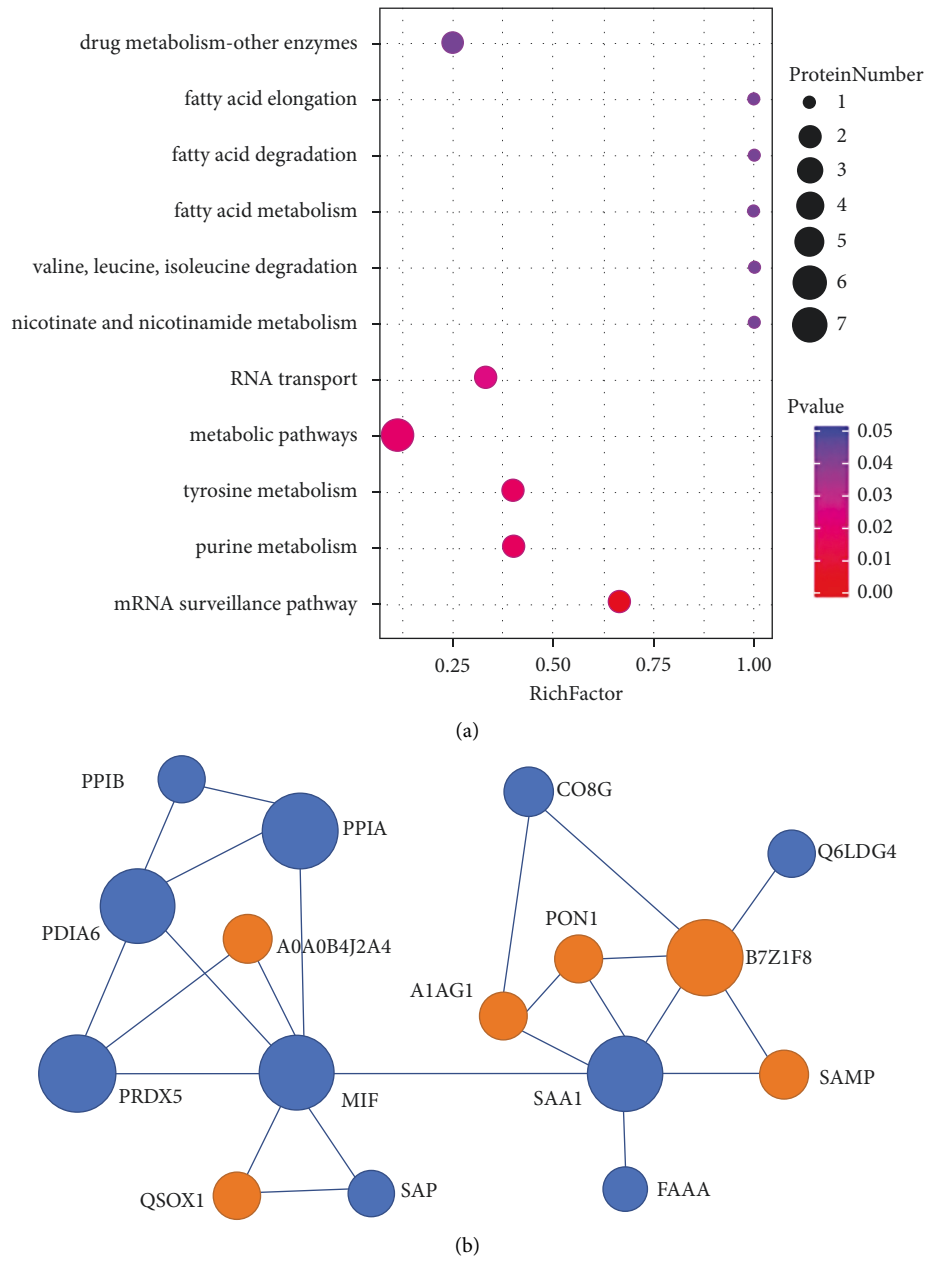

Figure 7: A bubble chart classification of the KEGG pathway analysis and the linking protein-protein interaction network between the KLP-treated group and the DOR group. (a) The bubble size shows the number of DEPs associated with each GO and KEGG term. (b) The red bubbles represent upregulated proteins, and the blue bubbles represent downregulated protein.

molecular functions accounted for 13 GO terms, such as binding, catalytic activity, and antioxidant activity. The most enriched cell components accounted for 19 GO terms, such as cell, cell part, and organelles. The most enriched biological processes accounted for 28 GO terms, such as cellular process, biological regulation, metabolic process, regulation of biological process, and response to stimuli.

According to the KEGG pathway enrichment analysis, the six highest ranked biological functions for the DEPs included cellular processes, environmental information processing, genetic information processing, human disease, metabolism, and organic systems (Figure 6). In the context of this experiment, the pathway enrichment analysis gave a snapshot of the significantly enriched metabolic pathways.

KEGG enrichment analysis of the DEPs identified in this study showed the involvement of multiple pathways, and infection, immune diseases, and lipid metabolism were considered to be endogenous signals affecting follicular development. Among these pathways, the 11 highest ranked biological functions for the DEPs in the KLP-treated group compared with the DOR group were mostly associated with fatty acid elongation, fatty acid degradation, fatty acid metabolism, nicotinate and nicotinamide metabolism, and valine, leucine, and isoleucine degradation (Figure 7(a)). The

Table 2: Proteins contained in the significantly enriched pathways.

Pathway ID	Pathway	Matched proteins	Functional description
map00062	Fatty acid elongation	A0A0B4J2A4, SAA1	Lipid metabolism
map00071	Fatty acid degradation		
map01212	Fatty acid metabolism		
map00280	Valine, leucine, and isoleucine degradation		
map00760	Nicotinate and nicotinamide metabolism	RET1	Metabolism of cofactors and vitamins
map03015	mRNA surveillance pathway	NTF2, Q6ZW19	Genetic information processing, translation
map03013	RNA transport	NTF2, Q6ZW19	
map00350	Tyrosine metabolism	MIF, FAAA	Amino acid metabolism
map00230	Purine metabolism	HPRT, NDKB	Nucleotide metabolism

linking protein information is listed in Table 2. We then imported the protein IDs into the STRING database to build a protein-protein network (Figure 7(b)). Three proteins (MIF, SAA1, and PRDX5) were core nodes and were connected to two subnetworks, one involving SAA1, FAAA, SAMP, A1AG1, PON1, B7Z1F8, CO8G, and Q6LDG4 and the other involving MIF, SAP, QSOX1, PRDX5, PPIA, PDIA6, A0A0B4J2A4, and PPIB.

4. Discussion

Infertility has become a worldwide public health problem, affecting about 12.5–15 in 100 couples of reproductive age annually in China [34]. DOR, generally defined as a decreased number of high-quality oocytes, is a predominant contributor to infertility [35–37]. In recent years, the incidence of DOR has been increasing and the trend is for it to present in younger patients. Thus, the development of effective treatment strategies has emerged as one of the preeminent topics in the reproductive health field.

KLP, also called Kunling Wan, has been demonstrated to increase endometrial blood flow, upregulate vascular endothelial growth factor A, and inhibit angiogenesis and endometriosis induced by controlled ovarian hyperstimulation [38]. Some published clinical studies have suggested that KLP has a better therapeutic effect on DOR and premature ovary failure (ref. in Chinese). Our experimental results partly support the existing arguments, but the pharmacological mechanisms of KLP in the treatment of DOR remain unclear.

A major strength of this study is that we used human FF collected from IVF patients with or without DOR and with DOR treated by KLP. The proteomic profiles of the FF samples were obtained by DIA-MS combined with bioinformatic analyses. The quantitative analysis results suggested that 27 proteins (10 upregulated and 17 downregulated) were differentially expressed between the NOR patients, DOR patients, and KLP-treated patients. Among these, 4 proteins were associated with protein degradation, 4 proteins were involved in the immune response, and 16 proteins were associated with various biological functions such as inflammatory response, free radical scavenging, protein modification, and energy conversion. It is expected therefore that these proteins have essential roles in DOR patients treated by KLP. Regarding these DEP functions, the results of GO and KEGG enrichment analyses indicated that most of the annotations belonged to binding, catalytic activity, cellular process, biological regulation, and metabolic process. Our datasets are in agreement with previous reports in similar projects [39, 40].

In our study, some signature proteins in the FF were mainly found after KLP treatment. Among them, macrophage migration inhibitory factor (MIF) is a soluble pro-inflammatory cytokine produced by activated T lymphocytes that triggers cell proliferation, migration, follicle growth, and ovulation [41]. MIF is also an important regulator of the host innate immunity induced by pro-inflammatory states such as PCOS and ovarian tumors [42, 43], and it has been associated with various immunological events in the process of oocyte development [44, 45]. SAA1 (serum amyloid A), an acute-phase protein, is produced mainly by the liver and ovarian granulosa cells [46]. The biological functions of SAA1 in the ovary are still not fully understood, but a previous study demonstrated that elevated follicular SAA1 is associated with decreased pregnancy rate [47], and our findings suggest that SAA1 is a potential target of KLP in the treatment of DOR. PRDXs (peroxiredoxins) are cytoprotective peroxidases that prevent oxidative stress by reducing peroxides. PRDXs constitute a large superfamily (PRDX1–6) of proteins that are involved in the processes of inflammation and tumor development, including ovarian cancer [48]. In particular, PRDX5 plays an important role in the Nrf2 signaling pathway [49]. Linking with the three core proteins, multiple proteins such as alpha-1-acid glycoprotein-1 (A1AG1), paraoxonase-1 (PON1), acyl-CoA synthetase gene (FAAA), serum amyloid P component (SAP), quiescin sulfhydryl oxidase-1 (QSOX1), protein disulfide-isomerase family-6 (PDIA6), and cyclophilins (PPIA and PPIB) co-regulate lipid acid metabolism, nicotinate and nicotinamide metabolism, and tyrosine and purine metabolism.

5. Conclusions

The FF is different from blood and is a unique biological fluid in which the critical events of oocyte and follicular maturation take place, and it provides a unique window into the processes occurring during follicular maturation. In summary, our study provides the proteome profiles of human FF from DOR patients with and without KLP treatment, and functional analyses of proteome datasets revealed a possible pharmacological mechanism of KLP for

the improvement of DOR. Several detected core proteins (SAA1, MIF, PRDX5) and related pathways (fatty acid metabolism, nicotinate and nicotinamide metabolism, and tyrosine and purine metabolism) are potential targets of KLP. Our proposed datasets provide a useful basis for future studies to better understand the pathological mechanisms of DOR and the pharmacological mechanisms of TCM preparations.

6. Limitations

Although DIA-MS in this study can provide the possibility to investigate proteomic changes in the FF, the conventional bioinformatic analysis methods might miss some possible valuable information. Adding the effect of the smaller sample size, caution should be taken in extrapolating this result to other studies and to clinical practice. In addition, experimental verification was not carried out in this study, and we are now designing a cross-validation using other omic methods such as transcriptomics or metabolomics.

Abbreviations

DOR: Diminished ovarian reserve
TCM: Traditional Chinese medicine
ART: Assisted reproductive technology
FF: Follicular fluid
DDA: Data-dependent acquisition
DIA: Data-independent acquisition
DEP: Differentially expressed proteins
GO: Gene ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes Database
PCOS: Polycystic ovary syndrome
MS: Mass spectrometry
IVF: In vitro fertilization
FSH: Follicle-stimulating hormone
E_2: Estradiol
AMH: Anti-Müllerian hormone
PCA: Principal component analysis
LH: Luteinizing hormone.

Data Availability

All datasets used to support the findings of the study are included within the article and supplementary files.

Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committee of Dalian Municipal Women and Children's Medical Center (no. 2020010). All participants signed informed consent forms.

Disclosure

Beijing University of Chinese Medicine is the first and corresponding address.

Conflicts of Interest

The authors have no financial or scientific conflicts of interest regarding the research described in this manuscript.

Authors' Contributions

Haiyan Wang and Dan Cao contributed equally to this work. HYW and DC conceived the project. ZQM and JL provided supervision. HYW, MXW, BWW, and XOX performed the research. YBS, YYJ, SYJ, and HL analyzed the data. DC, HYW, and JL wrote the article. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

This study was supported in part by the National Natural Science Foundation of China (no. 81873099) and the National Key Scientific and Technological Project Fund of Beijing University of Chinese Medicine (no. 2020-JYB-ZDGG-011).

Supplementary Materials

Table S1: components of KLP. Table S2: the baseline clinical parameters of the study participants before KLP treatment. Table S3: the main effective ingredients of KLP. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1397060, 13 pages
https://doi.org/10.1155/2022/1397060

Research Article

Effects of *Fagonia indica* on Letrozole-Induced Polycystic Ovarian Syndrome (PCOS) in Young Adult Female Rats

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Received 17 March 2022; Revised 15 April 2022; Accepted 19 April 2022; Published 26 May 2022

Academic Editor: Yong Wang

Polycystic ovarian syndrome is a multidisciplinary endocrinopathy of reproductive-aged women that provokes insulin resistance, hyperandrogenism, cardiovascular problems, obesity, and menstrual complications. The present study was designed to investigate the effectiveness of ethanolic extract of *Fagonia indica* in letrozole-induced PCOS young adult female rats. HPLC was carried out to find the phenolic and flavonoid content of the ethanolic extract of *Fagonia indica*. Twenty-five female rats were taken and initially divided into two groups: group I (control group) and group II (PCOS group). PCOS was induced by letrozole given orally by gavage. Body weight was recorded weekly and vaginal cytology was analyzed daily. After induction of disease, the PCOS group is further divided into four groups ($n = 5$): group II (positive control with PCOS), group III (metformin 20 mg/kg treated group), group IV (ethanolic extract of *Fagonia indica* 500 mg/kg treated group), and group V (metformin plus *Fagonia* extract). At the end of experimental period, the blood sample of each rat was collected and serum was separated by centrifugation. Afterwards hormonal analysis, lipid profile and liver functioning tests were performed. Ovaries were removed and preserved for histopathological findings while the liver of each rat was stored for the determination of antioxidant potential assessment. *Fagonia indica* was found to possess quercetin as one of the major flavonoid phytoconstituents. The plant extract exhibited its beneficial effects by restoring hormonal balance, lipid profile, and liver functioning markers. Treatment with *F. indica* reduced body weight, resolved ovarian cysts, and showed positive effects on follicular growth. Treatment with plant also increased the levels of antioxidant enzymes. This study validates the potential of *Fagonia indica* for the amelioration of metabolic, as well as, hormonal disturbances that occurred in PCOS.

1. Introduction

Hyperandrogenemia is a salient feature of PCOS, and a major contributor to cosmetic anomalies including hirsutism, acne, and male pattern alopecia in affected women. Polycystic ovarian syndrome is a complex endocrine and metabolic disorder [1] that is characterized by multiple follicular cysts, found on the periphery of ovaries and can be visualized ultrasonographically. Overweight and obesity further complicate the situation by accelerating insulin resistance [2]. Moreover, PCOS is associated with different endocrine, metabolic, and reproductive traits including hyperandrogenism, hyperinsulinism, obesity, hyperlipidemia, type 2 diabetes mellitus, cardiovascular abnormalities, depression, anxiety, anovulation, oligo-ovulation, infertility, hirsutism, and acne [3, 4]. This syndrome is so devastated that it can cause endometrial hyperplasia and subsequently the development of endometrial cancer [5]. Concurrently,

the PCOS patients are also suffering from elevated levels of low-density lipoprotein (LDL), triglycerides, total cholesterol, and decreased level of high-density lipoprotein (HDL) [6]. In PCOS women, the balance of sex hormones, e.g., luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, progesterone, and testosterone is disturbed [7]. Blood glucose levels are also elevated because of insulin insensitivity. Evidence suggested that increase in GnRH pulse frequency and amplitude from hypothalamus stimulates LH synthesis over FSH, which results in elevated LH/FSH ratio in PCOS women. Consequently, enhanced luteinizing hormone levels begin to develop metabolic and reproductive disorders such as increased androgen synthesis in theca cells of ovaries, impairment of FSH and estrogen synthesis, and promoting the secretion of insulin-like growth factor-1 (IGF-1). Nowadays, polycystic ovarian syndrome (PCOS) has become very common among females of reproductive ages. It is a multidisciplinary disorder and hence requires a multidisciplinary consultation to manage the symptoms of the disorder [1].

Lifestyle modifications for PCOS treatment in obese patients include moderate exercise (≥30 min/day), reduce psychological stressors, weight reduction, calorie deficit diet of 500–1000 kcal/day, decrease caffeine consumption, and low glycemic index (GI) diet [8]. Current treatment modalities include metformin or OCP (estrogen-progestin combinations), antiandrogens, flutamide, pioglitazone, spironolactone, cyclic-progestins, GnRH agonists, myo-inositol, and fertility treatments such as clomiphene citrate and letrozole [2, 9–11]. When used for longer duration, each of the above treatment has adverse effects that limit their use in the patients. For example, metformin causes fatal and nonfatal lactic acidosis in 1–17/100,000 patients per year. Oral contraceptive pills (OCPs) are associated with weight gain, thromboembolism, and cardiovascular events. Some side effects are more common in patients using OCPs such as abdominal pain, dysmenorrhea, nausea, headache, backache, and dizziness [12]. Antiandrogenic agents are hepatotoxic that could be very fatal [13].

Nowadays, many physicians and patients are inclined towards herbal treatment due to these abovementioned adverse effects of conventional treatment and cost of the treatment. The pharmacological and therapeutic effectiveness of medicinal plants is due to the presence of different phytoconstituents in these plants and these can be used for various ailments [14, 15]. In this respect, herbal remedies for PCOS are very effective and some of these herbal therapies reduce hyperandrogenism and improve ovulation and insulin sensitivity without causing the abovementioned adverse effects. This is why people tend to use these herbal remedies to treat PCOS. Some of these herbal interventions include *Glycyrrhiza glabra*, *Mentha spicata*, *Panax ginseng*, flaxseed, *Aloe vera*, *Chaste berry*, *White poeny*, cinnamon, milk thistle, *Matricaria chamomilla*, N-acetyle cysteine, D-chiro-inositol, Kacip fatimah, *Astragalus* polysaccharide, *Apium graveolens*, and *Cinnamon zeylanicum* [16, 17].

Fagonia indica is a member of Zygophyllaceae family. All of the species of this family are herbs and shrubs/shrublets [18]. *Fagonia* is commonly known as dhamasa booti. *Fagonia* has a great medicinal potential to treat a lot of diseases. It is known to possess following activities such as antioxidant, thrombolytic, antimicrobial, antidiabetic, antitumor, anti-inflammatory, hepatoprotective, antihaemorrhagic, laxative, and antiulcerogenic potential. *Fagonia* has been used traditionally for the treatment of menstrual problems for thousands of years. Practitioners of traditional medicine have used this plant for the treatment of PCOS in different areas of Pakistan. Due to its folklore use in PCOS, it is worthwhile to explore its potential in PCOS treatment. The current study investigates the efficacy of ethanolic extract of *Fagonia indica* for the treatment of letrozole-induced PCOS rats.

2. Material and Methods

2.1. Plant Collection and Authentication. *Fagonia indica* was collected from a town called Tarnol Phatak situated in Islamabad, Pakistan. The plant was collected in March 2021. It was identified and authenticated by the botanist, Dr. Mansoor Hameed at University of Agriculture Faisalabad and was preserved under a specific specimen number 071-21-01 in the herbarium of Department of Botany, University of Agriculture Faisalabad, for the further reference.

2.2. Preparation of Plant Extract. The extraction was carried out by the simple maceration method. The plant was air dried and soaked for 14 days in absolute ethanol [19] in 1 : 5 ratio. Subsequently, it was filtered by using a Whatman filter paper and the resultant filtrate was evaporated by using a rotatory evaporator (SCILOGEX RE100-pro 5 L). At the end of this process, a semisolid extract was obtained that was administered to PCOS-induced rats for experimental purpose.

2.3. Plant Characterization. HPLC was carried out to find the phenolic and flavonoid content of the ethanolic extract of *Fagonia indica*.

2.4. Total Phenolic Content (TPC). Total phenolic content was measured by using Folin–Ciocalteu reagent. 0.5 mL solution of the extract (0.05 g/5 mL) was admixed with 0.5 mL of Folin–Ciocalteu reagent and then added 7.5 mL of deionized water. This mixture was allowed to set at 25°C for 10 min after that 1.5 mL of 20% sodium carbonate (w/v) was added. Then, the mixture was heated in a water bath at 40°C for 20 min and cooled it in ice bath. Finally, the absorbance was recorded at 755 nm wavelength by using a spectrophotometer. Gallic acid was used as a standard, so the total phenolic contents were calculated by using the standard curve of gallic acid (100–1300 ppm). TPC was measured as gallic acid equivalent (GAE), and the results were expressed as mg/gm of dry matter [20].

2.5. Total Flavonoid Content (TFC). The method described by Dewanto [21] was adopted to determine total flavonoid contents. The mixture was kept at room temperature for 5 min and 0.6 mL of 10% $A1C1_3$ was added. The mixture was again kept for 5 min at room temperature. After 5 min, 2 mL

of 1 M NaOH was mixed and distilled water was used to make up the volume. The absorbance was recorded at 510 nm by using a spectrophotometer. Catechin was used as a standard, so the total flavonoid contents were calculated by a calibration curve for catechin (10–130 ppm). The TFC was measured as catechin equivalent (CE) [22].

2.6. Housing of Animals. Twenty-five healthy female Wistar albino rats (150–200 grams) were acquired and kept in the animal house of the Faculty of Pharmaceutical Sciences, Government College University of Faisalabad (GCUF). Standard conditions (12 hrs light/dark cycles at room temperature $25 \pm 5^{\circ}$C with 45–55% humidity) were maintained. Standard laboratory diet and water ad libitum were given to rats. The experiments on animals were performed according to the guidelines of the Ethical Review Committee ERC-21-886, Government College University, Faisalabad. The experimental design was approved by Advanced Studies and Research Board (ASRB) under the study number IRB-21-19886.

2.7. Induction of Disease. After one week of acclimatization period, letrozole (1 mg/kg) in suspension form (letrozole suspended in 0.5% carboxymethylcellulose (CMC)) was given for 7 weeks to rats for the induction of PCOS. This dose was chosen according to protocols suggested in a published article by Rezvanfar et al. [23]. During this period, the estrous cycle was assessed daily by vaginal cytology (relative proportion of cornified cells, leukocytes, and epithelial cells under light microscopy) and weight variations were recorded weekly. The leaving criteria of the study were based on the cyclicity of estrous cycle, % reduction in weight gain, and the restoration of biochemical parameters.

2.8. Experimental Design and Collection of Samples. Animals were divided randomly in 5 groups ($n = 5$). Groups were labeled as I, II, III, IV, and V (Table 1). All the doses were administered daily through oral gavage for seven weeks and vaginal smear was examined daily via colposcopy [24]. After seven weeks of dosing, all the animals were weighed and euthanized after 24 hours of the last dose. The blood sample was collected by cardiac puncture. The serum of each sample was separated out by centrifugation and freezed at -20°C for biochemical analysis and hormonal evaluation. Ovaries were removed and dissected to perform their histopathology. The liver of the rats was preserved for evaluation of antioxidant status [7, 25].

2.9. Estrous Cycle Monitoring and Vaginal Smear Cytology. Estrous cycle consists of four stages, e.g., proestrus, estrus, metestrus, and diestrus. To determine these stages of the estrous cycle, the already-reported method was adopted. The proestrus stage consists of nucleated epithelial cells predominated with a small number of leukocytes. The estrus stage of the estrous cycle comprises cornified epithelial cells. Metestrus stages embraced with leukocytes, cornified epithelial cells, and nucleated cornified cells while the diestrus stage consists of mostly leukocytes with more mucus. To assess the different stages of estrous cycle, a small piece of cotton swab was moistened with 0.9% normal saline and samples from the rat vagina were taken. Slides were prepared and stained with methylene blue dye. Vaginal cytology assessment was performed by using a light microscope [26].

2.10. Ovarian Histopathological Examination. Rat ovaries of all groups were dissected out and fixed in 10% buffer formalin solution. After fixation, paraffin blocks were made and $5\,\mu$ thick slices were cut using microtome. Slides were prepared and stained with eosin and hematoxylin stains.

2.11. Serum Hormone Analysis. Serum hormone analysis was performed by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) kit methods. The blood sample was taken by cardiac puncture and then, the serum was separated out by cold centrifugation. Serum follicle-stimulating hormone (FSH; mμ/mL), progesterone (ng/dL), prolactin (ng/mL), and testosterone (ng/dL) levels were measured by RIA utilizing the kit of Beckman Coulter, Inc. USA. The serum luteinizing hormone (LH; mμ/mL) concentration was determined by the ELISA method utilizing the kit of Pointe Scientific Inc. USA. Serum estrogen (pg/mL) levels were evaluated by using the kit of ALPCO, USA, and serum insulin (μlU/ml) levels were determined by using the kit of Calbiotech Inc., USA.

2.12. Lipid Profile and Liver Function Tests. Various markers of lipid profile, i.e., total serum cholesterol, triglycerides, HDL, and LDL levels were determined. Similarly, different markers of liver function test, i.e., levels of ALT, AST, and ALP along with total and direct bilirubin levels were also evaluated. All these tests were conducted using an automated chemistry analyzer (Microlab-300).

2.13. Antioxidant Activity Assays

2.13.1. Determination of Enzyme Activities using Liver Homogenates. It is commonly observed that in polycystic ovarian syndrome, the levels of antioxidant enzymes, e.g., catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) are altered and therefore were evaluated in the current study. The liver tissues were homogenized to prepare liver homogenates by using 0.1 M phosphate buffer saline (pH 7.4). Supernatant was obtained by centrifuging the mixture at 800 rpm at 4°C for 30 min. This supernatant solution was used for the determination of different antioxidant parameters (CAT, SOD, and GSH) [27].

2.13.2. DPPH Radical Scavenging Assay. Stock solution of DPPH (2.4 mg/100 mL methanol) was prepared. *F. indica* extract stock solution (5 mg/mL in methanol) was also made. Different sample dilutions (20, 40, 60, 80, and 100 ppm) were made using the *F. indica* extract stock solution. Similarly, 200 mg/L stock solution of BHT (butylated hydroxytoluene) was prepared and using this BHT stock solution, different sample dilutions (20, 40, 60, 80, and 100 ppm) were

Table 1: Experimental design.

Groups	Treatment
Group I (vehicle control)	Aqueous solution of carboxymethyl cellulose (CMC 0.5%)
Group II (PCOS/disease control)	Letrozole (1 mg/kg b.w.) dissolved in 0.5% carboxymethyl cellulose
Group III (metformin/standard)	Metformin (20 mg/kg b.w.) to letrozole-induced PCOS rats
Group IV (*Fagonia* extract)	*F. indica* (500 mg/kg b.w.) to letrozole-induced PCOS rats
Group V (*Fagonia* + metformin)	*F. indica* (500 mg/kg b.w.) + metformin (20 mg/kg b.w.) to letrozole-induced PCOS rats

prepared. Subsequently, added 4 mL of DPPH solution in both plant samples and BHT samples. The mixture was kept at room temperature for 30 min in dark, and the absorbance was recorded at 515 nm using a spectrophotometer. The absorbance of the blank (DPPH radicle without antioxidant) was also recorded [28].

2.14. Statistical Analysis. The data were demonstrated as mean ± SEM, and the results of different groups were compared by analysis of variance (ANOVA) using GraphPad Prism 7.04 followed by the Tukey's multiple comparisons test. The results were considered statistically significant when the p value was less than 0.05.

3. Results

3.1. Plant Characterization

3.1.1. Analysis of Phytoconstituents of F. indica using HPLC. HPLC analysis of ethanolic extract of *F. indica* showed the presence of phenolic contents, i.e., synaptic acid (1.603 mg/gm), benzoic acid (2.785 mg/gm), and traces of chlorogenic acid. While, flavonoid contents in ethanolic extract of *F. indica* included myricetin (0.672 mg/gm) and quercetin (0.904 mg/gm) (Figure 1)

3.1.2. Total Phenolic Content. The TPC of the ethanolic extract of *F. indica* was determined as 109.56 ± 0.124. The TPC is expressed as gallic acid equivalent (GAE).

3.1.3. Total Flavonoid Content. The TFC of the ethanolic extract of *F. indica* was determined as 35.10 ± 0.08. The TFC is expressed as catechin equivalent (CE).

3.2. Estrous Cycle Monitoring and Vaginal Smear Cytology. Throughout the study period, changes of the estrous cycle (proestrus, estrous, metestrus, and diestrus phases) were regular at regular time periods in group I, indicating a normal estrous cycle. Figure 2 shows the four stages of estrous cycle of a normal healthy rat.

Animals with PCOS delayed the estrous cycle and remained in the diestrus phase for a prolonged time. Animals of group III who were treated with metformin remained in the diestrus phase as most of the cells found in vaginal smear were leukocytes, a characteristic feature of the diestrus phase. Examination of groups IV and V showed the estrous phase of cycle that consists majorly of cornified epithelial cells (Figure 3).

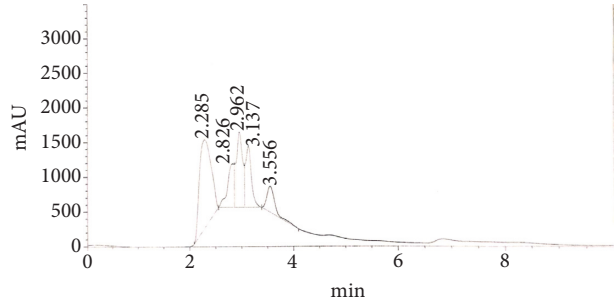

Figure 1: HPLC analysis of ethanolic extract of *Fagonia indica*.

3.3. Effect of Fagonia indica on Histopathology of Rat Ovaries. Histopathological slides of group I ovary exhibited normal architecture with small- to medium-sized anteral follicles, multiple corpus luteum, granulosa cells, oocyte, and various stages of developing follicles (Figure 4(a)). Ovary slides of group II rats presented with cystic follicles and disorganized granulosa cell compartment with irregular thickness of granulosa cells that is the characteristic of atretic anteral follicles. Letrozole-treated ovaries lacked corpus luteum (Figure 4(b)). These changes may be explained by decreased FSH and increased testosterone level in the letrozole-treated group. However, reduction in number of cystic follicles was found metformin and *Fagonia indica*-treated groups, respectively (Figures 4(c) and 4(d)). While, appreciably restored normal anatomy of ovaries were found in the animals of group V (metformin + *Fagonia*) (Figure 4(e)).

3.4. Body Weight Changes. At the end of the study period, group II rats generally gained more weight than group I rats. Body weight of control rats were increased by 18% of its original weight, while the weight of PCOS rats was increased by 35%, i.e., almost the double of weight gain of the rats in the control group. The weight difference among all groups was mostly statistically nonsignificant (Figure 5).

3.5. Effect of F. indica on Hormonal Status. Group II (PCOS) showed significant increase ($p < 0.05$) in LH levels as compared to group I (normal control). LH levels were found decreased significantly in group III ($p < 0.05$) and group V, ($p > 0.05$) while a nonsignificant decrease was observed in group IV, when compared with group II (Figure 6(a)).

A significant ($p < 0.0001$) reduction was observed in FSH, estradiol, and progesterone concentrations in group II, when compared with group I. The levels were significantly improved ($p < 0.0001$) in all treated groups as compared with group II (Figures 6(b), 6(f), and 6(g)).

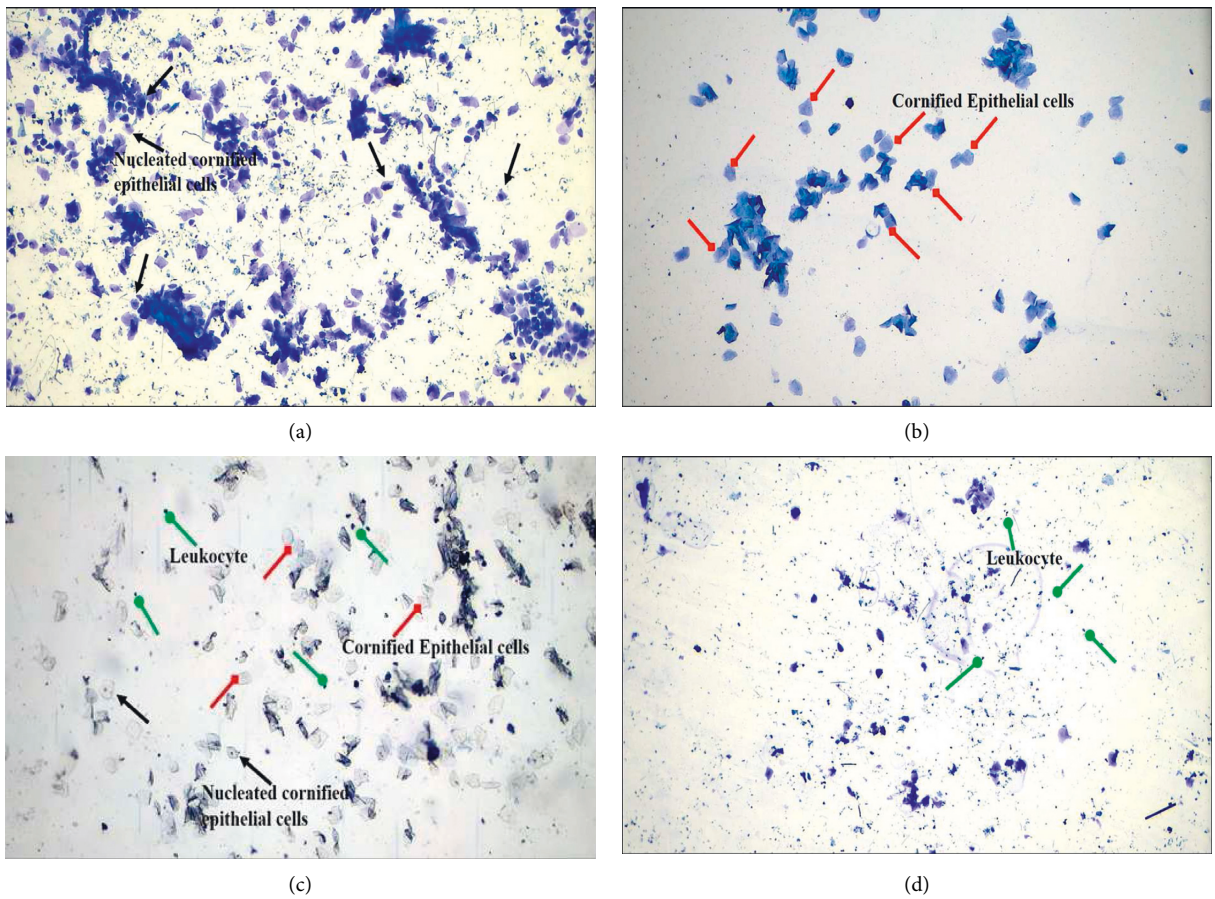

Figure 2: Vaginal smear of healthy rat (normal control group) showing different stages of the estrous cycle. (a) Proestrous phase containing dominantly nucleated cornified epithelial cells (black arrow). (b) Estrous phase characterized by cornified epithelial cells (red arrow). (c) Metestrous phase: in this phase, leukocytes (green arrow), cornified epithelial cells, and nucleated cornified cells are present. (d) Diestrous phase comprises of mostly leukocytes (green arrow).

A significant ($p < 0.01$) decrease in serum prolactin concentration was observed in group II (PCOS-induced group) as compared to group I (control group). There was a significant increase in the prolactin level in group III ($p < 0.01$); however, nonsignificant elevation was found in other treated groups (Figure 6(c)).

A significant ($p < 0.05$) increase was observed in testosterone concentration of group II (PCOS control rats) when compared with group I (normal control). The data showed significant reduction in testosterone levels in all treated groups as compared with group II (Figure 6(d)).

A significant ($p < 0.001$) increase was observed in serum insulin concentration of group II, when compared with group I. The data showed significant reduction in group III and IV ($P < 0.001$), while nonsignificant reduction was found in group V, when compared with group II (Figure 6(e)).

3.6. Effect of Ethanolic Extract of F. indica on Lipid Profile. In group II, total cholesterol (TC) levels were significantly increased ($p < 0.0001$) as compared to group I. While treatment groups showed significantly lessened levels of total cholesterol as compared with group II.

In group II, significant increase ($p < 0.0349$) was noticed in the triglycerides level compared with group I. However, there was a significant reduction ($p < 0.0001$) in triglycerides (TG) of group III and nonsignificant reduction was noticed in group IV and V in comparison with group II.

There was a significant decrease ($p < 0.0001$) in high-density lipoprotein (HDL) level of group II in comparison with group I. While a nonsignificant increase in the HDL level was observed in group III, IV, and V when compared with group II.

Low-density lipoprotein (LDL) level in group II was significantly elevated ($p < 0.0001$) as compare to group I. A significant reduction ($p < 0.0001$) was found in the levels of LDL in group III, IV, and V, when compared with group II (Figure 7).

3.7. Effects of F. indica on Liver Functioning Test (LFT). There was not any statistical difference found in the concentrations of total bilirubin, direct and indirect bilirubin in all groups. Aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase levels were elevated significantly in group II as compared to group I. There was a significant reduction in the levels of AST and ALT in group III, IV, and V when compared with group II.

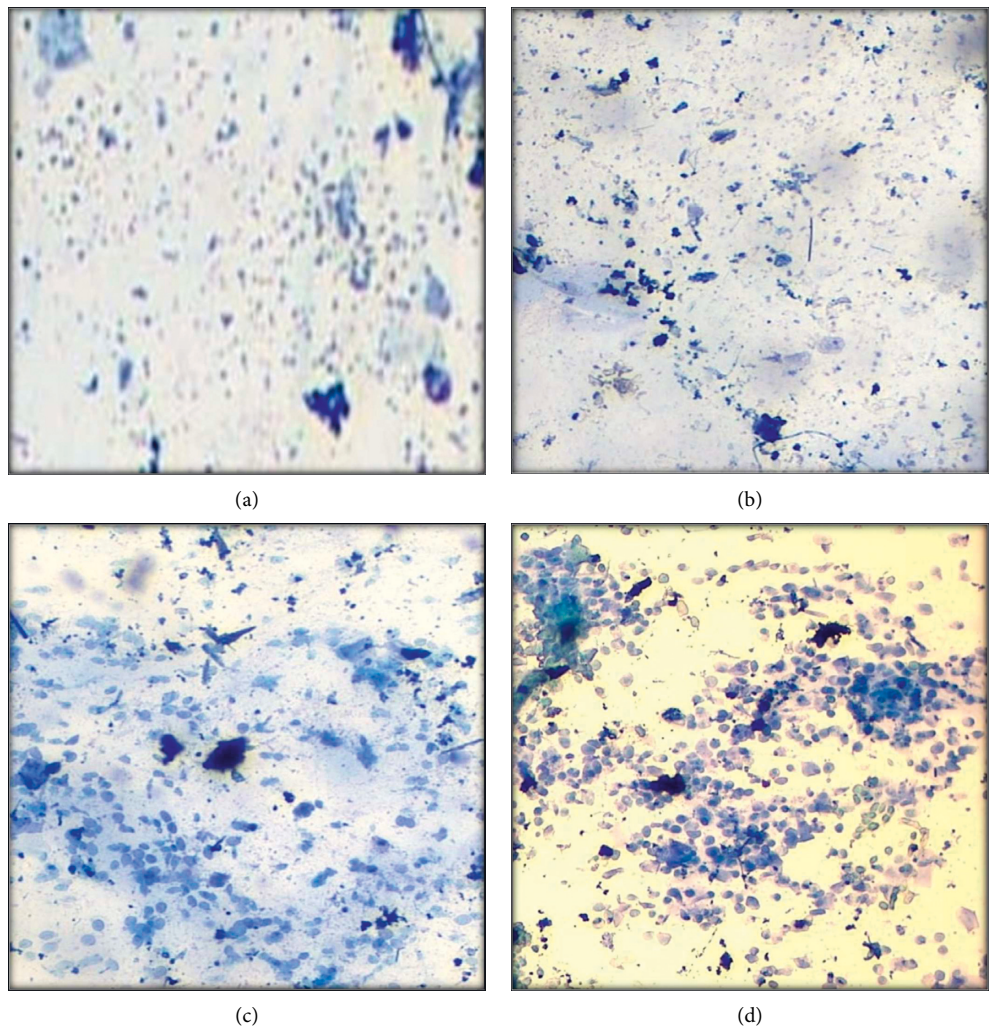

Figure 3: Vaginal smear at different stages of estrous cycle after PCOS induction and treatment. (a) PCOS group with diestrous phase. (b) Metformin treated group with diestrous phase. (c) *Fagonia* extract treated group with estrous phase. (d) Metformin plus *Fagonia*-treated group with an estrous phase of the cycle.

The levels of alkaline phosphatase is found ameliorated in group V while a nonsignificant difference was observed in group III and V as compared to group II (Table 2).

3.8. Effects of F. indica on Antioxidant Enzymes. The levels of antioxidant biomarkers such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were estimated in liver samples. The levels of all these parameters were decreased ($p < 0.001$) in group II as compared to group I. Results showed that in all the treated groups, oxidative stress biomarkers were increased significantly ($p < 0.05$) when compared with group II (Figures 8(a)–8(c)).

3.9. DPPH Assay. Percent inhibition of DPPH by *Fagonia* extract is mentioned in Table 3. The sample is taken in parts per million (ppm). Butylated hydroxytoluene (BHT) was used as a standard for comparison of results.

4. Discussion

The ovaries are highly organized female reproductive organs composed of germ cells (eggs or oocytes) and somatic cells (stromal cells, theca cells, and granulosa cells). Interaction of these cells decides the development and formation of oocyte-containing follicles, ovulation of egg, and formation of corpus luteum. Follicular development is regulated by hypothalamic-pituitary-ovarian axis (HPO). In this axis, hypothalamic gonadotropin-releasing hormone (GnRH) stimulates the secretion of pituitary hormones, e.g., FSH and LH [29]. Follicular development is regulated by hypothalamic-pituitary-ovarian axis (HPO). In this axis, hypothalamic gonadotropin-releasing hormone (GnRH) stimulates the secretion of pituitary hormones, e.g., FSH and LH. Hypothalamus stimulates LH synthesis over FSH, results in elevated LH/FSH ratio in PCOS women. Increased level of LH causes the increased production of testosterone that consequently leads to follicular arrest and increased AMH

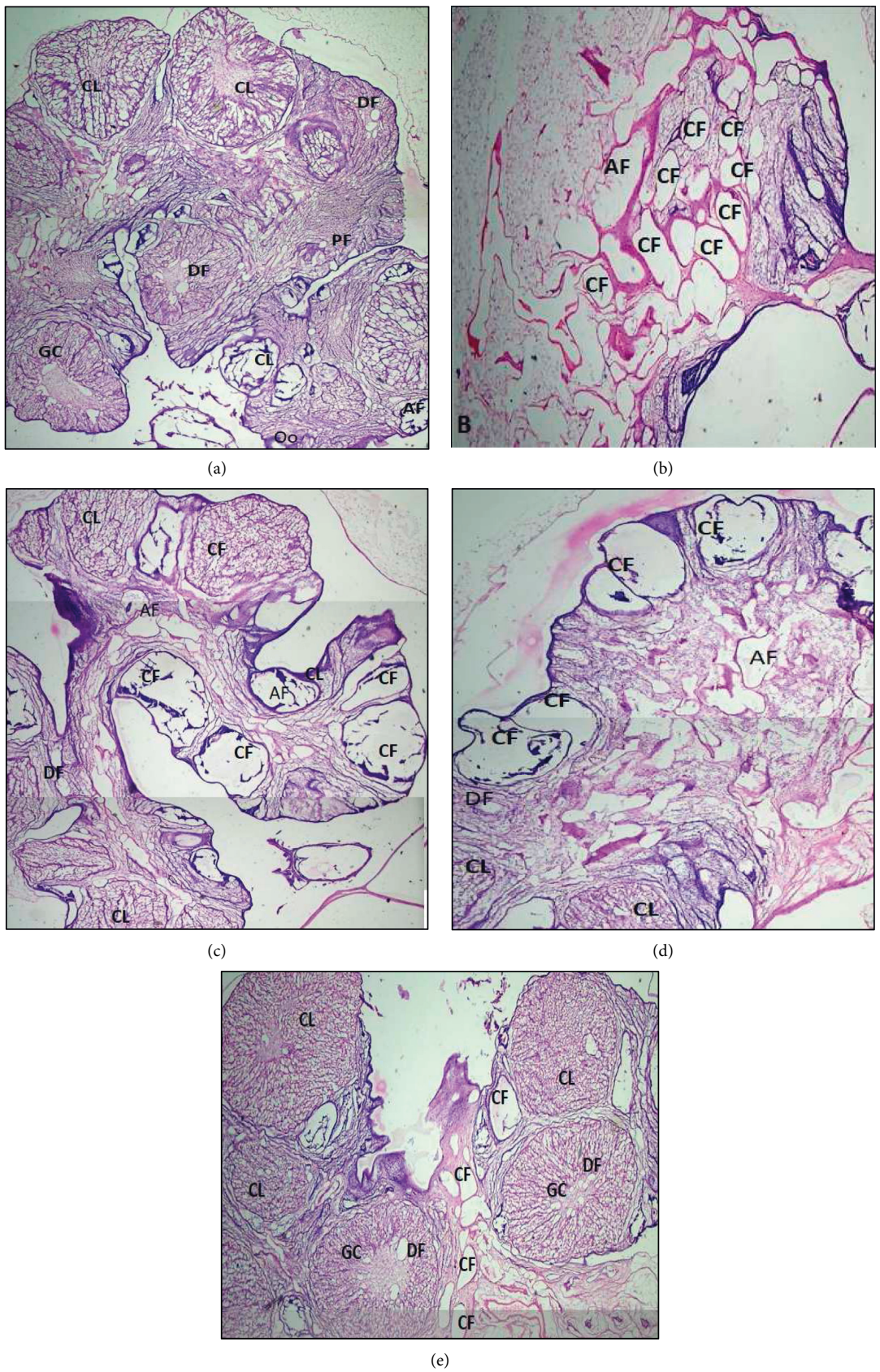

Figure 4: Ovarian cross section stained by hematoxylin-eosin. (a) Section from the control group showing normal morphology with different stages of ovarian follicles. (b) Ovary from PCOS rat containing various cystic follicles. (c) Ovarian section of PCOS rat treated with metformin. (d) Ovary of PCOS rat treated with *Fagonia* extract. (e) Ovary of PCOS rat treated with metformin plus *Fagonia*. CL: corpus luteum; CF: cystic follicles; AF: atretic follicles; DF: developing follicles; PF: primary follicles; GC: granulosa cells; Oo: oocyte. Scale bar is equal to 100 μM. Magnification: 4X.

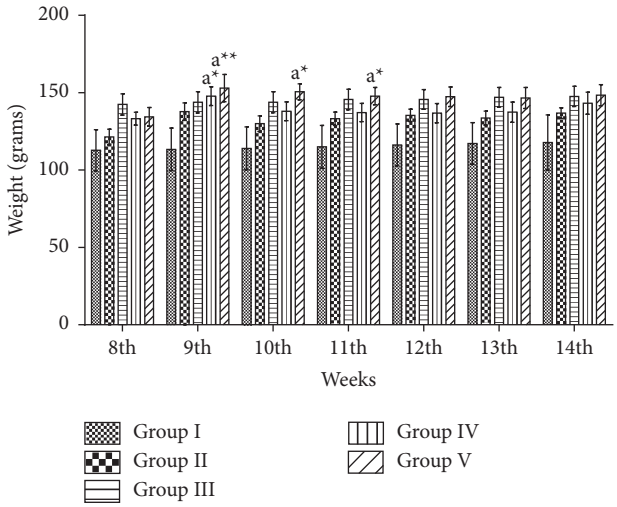

FIGURE 5: Effect of ethanolic extract of *Fagonia indica* on weekly body weight (grams) in letrozole-induced PCOS rat model. Values are expressed as mean ± SEM ($n = 5$). Comparison among groups were performed by using two-way ANOVA followed by Tukey's multiple comparisons test. ns $p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

levels [30]. Polycystic ovarian syndrome is very complex endocrine disorder that is most frequently encountered gynecological endocrinopathy among reproductive aged women [31]. Many genetic and environmental factors are responsible for the etiology of this syndrome. Unhealthy lifestyle and diet or any infectious mediators elevate the probability of PCOS. In the case of PCOS, LH pulse frequency is significantly increased, while the FSH production is reduced causing positive feedback on the GnRH pulse frequency. It worsen the condition by releasing more LH [32]. In the case of treatment group, the LH/FSH is decreased and has negative feedback on the GnRH pulse frequency which ultimately decreased the LH levels and increased the production of FSH. One of the factors is insulin resistance that disturbs ovarian function and raises androgen levels leading to anovulation. Gonadotropin-releasing hormone (GnRH), FSH, LH, and prolactin levels are also disturbed in PCOS [33]. Cytochrome P450c17 (CYP17A1) is the key enzyme (rate-limiting enzyme) that limits the sex steroidal production in theca cells of ovary and adrenal cortex of adrenal gland. Strong evidences suggested the ovary as the main source of androgens in PCOS women although in minority of cases, the adrenal gland might also contributed for androgen excess. Neuroendocrine dysregulation may also participate in hyperandrogenism in ovaries that contribute to the pathogenesis of PCOS [32]. There is growing literature illustrating the effect of oxidative stress in pathophysiology of PCOS. Oxidative stress markers are elevated in the body of PCOS women [23]. Oxidative stress is defined as the imbalance between the overproduction of oxidants and the limited reserves of antioxidant defenses. PCOS is also considered as an oxidative state. Oxidative stress is partly associated with the various characteristics of this disease including IR, obesity, hyperandrogenism, and abdominal adiposity. These conditions lead towards the development of oxidative stress [34]. Evidences suggested that many herbal remedies were effective in reducing the oxidative stress, e.g., *Panax ginseng*, *Olea europaea*, and many other plants that have the antioxidant potential [35, 36]. A vitamin-like nutrient called coenzyme Q10 (CoQ10) also has a remarkable antioxidant potential. It has a positive effect on the reproductive health. CoQ10 is also involved in the expression of genes such as proliferation cell nuclear antigen (PCNA) and follicle-stimulating hormone receptor (FSHR) that are responsible for the folliculogenesis [37].

Herbal drugs have shown excellent results in management of PCOS having steady therapeutic effects with least side effects. They increase immunity of body and regularize menstrual cycle [16]. The ethanolic extract of *Fagonia indica* contains different phenolic and flavonoid phytoconstituents that have potential to treat signs and symptoms of PCOS.

The PCOS rat model was successfully developed by administration of letrozole. It is a nonsteroidal and highly potent aromatase inhibitor [38]. Current study showed an increase in weight gain of PCOS rats as compared to normal control rats and clear difference between normal control rat ovaries and PCOS rat ovaries was observed in histopathological evaluation. Furthermore, vaginal smear cytology expressed that letrozole induced the cysts in ovaries of rats as PCOS rats remained in diestrus phase for a prolonged time. Letrozole also reduced the levels of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) in the body [25]. Previously, increased oxidative status has been determined in the systemic circulation and follicular fluids of PCOS rats [39]. Imbalanced reproductive hormones and insulin resistance is the key characteristics in PCOS patients and were responsible for the imbalance in energy homeostasis, development of adiposity, and weight gain [40].

Previously, *F. indica* has shown antioxidant, antiobesity, antilipoprotein and antidiabetic activities. It regularized the imbalanced reproductive hormones as well. So, the present study was aimed to explore its therapeutic potential on PCOS rat model. *F. indica* have antioxidant potential due to the presence of its flavonoid phytoconstituents [41]. Quercetin and myricetin are two flavonoids that were identified in the ethanolic extract of *Fagonia indica* and are known potent antioxidants [25, 42]. Antioxidant activity is due to the presence of OH group on the phenyl ring of quercetin and myricetin and is dependent upon the presence of OH groups [43]. Quercetin is known to arrest the oxidative stress by triggering the nuclear factor (erythroid-derived 2)-like 2/antioxidant response element (Nrf2-ARE) pathway and stimulating the expression of phase II antioxidant enzymes such as glutathione s-transferase (GST), CAT, NAD(P)H: quinone oxidoreductase 1 (NQO1), SOD, and glutathione peroxidase (GPx) [44]. The results of current study are in-line with the abovementioned inferences and showed that *F. indica* elevated the levels of CAT, GSH, and SOD enzymes.

Histology of vaginal smear is the key determinant of ovarian physiology. Vaginal smear test is indicative of disease induction in group II as the leukocytes were present abundantly showing the diestrus stage of estrous cycle.

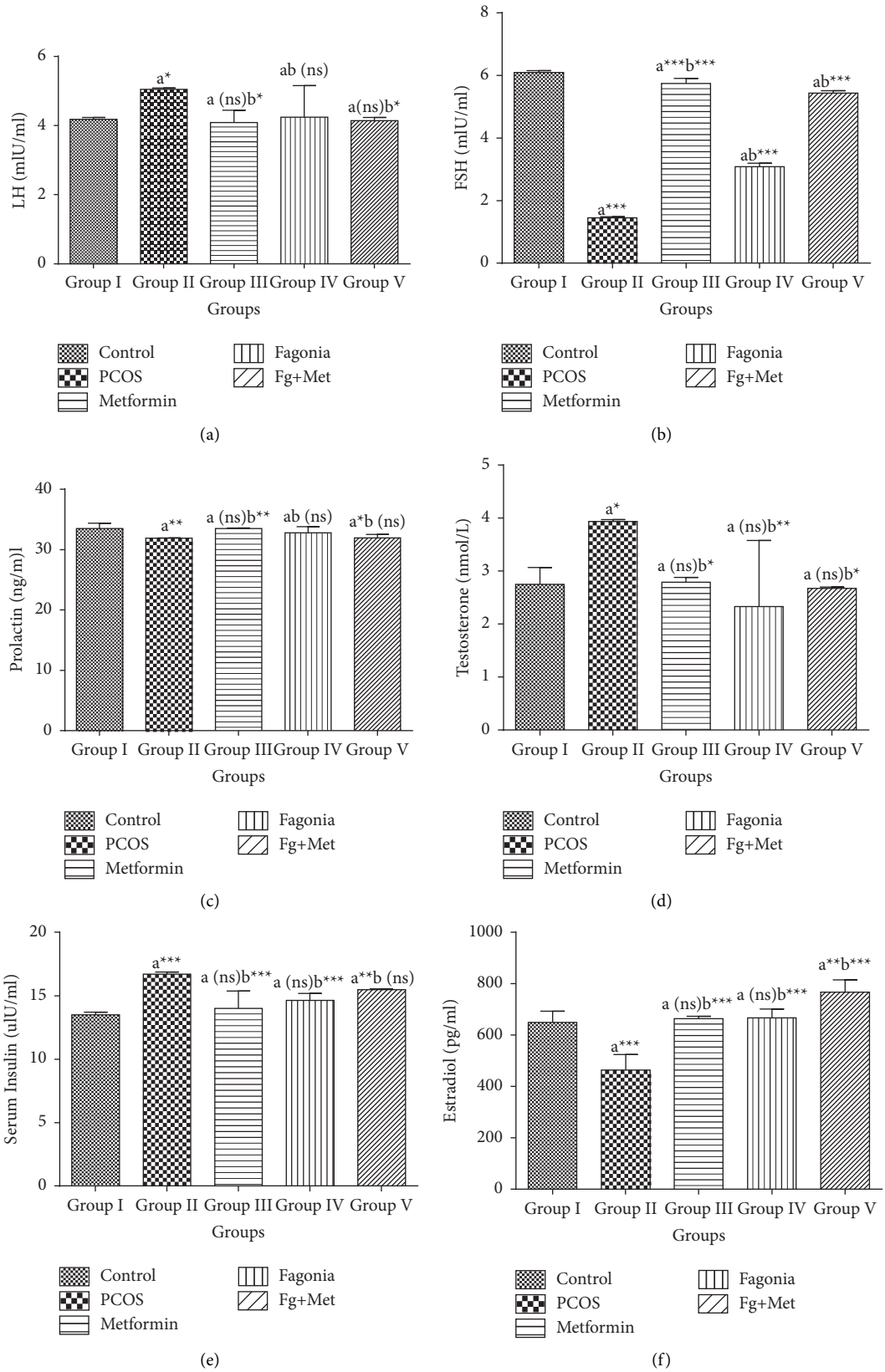

Figure 6: Continued.

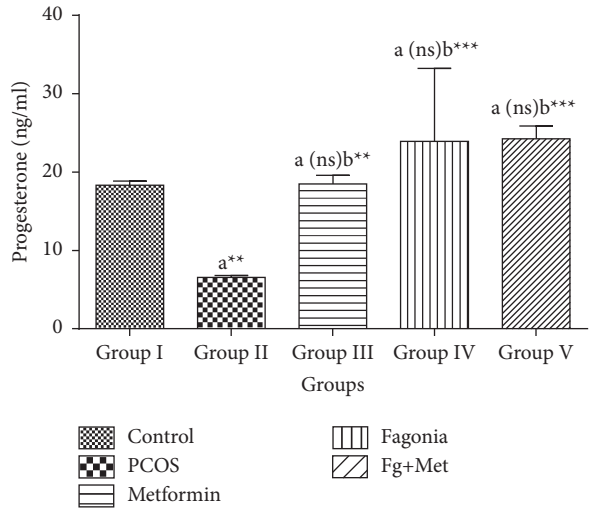

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Figure 6: Effects of ethanolic extract of *F. indica* on (a) LH, (b) FSH, (c) prolactin, (d) testosterone, (e) insulin, (f) estradiol, and (g) progesterone level in letrozole-induced PCOS rats. Values are expressed as mean ± SEM ($n = 5$). ns: nonsignificant. [a]Significantly different from Group I (control); [b]Significantly different from Group II (PCOS). Comparison among groups was made by one-way ANOVA followed by Tukey's multiple comparisons test. ns $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

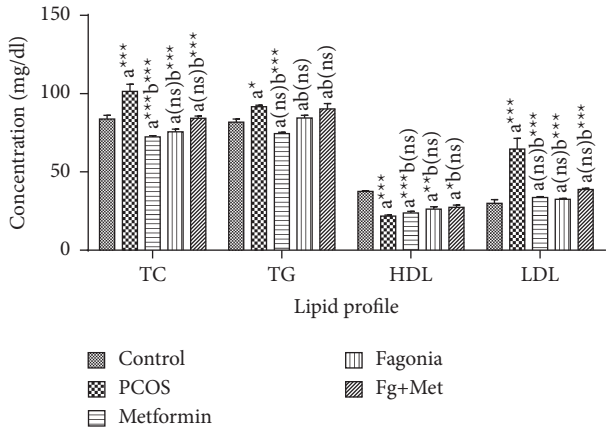

Figure 7: Effect of ethanolic extract of *Fagonia indica* on lipid profile in letrozole-induced PCOS rat model. The data are presented as mean ± SEM ($n = 5$). ns: nonsignificant. [a]Significantly different from group I (control), [b]Significantly different from group II (PCOS). Comparisons among groups were made by two-way ANOVA followed by Tukey's multiple comparison test. ns $p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

PCOS rats remained for a prolonged period in their diestrus stage. While, treatment with *F. indica* abolished the irregularity of estrous cycle and controlled the cyclicity. These changes might be attributed to decreased FSH leveled and increased testosterone leveled in PCOS group. Treatment with *F. indica* enhanced the levels of FSH and reduced the levels of testosterone. Furthermore, combination treatment exhibited remarkable outcomes in group V by restoring the nearly normal physiology of ovaries. Previous evidences suggested that quercetin possessed innate ability to rectify hormonal imbalance and subsequent metabolic disorder that occurred in PCOS [31].

Serum LH levels are increased in PCOS because of disrupted hypothalamic-pituitary axis that activates PI3K/Akt pathway causing the overexpression of ovarian CYP17A1 gene along with 17-α hydroxylase enzyme levels, which is used in the reaction to synthesize the androgen from progesterone [45]. Previous research data have shown the ability of quercetin to inhibit the pathway of androgen biosynthesis due to the LH. Lowering effects of quercetin on the levels of LH and testosterone are associated to resistin that is important for steroidogenesis [31]. Current study showed that treatment with *F. indica* nearly normalized the LH levels.

Current study showed that *F. indica* reduced serum levels of total cholesterol, TGs, and LDL while, caused an increase in HDL levels. This may be attributed to quercetin content of the ethanolic extract of *Fagonia indica* [31]. Proposed mechanism for quercetin to lower the triglyceride level in obese patient who consumes diet overloaded with free fatty acids (FFAs) (highly saturated food) has been reported by Kim et al. [46]. Quercetin is known to restrict the hepatic triglycerides accumulation by enhancing the hepatic mitochondrial oxidative metabolic capacity which subsequently causes the reduction of FFA-induced lipid peroxidation. It also prevents mitochondrial damage and hepatic lipid accumulation [46]. PCOS patients are hyperglycemic because of insulin resistance. Evidence suggested that *F. indica* also have hypoglycemic effect [47]. Current study showed that treatment with *F. indica* reduced insulin resistance.

Furthermore, this study can be extended to find out the effects of ethanolic extract of *Fagonia indica* at the molecular and genetic levels. Future studies may also emphasize on identification of phytochemical constituents responsible for the found effect.

Table 2: Effect of *F. indica* on bilirubin (total, direct, and indirect), ALT, AST, and alkaline phosphatase levels. ns: nonsignificant. [a]Significantly different from group I; [b]Significantly different from group II. The data are presented as mean ± SEM ($n = 5$). Comparisons among groups were made by two-way ANOVA followed by Tukey's multiple comparison test. ns $p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Parameters	Group I	Group II	Group III	Group IV	Group V
Total bilirubin (mg/dl)	2.70 ± 0.07	2.73 ± 0.01	2.73 ± 0.01	2.73 ± 0.01	2.70 ± 0.07
Direct bilirubin (mg/dl)	2.57 ± 0.01	2.87 ± 0.01	2.57 ± 0.01	2.87 ± 0.01	2.57 ± 0.01
Indirect bilirubin (mg/dl)	0.70 ± 0.002	0.69 ± 0.002	0.69 ± 0.002	0.70 ± 0.002	0.69 ± 0.002
(ALT) (U/l)	22.26 ± 0.02	28.80 ± 0.01^{a***}	25.78 ± 0.2^{ab***}	18.47 ± 0.02^{ab***}	27.69 ± 0.02^{ab***}
(AST) (U/l)	179.62 ± 0.09	189.62 ± 0.09^{a***}	175.20 ± 0.88^{ab***}	178.50 ± 0.01^{ab***}	174.90 ± 0.01^{ab***}
Alkaline phosphatase (U/l)	20.45 ± 0.02	24.15 ± 0.11^{a***}	23.88 ± 0.06^{a***b} (ns)	18.60 ± 0.01^{ab***}	23.81 ± 0.01^{a***b} (ns)

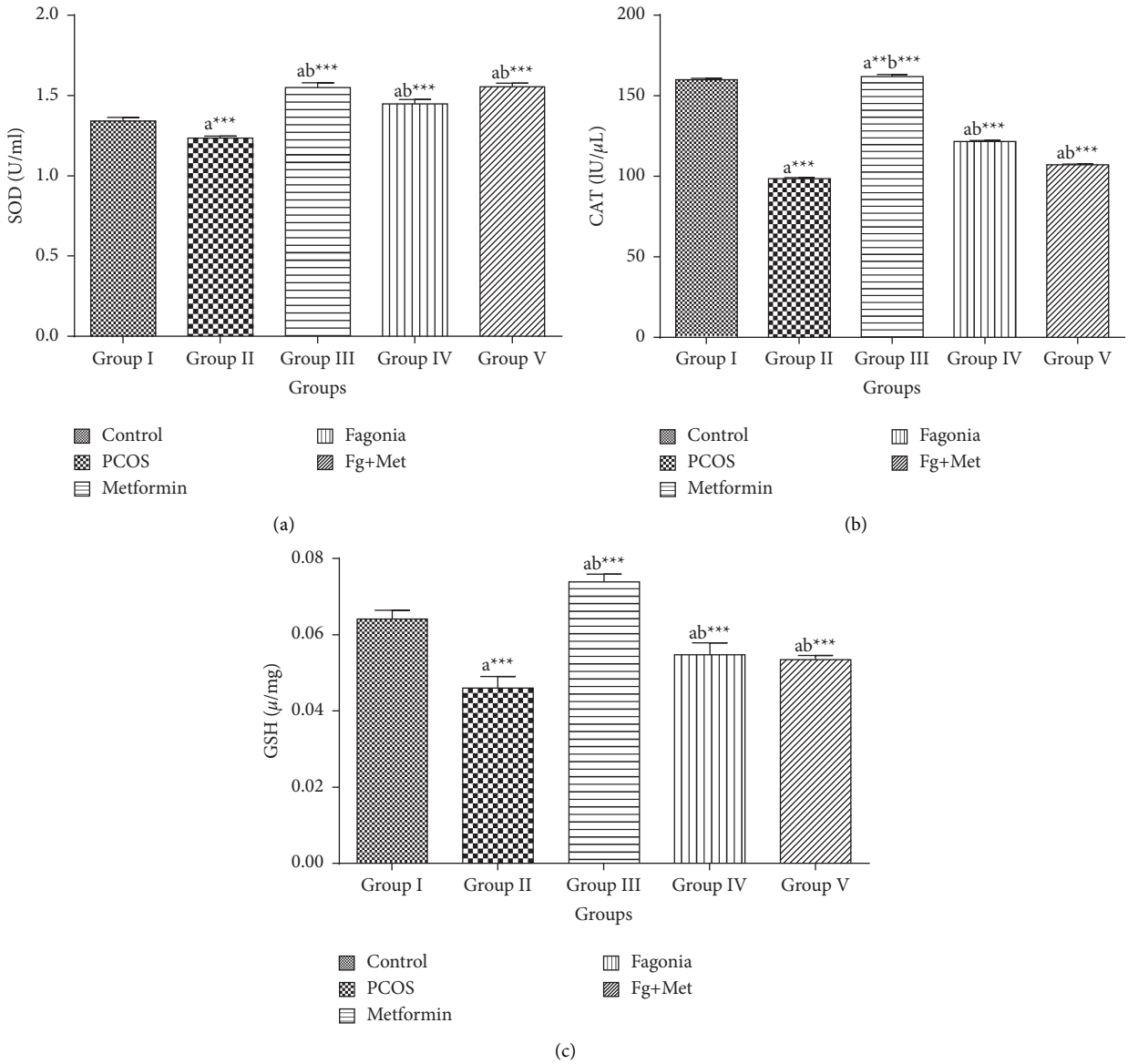

Figure 8: *Fagonia indica* improved the levels of hepatic enzymes (a) SOD, (b) CAT, and (c) GSH. [a]Significantly different from group I, [b]Significantly different from group II. The data are presented as mean ± SEM ($n = 5$). Comparison among groups was made by one-way ANOVA followed by Tukey's multiple comparisons test. ns $p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 3: DPPH scavenging activity of ethanolic extract of *F. indica*.

BHT standard		Ethanolic extract of *Fagonia indica*	
Sample ppm	Scavenging %	Sample ppm	Scavenging %
20	55.27	20	46.83
40	63.5	40	49.75
60	76.31	60	54.23
80	84.64	80	57.56
100	88.6	100	59.33

BHT: butylated hydroxytoluene; DPPH: 2,2-diphenylpicrylhydrazyl.

5. Conclusion

Current study showed that the ethanolic extract of *Fagonia indica* had the potential to ameliorate polycystic ovarian syndrome at the dose of 500 mg/kg which is statistically comparable with metformin. This property might be attributed to antioxidant activity and improvement in liver enzymes and lipid profile, along with amelioration of hormonal enzymes by *F. indica*.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 4395612, 13 pages
https://doi.org/10.1155/2022/4395612

Research Article

Efficacy and Safety of Cangfu Daotan Decoction in Patients with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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Received 1 February 2022; Accepted 18 April 2022; Published 17 May 2022

Academic Editor: Yue Hui Zhang

Background. The infertility caused by polycystic ovary syndrome (PCOS) has received considerable attention. Considerable efforts have been made to improve the rates of pregnancy and live birth. Cangfu Daotan Decoction (CFDTD) is a classic prescription for treating infertility in obese women. The efficacy of CFDTD in PCOS is controversial. *Objective*. To evaluate the effectiveness and safety of CFDTD in treating infertility with PCOS. *Methods*. A literature search was performed in the Cochrane Library, PubMed, Embase, the China National Knowledge Infrastructure, the Wanfang Database, the VIP Chinese Biomedical science journal database, and the Chinese BioMedical database from the date of each database establishment to December 2021. Only randomized controlled trials, which were used to evaluate the efficacy of CFDTD in treating subjects with PCOS, were included in the present study. The quality of evidence was assessed using the Cochrane Reviewer Handbook 5.0.0, and meta-analysis was performed using RevMan 5.3.5 software. *Results*. Fourteen studies with a total of 1,433 patients were included in this analysis. The present study indicated that CFDTD could significantly improve pregnancy rate (RR = 1.62, 95% CI (1.44, 1.83), $P < 0.00001$), ovulation rate (RR = 1.40, 95% CI (1.25, 1.56), $P < 0.00001$), and estradiol levels (SMD = 0.80, 95% CI (0.03, 1.58), $P = 0.04$), while testosterone levels (SMD = −0.92, 95% CI (−1.52, −0.31), $P = 0.003$), homeostatic model assessment for insulin resistance (MD = −0.56, 95% CI (−0.99, −0.12), $P = 0.01$), total cholesterol levels (MD = −0.60, 95% CI (−0.76, −0.44), $P < 0.00001$), triglyceride levels (MD = −0.48, 95% CI (−0.60, −0.36), $P < 0.00001$), body mass index (MD = −2.96, 95% CI (−3.88, −2.03), $P < 0.00001$), and the incidence of adverse reactions (RR = 0.47, 95% CI (0.34, 0.65), $P < 0.00001$) were significantly reduced. *Conclusions*. Evidence from the meta-analysis suggested that CFDTD appeared to be an effective and relatively safe treatment for PCOS. However, the influence of CFDTD on reproductive hormones, glucose metabolism, and blood lipids should be carefully concluded. Due to the low quality of the methods used in the included randomized controlled trials, further studies are required with larger sample sizes and well-designed models to confirm our findings.

1. Introduction

As the most common reproductive endocrine disorder, polycystic ovary syndrome (PCOS) affects approximately 7–12% of women with reproductive age [1]. PCOS not only includes symptoms of sparse menstruation, obesity, hyperandrogenemia, abnormal glucose tolerance, and hyperlipidemia but is also associated with infertility [2]. Approximately 90% of PCOS patients are infertile, and 80% of cases with ovulatory dysfunction infertility are caused by PCOS [3]. In addition, PCOS is costly, with an estimated $93 million spent annually on PCOS treatment in the United States, including an average annual cost of $533 million for infertility care [4]. Although the therapies for PCOS have been considerably optimized, the costs remain high ($669.78 per normal pregnancy) [5].

As a common cause of infertility, PCOS has attracted considerable attention [6]. At present, ovulation induction is still the mainstream treatment for PCOS

infertility. Ovulation induction therapy has improved the pregnancy rate of infertility patients with PCOS; however, the efficacy of ovulation induction therapy requires further improvement [7]. Ovulation regimens include oral use of drugs, such as clomiphene and letrozole, as well as in vitro fertilization (IVF) [8]. However, neither clomiphene nor letrozole can completely eliminate the problem of infertility (live birth rate clomiphene 25.2%, letrozole 30.2–37.6%) [9]. The improvement of the effectiveness of ovulation induction therapy is imperative to reduce the incidence of infertility. Ovulation induction can also cause side effects, including luteinized unruptured follicle (LUF) and ovarian hyperstimulation syndrome [10]. The majority of the patients with infertility exhibit complications such as insulin resistance and hyperandrogenemia. Previous studies have shown that the effect of clomiphene is affected by the serum levels of insulin, and the improvement of insulin resistance can increase clinical pregnancy and possibly shorten the duration of pregnancy [11, 12]. Similarly, the efficacy of letrozole is influenced by the total serum testosterone levels. Therefore, it is imperative to identify a safe, effective, and multitargeted drug to regulate the reproduction and metabolism of PCOS as an adjuvant therapy.

In the past 50 years, the use of complementary and alternative medicine and the study of Cangfu Daotan Decoction (CFDTD) for PCOS have been gradually increased [13, 14]. In China, CFDTD was first reported to be used in the mid-16th century in Guangsi Ji Yao. According to this book, CFDTD was used for treating infertility in obese women [15]. In addition, CFDTD can also be used in obese women with oligomenorrhea. Since no records of PCOS have been reported in ancient China, ancient books have been used for prescriptions based on the typical symptoms of PCOS, including irregular menstruation, obesity, and infertility. In traditional medicine, CFDTD has been used to treat the phlegm-dampness syndrome, which is very similar to the typical symptoms of obese PCOS, including obesity, irregular menstruation, and acanthosis nigricans. An accumulated number of studies have shown that CFDTD can be used to treat PCOS, notably in obese PCOS cases [16–18]. Concomitantly, a high number of studies have been conducted to explore the effect and mechanism of CFDTD. These studies have suggested that CFDTD can improve infertility by improving insulin resistance, reducing hyperlipidemia, hyperandrogenemia, and body weight [19–22]. The research on CFDTD is not consistent. Zhao et al. [23] suggested that CFDTD did not significantly increase the pregnancy rate compared with that of the placebo. Similar conclusions were reported in a previous study by Chen et al. which suggested that CFDTD did not significantly improve the ovulation rate [24]. However, other studies have shown that CFDTD can significantly increase pregnancy and ovulation rates [25–27]. Moreover, the conclusions drawn regarding the safety of CFDTD are contradictory [28, 29]. Therefore, a meta-analysis was performed to systematically review and evaluate the efficacy and safety of CFDTD as an adjunctive drug for PCOS.

2. Materials and Methods

2.1. Search Strategy. This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the Cochrane Handbook for Systematic Reviews of Interventions.

2.2. Inclusion and Exclusion Criteria

2.2.1. Type of Study. A randomized controlled trial (RCT) study design was used, which was based on the treatment of CFDTD in PCOS.

2.2.2. Objective of the Study. The patients were diagnosed with PCOS according to the Rotterdam conference recommendations of 2003. There was no restriction on age, race, or region of study subjects.

2.2.3. Intervention Measures. CFDTD alone or CFDTD combined with Western medicine or CFDTD combined with complementary and alternative medical therapies (including drugs, acupuncture, exercise, and diet change) was used. There was no limit to the dosage and course of CFDTD. The control group included blank control, placebo control, Western medicine, and complementary and alternative medicine therapy groups.

2.2.4. Outcome Indicators. The primary outcome measured was the pregnancy rate; the secondary outcomes were the following: rate of ovulation, reproductive hormone levels (including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), and testosterone (T) levels), assessment of glucose metabolism (homeostatic model assessment insulin resistance (HOMA-IR)), assessment of lipid metabolism (including total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels, as well as low-density lipoprotein (LDL) concentration), body mass index (BMI), and the gestational safety as the incidence of adverse events.

This systematic evaluation was used to explore the efficacy of CFDTD in treating subjects with PCOS. Therefore, only the evaluation results of each study at the end of the course of treatment were extracted, without considering the periodic evaluation results.

2.2.5. Exclusion Criteria. The following exclusion criteria were used: (1) literature that contained incomplete data and could not be analyzed, (2) literature, which was published repeatedly, (3) subjects with PCOS and other diseases or serious complications, (4) subjects of the experimental and the control groups treated with CFDTD and lack of the aim of the study to verify the effectiveness of CFDTD, and (5) the addition of CFDTD and the reduced prescription for the study intervention measures were not included.

Table 1: Literature retrieval strategy of PubMed.

#1 "polycystic ovary syndrome" [MeSH Terms] OR "polycystic ovary syndrome" [Title/Abstract] OR "polycystic ovarian syndrome" [Title/Abstract] OR "PCOS" [Title/Abstract]
#2 "Cangfudaotan" [Title/Abstract] OR "Cangfu daotan" [Title/Abstract] OR "cang fu dao tan" [Title/Abstract]
#3 "randomized controlled trials as topic" [MeSH terms] OR "randomized controlled trial" [Title/Abstract] OR "randomized" [Title/Abstract] OR "randomly" [Title/Abstract] OR "clinical trial" [Title/Abstract]

2.3. Literature Retrieval Strategy. The following databases were searched: Cochrane Library, PubMed, Embase, VIP Chinese Biomedical science journal database, the China National Knowledge Infrastructure (CNKI), WanFang Data databases, and the Chinese BioMedical database (CBM) from the date of inception to December 31, 2021. Only RCTs, which were used to evaluate the efficacy of CFDTD in PCOS, were included in the present study. The keywords and free words were used to search. The English search terms included the following: Polycystic Ovary Syndrome, Polycystic Ovarian Syndrome, PCOS, cangfu daotan, cangfu daotan, cang fu dao tan, randomized, randomly, randomized controlled trial, and clinical trial. For example, Table 1 is the specific retrieval strategy used in PubMed. To avoid missing information, a manual search of the references to similar studies was conducted.

2.4. Literature Selection, Data Extraction, and Bias Risk Assessment of the Included Studies. Literature screening and data extraction were independently completed by two researchers and cross-checked. Any discrepancy was solved by consultation with a third reviewer. A form was created for data extraction, which included the summary of general data (including title, author, research location, and publication time), the basic characteristics of the study (including the number of cases, age, and intervention measures of the experimental group and the control group), the key elements of risk assessment for bias, the outcome indicators, and the outcome measurement data. Cochrane systematic assessor manual 5.3.0 was used as a risk assessment tool for RCTs to evaluate the risk of bias in the included studies.

2.5. Statistical Analysis. The RevMan 5.3 software was used for meta-analysis. The relative risk was used as the effect index for the enumeration data. Mean difference (MD) or standardized mean difference (SMD) was used as the effect index for measurement data. The confidence interval was set at 95%. The χ^2 test was used to analyze significant differences between the results, and $P < 0.05$ was considered to indicate a statistically significant difference. The heterogeneity was quantitatively assessed by I^2. In the absence of statistical heterogeneity among the results, the fixed effect model was used for meta-analysis. In case statistical heterogeneity was noted among the results of each study, the source of heterogeneity was analyzed, and the influence of significant clinical heterogeneity was excluded. Finally, a random-effect model was used for meta-analysis. Significant clinical heterogeneity was treated with subgroup analysis or sensitivity analysis or only descriptive analysis.

3. Results

3.1. Study Characteristics. The search strategy identified 480 studies. A total of 466 publications were excluded due to the repetition of data or due to being irrelevant or nonspecific to the study topic. This classification was performed based on the title, abstract, and full text. Finally, 14 studies with a total of 1,433 patients were included in the quantitative analyses (Figure 1).

3.2. Basic Characteristics and Bias Risk Assessment of the Included Studies. All included studies indicated no significant differences at baseline between the experimental and treatment groups. Eight studies reported randomization using a random number table, while the other six studies did not report specific methods for achieving randomization. None of the 14 trials described allocation hiding. Only one study included a blind study design; however, following the reading of its full text, it was found to be falsely blinded. None of the 14 studies mentioned withdrawal or follow-up. Due to the lack of detail and specific information, it was impossible to determine whether sufficient implementation had taken place during random sequence generation, blinding, or allocation hiding. As a result, the trials included in the present study were of poor quality (Figure 2). The basic characteristics of the included studies are shown in Table 2.

3.3. Outcomes

3.3.1. Pregnancy Rate. A total of 14 studies including 1,433 patients (717 in the experimental and 716 in the control groups, respectively) assessed pregnancy rates [23–36]. The literature indicated homogeneity (heterogeneity test severity $\chi^2 = 13.78$, $P = 0.39$, $I^2 = 6\%$), and the combined effect amount adopted the fixed effect model. The analysis indicated that CFDTD could significantly increase the pregnancy rate of PCOS (RR = 1.62, 95% CI (1.44, 1.83), $P < 0.00001$) (Figure 3). Funnel plot was used to estimate publication bias, and the results demonstrated no significant publication bias (Figure 4).

A subgroup analysis was performed for the included trials based on different interventions. Two RCTs [23, 33] compared the efficacy of CFDTD + Western medicine with placebo + Western medicine on the pregnancy rate. The literature indicated homogeneity (heterogeneity test severity $\chi^2 = 1.09$, $P = 0.30$, $I^2 = 8\%$), and the combined effect adopted the fixed effect model. The results indicated that CFDTD + Western medicine exhibited a significant effect on improving pregnancy rate than that of placebo + Western medicine in the PCOS subjects (RR = 1.43, 95% CI (1.06, 1.94), $P = 0.02$) (Figure 3.1.1.1).

A total of 11 RCTs [24–30, 32, 34–36] compared the efficacy between CFDTD + Western medicine with Western medicine on the pregnancy rate. The literature indicated

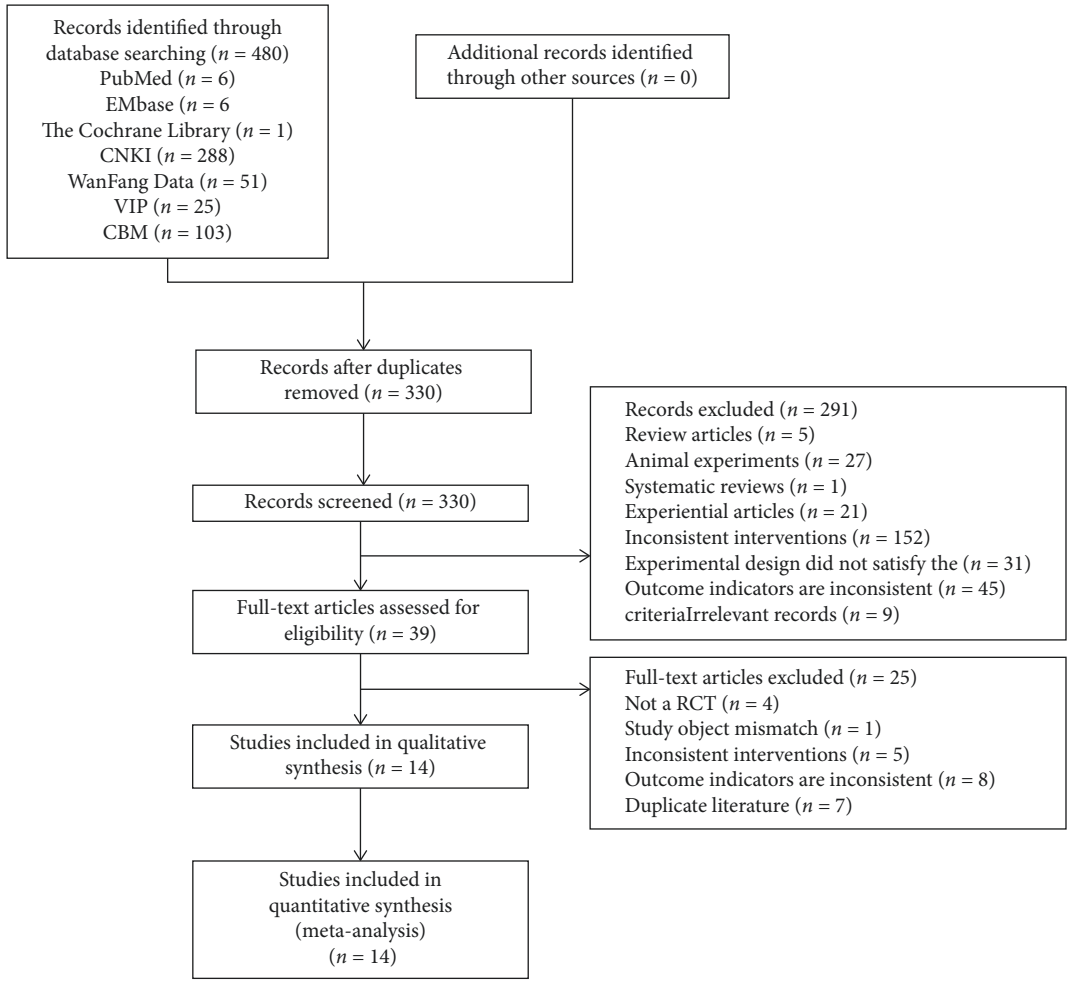


Figure 1: Flow diagram of the study selection process.

homogeneity (heterogeneity test severity $\chi^2 = 12.57$, $P = 0.25$, $I^2 = 20\%$), and the combined effect followed the fixed effect model. The results indicated that CFDTD + Western medicine used for PCOS was more effective than Western medicine used to improve pregnancy rate (RR = 1.66, 95% CI (1.45, 1.90), $P < 0.00001$) (Figure 3.1.1.2).

Only one RCT [31] reported the incidence of the pregnancy rate in CFDTD compared with that noted in Western medicine. Meta-analysis indicated that CFDTD increased the pregnancy rate of PCOS compared with that noted following Western medicine (RR = 1.67, 95% CI (0.87, 3.20), $P = 0.13$); however, these results were not statistically significant (Figure 3.1.1.3).

3.3.2. Ovulation Rate. The ovulation rate was assessed in 584 patients (experimental group 292, control group 292) derived from six studies [24–27, 31, 36]. The literature was homogenous (heterogeneity test Chi $^2 = 4.91$, $P = 0.43$, $I^2 = 0\%$), and the combined effect size followed the fixed effect model. CFDTD significantly increased the ovulation rate of PCOS compared with that noted in the control group (RR = 1.40, 95% CI (1.25, 1.56), $P < 0.00001$) (Figure 5).

A subgroup analysis was also performed in the included trials based on interventions. Five RCTs [24–27, 36] compared the effects of CFDTD + Western medicine with Western medicine on the ovulation rate. Heterogeneity was not significant in the literature (heterogeneity test Chi$^2 = 3.97$, $P = 0.41$, $I^2 = 0\%$), and the fixed-effect model was adopted. Compared with Western medicine, CFDTD + Western medicine significantly increased the ovulation rate of PCOS subjects (RR = 1.38, 95% CI (1.23, 1.54), $P < 0.00001$) (Figure 5.1.2.1).

Only one trial [31] compared the efficacy of CFDTD with that of Western medicine. The meta-analysis results indicated that CFDTD significantly increased the ovulation rate of PCOS compared with that of the Western medicine group (RR = 1.64, 95% CI (1.07, 2.53), $P = 0.02$) (Figure 5.1.2.2).

3.3.3. Reproductive Hormones. Three studies evaluated the influence of CFDTD on FSH [26, 32, 36]. The heterogeneity of the literature was statistically significant (heterogeneity test Chi $^2 = 8.51$, $P = 0.01$, $I^2 = 77\%$), and the random effect model was used. The results indicated that CFDTD treatment did not cause significant changes in FSH levels in PCOS compared with those noted in the control group (MD = −0.26, 95% CI (−0.77, 0.25), $P = 0.32$) (Figure 6(a)).

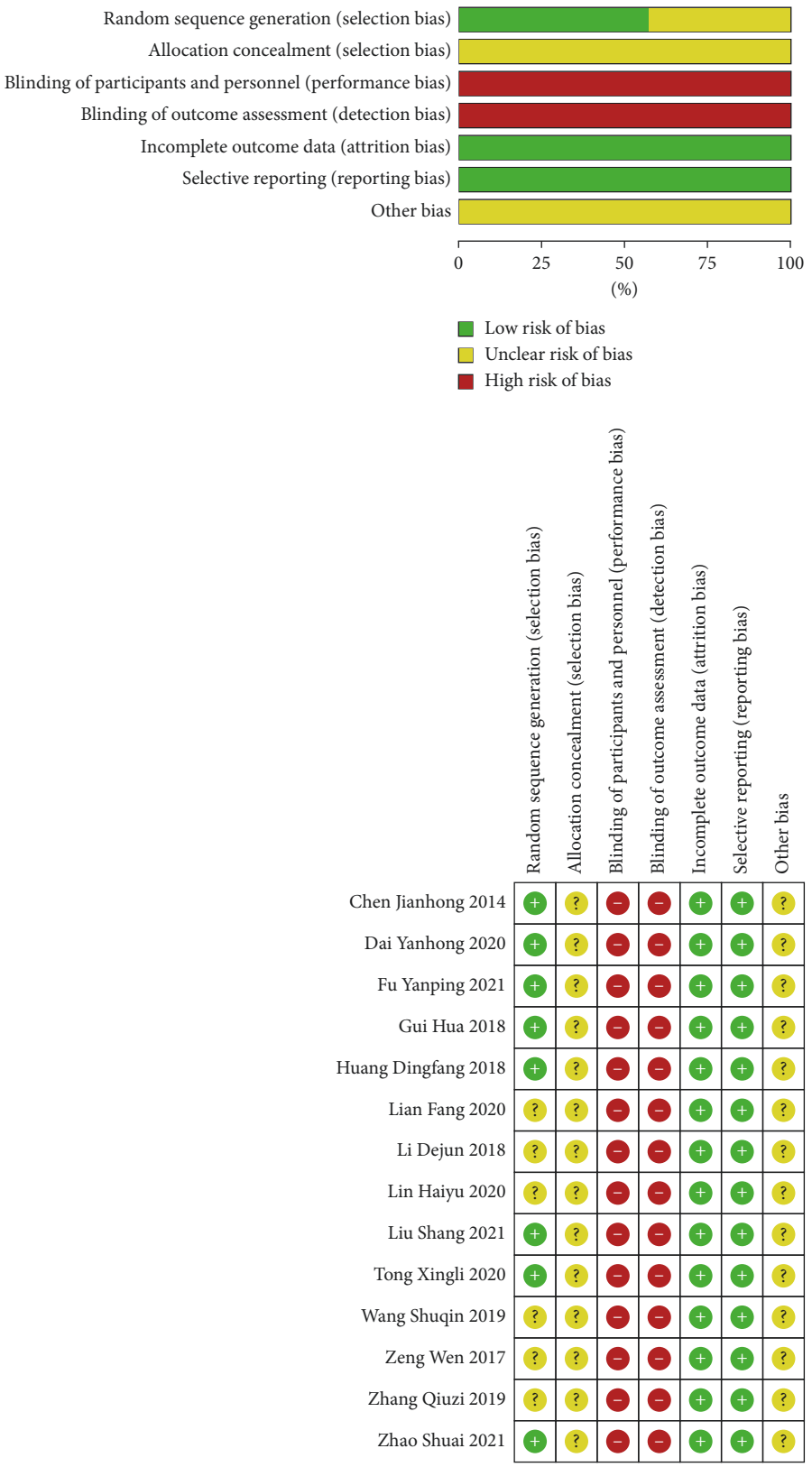


Figure 2: The risk of bias of the included studies.

Due to the large heterogeneity, sensitivity analysis was performed. We excluded Zhang Qiuzi 2019 [36], which indicated minimal heterogeneity (heterogeneity test $\chi^2 = 0.22$, $P = 0.64$, $I^2 = 0\%$). The combined effect test indicated no significant difference between the groups (MD = 0.01, 95%CI (−0.36, 0.38), $P = 0.97$). Sensitivity

Table 2: The details of the included studies.

Studies	Study location	Sample size		Age (T/C)	BMI (kg/m2) (T/C)	Intervention		Course of treatment	Outcomes
		T	C			T	C		
Zhao Shuai 2021	Shandong province, China	60	60	32.22 ± 4.35/ 30.80 ± 4.69	28.05 ± 1.88/ 28.63 ± 2.78	CFDTD + Western medicine	Placebo + Western medicine	21 days	①④
Lian Fang 2020	Shandong province, China	33	33	30.00 ± 3.07/ 29.70 ± 3.74	—	CFDTD + Western medicine	Placebo + Western medicine	21 days	①③
Liu Shang 2021	Zhejiang province, China	98	98	27.60 ± 3.50/ 27.80 ± 3.30	—	CFDTD + Western medicine	Western medicine	3 months	①②③⑥
Lin Haiyu 2020	Shandong province, China	40	40	31.19 ± 5.42/ 31.04 ± 5.31	—	CFDTD + Western medicine	Western medicine	30 days	①③⑥
Huang Dingfang 2018	Jiangxi province, China	42	42	24.87 ± 4.67/ 24.59 ± 4.92	32.20 ± 4.23/ 31.32 ± 4.65	CFDTD + Western medicine	Western medicine	3 months	①②⑥
Zeng Wen 2017	Hunan province, China	60	60	32.35 ± 5.64/ 32.23 ± 5.60	—	CFDTD + Western medicine	Western medicine	4 months	①②③④
Chen Jianhong 2014	Zhejiang province, China	24	24	28.50 ± 8.90/ 27.20 ± 8.50	—	CFDTD + Western medicine	Western medicine	6 months	①②③⑥
Dai Yanhong 2020	Henan province, China	46	46	29.62 ± 3.57/ 29.84 ± 3.32	—	CFDTD + Western medicine	Western medicine	4 months	①③
Gui Hua 2018	Zhejiang province, China	35	35	28.12 ± 2.23/ 27.56 ± 2.31	22.24 ± 1.18/ 22.15 ± 1.24	CFDTD + Western medicine	Western medicine	4 months	①③
Li Dejun 2018	Xinjiang Uygur autonomous region, China	124	124	25.64 ± 1.30/ 26.17 ± 1.82	—	CFDTD + Western medicine	Western medicine	3 months	①⑥
Zhang Qiuzi 2019	Fujian province, China	38	38	27.50 ± 3.60/ 28.40 ± 5.60	25.70 ± 2.52/ 25.02 ± 2.96	CFDTD + Western medicine	Western medicine	3 months	①②③⑥
Wang Shuqin 2019	Henan province, China	57	56	31.46 ± 4.43/ 31.51 ± 4.40	22.18 ± 1.02/ 22.43 ± 1.01	CFDTD + Western medicine	Western medicine	3 months	①③⑥
Tong Xingli 2020	Jiangsu province, China	30	30	28.80 ± 3.43/ 29.57 ± 2.61	26.50 ± 1.14/ 27.23 ± 1.83	CFDTD + Western medicine	Western medicine	3 months	①⑤⑥
Fu Yanping 2021	Hunan province, China	30	30	27.87 ± 3.93/ 28.07 ± 3.81	—	CFDTD	Western medicine	3 months	①②⑥

T: Treatment group; C: Control group; —: Unreported; ①: Pregnancy rate; ②: Ovulation rate; ③: Reproductive Hormones; ④: Glucose Metabolism; ⑤: Lipid Metabolism; ⑥: Incidence of adverse reactions; CFDTD: Cangfu Daotan Decoction.

analysis indicated no statistical differences in FSH levels between the two groups.

A total of 9 studies [24, 26, 27, 29, 30, 32–34, 36] evaluated the impact of CFDTD on LH levels. The literature did not contain homogenous data (heterogeneity test $\chi^2 = 322.49$, $P < 0.00001$, $I^2 = 98\%$), and the effect size was combined by the random effect model. The results indicated that CFDTD exhibited no significant differences in the treatment of LH in PCOS (SMD = −0.92, 95% CI (−1.91, 0.08), $P = 0.07$) (Figure 6(b)). Due to the large heterogeneity, a sensitivity analysis was performed. Following the removal of the study by Dai Yanhong 2020 [30], the heterogeneity was still large (heterogeneity test $\chi^2 = 228.03$, $P < 0.00001$, $I^2 = 97\%$); however, the combined effect test indicated significant differences between the groups (SMD = −1.21, 95% CI (−2.17, −0.25), $P = 0.01$). Sensitivity analysis indicated that compared with the control group, the effect of CFDTD on LH levels was not consistent.

A total of 5 studies [24, 26, 29, 30, 33] evaluated the effect of CFDTD on E_2 levels. The literature did not reveal homogeneity (heterogeneity test $\chi^2 = 65.80$, $P < 0.00001$, $I^2 = 94\%$), and the combined effect size followed the random effect model. The results indicated that CFDTD could significantly increase the E_2 levels of PCOS (SMD = 0.80, 95%

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.1.1 CFDTD+Western medicine vs placebo+ Western medicine						
Lian Fang 2020	17	33	9	33	3.9	1.89 [0.99, 3.61]
Zhao Shuai 2021	36	60	28	60	12.0	1.29 [0.91, 1.81]
Subtotal (95% CI)		*93*		*93*	*15.9*	*1.43 [1.06, 1.94]*
Total events	53		37			
Heterogeneity: chi^2 = 1.09, df = 1 (P = 0.30); I^2 = 8%						
Test for overall effect: Z = 2.32 (P = 0.02)						
1.1.2 CFDTD+Western medicine vs Western medicine						
Chen Jianhong 2014	9	24	2	24	0.9	4.50 [1.08, 18.69]
Dai Yanhong 2020	22	46	10	46	4.3	2.20 [1.18, 4.11]
Gui Hua 2018	31	35	23	35	9.9	1.35 [1.03, 1. 76]
Huang Dingfang 2018	32	42	18	42	7.7	1.78 [1.21, 2.62]
Li Dejun 2018	45	124	31	124	13.3	1.45 [0.99, 2.13]
Lin Haiyu 2020	17	40	8	40	3.4	2.13 [1.04, 4.35]
Liu Shang 2021	40	98	19	98	8.2	2 .11 [1.32, 3.36]
Tong Xingli 2020	19	30	11	30	4.7	1.73 [1.00, 2.97]
Wang Shuqin 2019	25	57	14	56	6.1	1.75 [1.02, 3.01]
Zeng Wen 2017	50	60	39	60	16.7	1.28 [1.03, 1.59]
Zhang Qiuzi 2019	21	38	12	38	5.1	1.75 [1.01, 3.03]
Subtotal (95% CI)		*594*		*593*	*80.3*	*1.66 [1.45, 1.90]*
Total events	311		187			
Heterogeneity: chi^2 = 12.57, df = 10 (P = 0.25); I^2 = 20%						
Test for overall effect: Z = 7.45 (P < 0.00001)						
1.1.3 CFDTD vs Western medicine						
Fu Yanping 2021	15	30	9	30	3.9	1.67 [0.87, 3.20]
Subtotal (95% CI)		*30*		*30*	*3.9*	*1.67 [0.87, 3.20]*
Total events	15		9			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.53 (P = 0.13)						
Total (95% CI)		*717*		*716*	*100.0*	*1.62 [1.44, 1.83]*
Total events	379		233			
Heterogeneity: chi^2 = 13.78, df = 13 (P = 0.39); I^2 = 6%						
Test for overall effect: Z = 7.92 (P < 0.00001)						
Test for subgroup differences: chi^2 = 0.77, df = 2 (P = 0.69); I^2 = 0%						

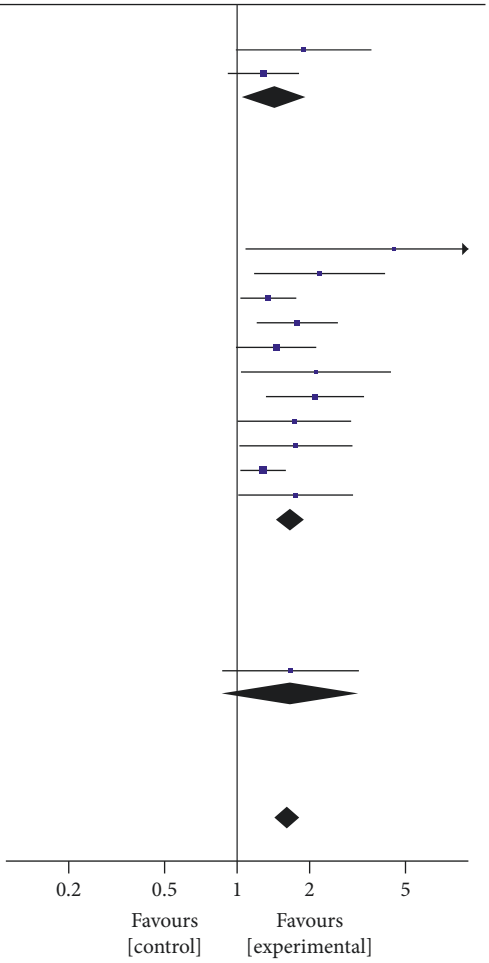


Figure 3: Meta-analyses of the effect of CFDTD on pregnancy rate.

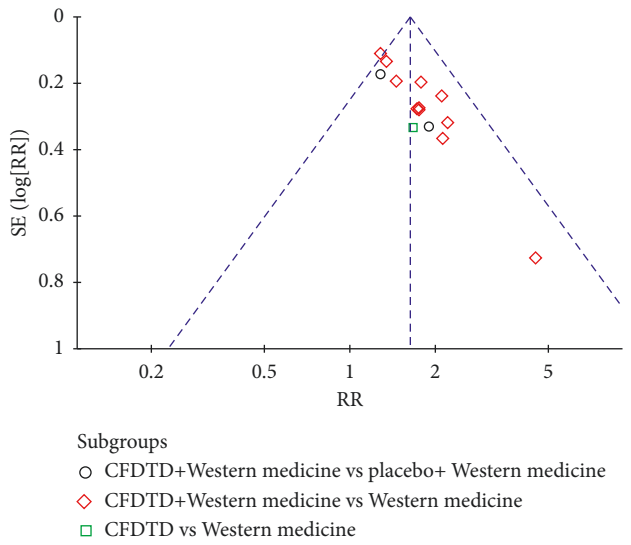


Figure 4: Funnel plot of pregnancy rate for the publication bias.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.2.1 CFDTD+Western medicine vs Westem medicine						
Chen Jianhong 2014	16	24	9	24	5.3	1.78 [0.99, 3.20]
Huang Dingfang 2018	35	42	20	42	11.7	1.75 [1.24, 2.47]
Liu Shang 2021	75	98	59	98	34.5	1.27 [1.05, 1.54]
Zeng Wen 2017	58	60	45	60	26.3	1.29 [1.11, 1.50]
Zhang Qiuzi 2019	32	38	24	38	14.0	1.33 [1.01, 1.76]
Subtotal (95% CI)		*262*		*262*	*91.8*	*1.38 [1.23, 1.54]*
Total events	216		157			
Heterogeneity: chi^2 = 3.97, df = 4 (P = 0.41); I^2 = 0%						
Test for overall effect: Z = 5.60 (P < 0.00001)						
1.2.2 CFDTD vs Western medicine						
Fu Yanping 2021	23	30	14	30	8.2	1.64 [1.07, 2.53]
Subtotal (95% CI)		*30*		*30*	*8.2*	*1.64 [1.07, 2.53]*
Total events	23		14			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.26 (P = 0.02)						
Total (95% CI)		*292*		*292*	*100.0*	*1.40 [1.25, 1.56]*
Total events	239		171			
Heterogeneity: chi^2 = 4.91, df = 5 (P = 0.43); I^2 = 0%						
Test for overall effect: Z = 6.04 (P < 0.00001)						
Test for subgroup differences: chi^2 = 0.61, df = 1 (P = 0.43); I^2 = 0%						

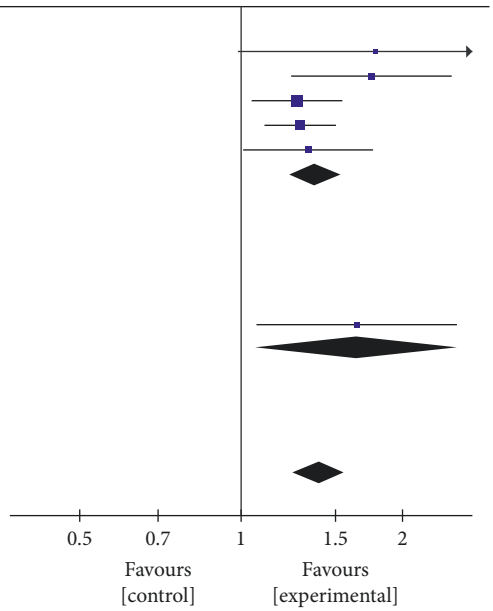
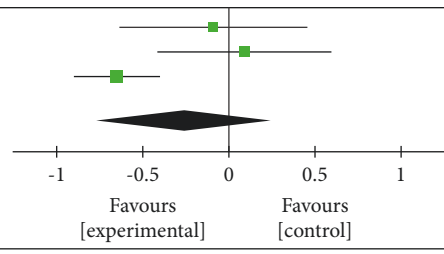


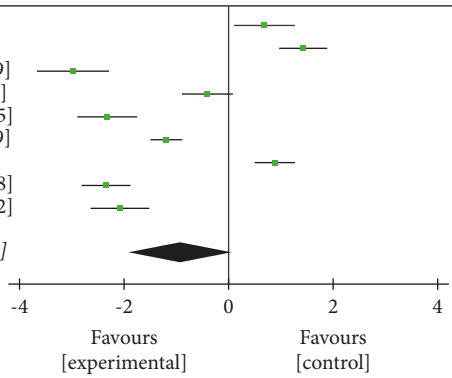
Figure 5: Meta-analyses of the effect of CFDTD on the ovulation rate.

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight (%)	Mean Difference IV, Random, 95% CI
Gui Hua 2018	5.09	1.36	35	5.16	0.93	35	29.3	-0.09 [-0.64, 0.46]
Liu Shang 2021	5.13	1.79	98	5.04	1.82	98	30.8	0.09 [-0.42, 0.60]
Zhang Qiuzi 2019	5.03	0.52	38	5.68	0.59	38	39.9	-0.65 [-0.90, -0.40]
Total (95% CI)			*171*			*171*	*100.0*	*-0.26 [-0.77, 0.25]*
Heterogeneity: tau^2 = 0.15; chi^2 = 8.51, df = 2 (P = 0.01); I^2 = 77%								
Test for overall effect: Z = 1.00 (P = 0.32)								



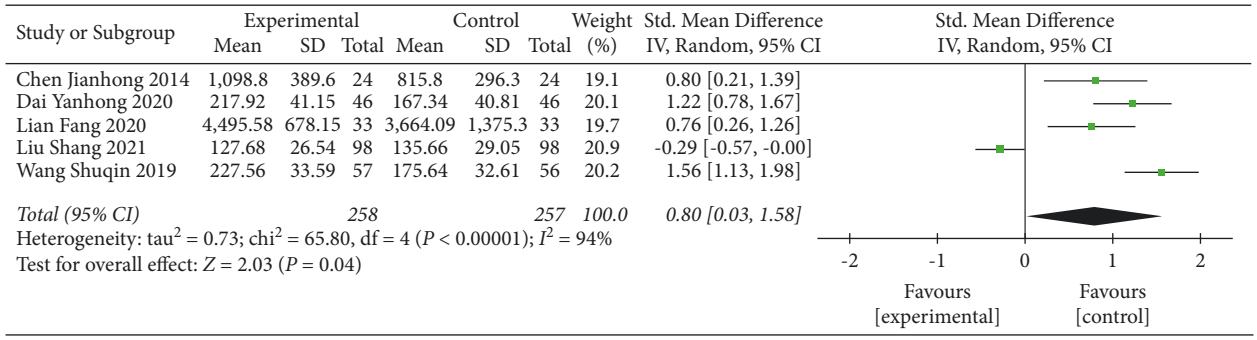
(a)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight (%)	Std. Mean Difference IV, Random, 95% CI
Chen Jianhong 2014	12.8	6.5	24	8.9	4.5	24	11.0	0.69 [0.10, 1.27]
Dai Yanhong 2020	29.54	3.87	46	23.67	4.28	46	11.2	1.43 [0.97, 1.89]
Gui Hua 2018	7.87	0.82	35	11.36	1.42	35	10.9	-2.98 [-3.67, -2.29]
Lian Fang 2020	1.72	0.28	33	1.85	0.35	33	11.1	-0.41 [-0.89, 0.08]
Lin Haiyu 2020	6.38	1.17	40	9.52	1.49	40	11.0	-2.32 [-2 .89, -1.75]
Liu Shang 2021	8.25	2.63	98	12.31	4.02	98	11.3	-1.19 [-1.49, -0.89]
Wang Shuqin 2019	41.02	4.97	57	36.58	5.01	56	11.3	0.88 [0.50, 1.27]
Zeng Wen 2017	6.38	1.05	60	9.45	1.51	60	11.2	-2.35 [-2 .81, -1.88]
Zhang Qiuzi 2019	6.48	1.29	38	9.44	1.52	38	11.0	-2.08 [-2 .64, -1.52]
Total (95% CI)			*431*			*430*	*100.0*	*-0.92 [-1.91, 0.08]*
Heterogeneity: tau^2 = 2.26; chi^2 = 322.49, df = 8 (P < 0.00001); I^2 = 98%								
Test for overall effect: Z = 1.80 (P = 0.07)								

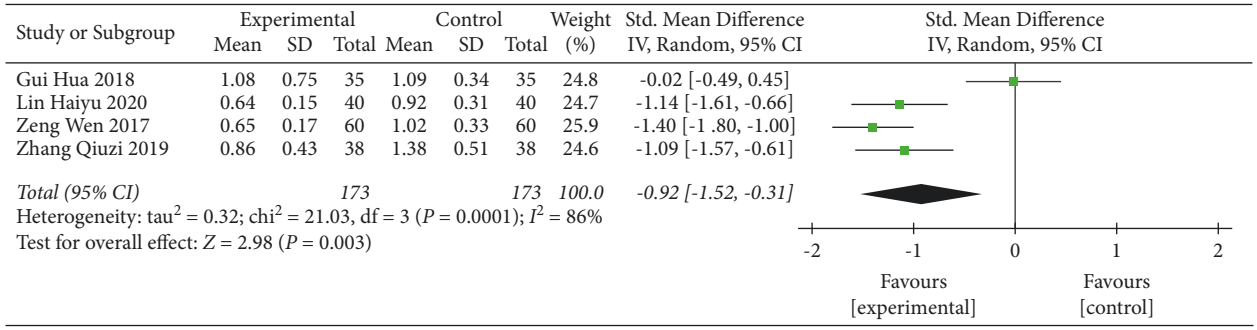


(b)

Figure 6: Continued.



(c)



(d)

Figure 6: Meta-analyses of the effect of CFDTD on reproductive hormones: (a) FSH; (b) LH; (c) E_2; (d) T.

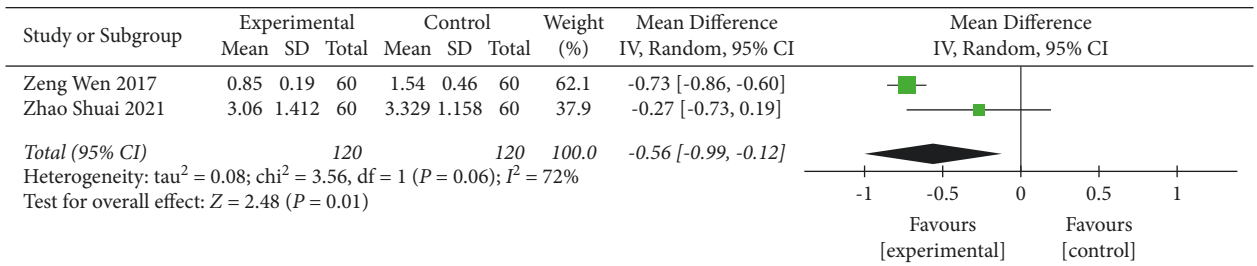


Figure 7: Meta-analyses of the effect of CFDTD on HOMA-IR.

CI (0.03, 1.58), $P = 0.04$) (Figure 6(c)). Due to the large heterogeneity present, sensitivity analysis was performed. Following the exclusion of two articles [26, 29], it was shown that heterogeneity was small (heterogeneity test $\chi^2 = 2.23$, $P = 0.33$, $I^2 = 10\%$). The combined effect test indicated that the difference between the two groups was statistically significant (SMD = 0.96, 95% CI (0.65, 1.27), $P < 0.00001$). The sensitivity analysis indicated that compared with the control group, CFDTD could significantly increase E_2 levels; in addition, the results were consistent.

A total of 4 studies [27, 32, 34, 36] evaluated the influence of CFDTD on the T levels. The literature did not contain homogenous data (heterogeneity test $\chi^2 = 21.03$, $P = 0.0001$, $I^2 = 86\%$), and the effect size followed the random effect model. The results indicated that CFDTD could significantly reduce the T levels of the PCOS subjects (SMD = −0.92, 95% CI (−1.52, −0.31), $P = 0.003$) (Figure 6(d)). Due to the large heterogeneity, sensitivity analysis was performed. The study by Gui Hua et al. in 2018 was excluded [32], and the heterogeneity was small (heterogeneity test $\chi^2 = 1.15$, $P = 0.56$, $I^2 = 0\%$). The combined effect test indicated significant differences between the two groups (SMD = −1.23, 95% CI (−1.49, −0.98), $P < 0.00001$). Sensitivity analysis indicated that CFDTD could significantly reduce T levels; the results were consistent.

3.3.4. Glucose Metabolism. Two studies [23, 27] evaluated the influence of CFDTD on HOMA-IR. The heterogeneity of the literature was statistically significant (heterogeneity test $\chi^2 = 3.56$, $P = 0.06$, $I^2 = 72\%$), and the random effect model was used. The results suggested that CFDTD could significantly reduce HOMA-IR levels of PCOS subjects (MD = −0.56, 95%CI (−0.99, −0.12), $P = 0.01$) (Figure 7).

3.3.5. Lipid Metabolism. Only one trial investigated the effect of CFDTD on blood lipid levels [35]. Meta-analysis indicated that CFDTD significantly reduced TC

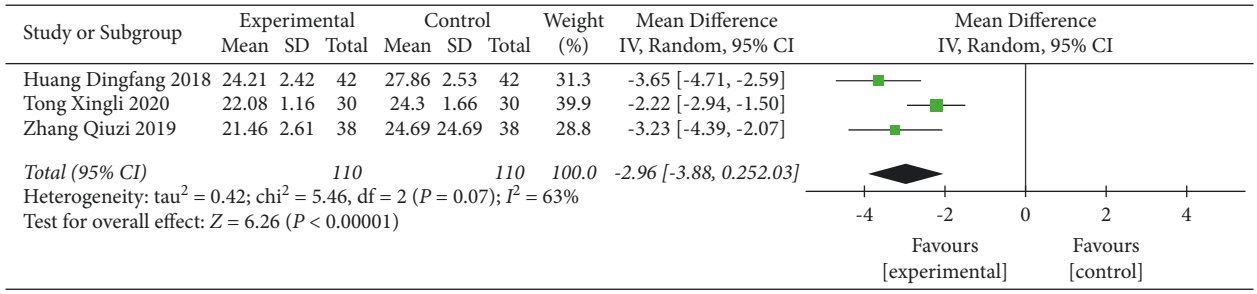


Figure 8: Meta-analyses of the effect of CFDTD on BMI.

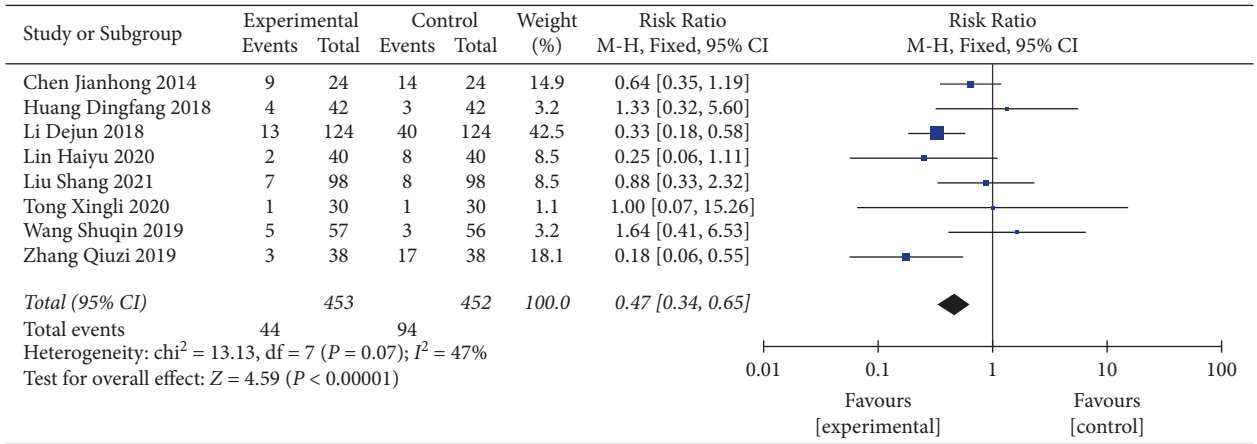


Figure 9: Meta-analyses of the effect of CFDTD on the incidence of adverse reactions.

(MD = −0.60, 95% CI (−0.76, −0.44), $P < 0.00001$) and TG (MD = −0.48, 95% CI (−0.60, −0.36), $P < 0.00001$) levels. No significant differences were noted in LDL and HDL levels between the two groups.

3.3.6. BMI. Three studies [25, 35, 36] evaluated the influence of CFDTD + Western medicine compared with that of Western medicine on BMI. The heterogeneity of the literature was statistically significant ($Chi^2 = 5.46$, $P = 0.07$, $I^2 = 63\%$), and the random effect model was used (MD = −2.96, 95% CI (−3.88, −2.03), $P < 0.00001$). The results suggested that CFDTD + Western medicine could significantly reduce the BMI of PCOS subjects (Figure 8).

3.3.7. Incidence of Adverse Reactions. Nine studies reported adverse reactions [24–26, 28, 29, 31, 34–36]. The incidence of adverse reactions between CFDTD + Western medicine and Western medicine was compared in eight RCTs. The literature exhibited homogeneity (heterogeneity test $\chi^2 = 13.13$, $P = 0.07$, $I^2 = 47\%$), and the fixed-effect model was adopted. The results indicated that CFDTD + Western medicine significantly reduced the incidence of adverse reactions in PCOS subjects compared with that of the Western medicine treatment group (RR = 0.47, 95% CI (0.34, 0.65), $P < 0.00001$) (Figure 9). In addition, a comparison between CFDTD and Western medicine was reported in one trial, and no adverse reactions occurred in both groups [31].

4. Discussion

In the present study, the differences in reproduction (pregnancy rate and ovulation rate), metabolism (reproductive hormones, glucose metabolism, and lipid metabolism), and safety of PCOS between CFDTD and different intervention programs were assessed. A total of 1,433 people were included in 14 studies. The function of CFDTD was summarized as follows: removing dampness, reducing phlegm, and strengthening the spleen. It was composed of the following constituents: *Rhizoma atractylodis*, *sweet attached*, *tangerine peel*, *Bile south star*, *Hovenia dulcis*, *pinellia*, *Rhizoma ligustici wallichii*, *talc*, *Poria cocos*, and *medicated leaven. Rhizoma atractylodis* can clear damp phlegm, and its function is to reduce blood glucose levels and the development of obesity [37]. Previous studies have shown that the reduction in the blood lipid levels is an important effect of *tangerine peel* and *Poria cocos* [38, 39]. *Sweet attached, Hovenia dulcis*, and *pinellia* can improve gastrointestinal function [40–42]. CFDTD has been used to treat infertility in ancient China, and modern studies have also confirmed this effect. Network pharmacological analysis suggested that the combination of *Rhizoma atractylodis*, *sweet attached*, and *pinellia* could improve insulin resistance, reduce the levels of inflammatory cytokines, regulate endocrine hormones and the reproductive axis, promote follicular maturation and ovulation, and ultimately improve reproductive ability [43]. The results of this meta-analysis demonstrated that CFDTD could significantly

improve the pregnancy rate of PCOS. Subgroup analysis indicated that CFDTD significantly increased the pregnancy rate of PCOS subjects compared with that of the placebo and the blank control group. Compared with Western medicine, CFDTD did not exhibit a statistically significant effect on the pregnancy rate. In terms of the ovulation rate, CFDTD has also been shown to significantly increase the ovulation rate. The exclusion of individual studies did not significantly affect the overall effect size of the pregnancy and ovulation rates. This confirmed that CFDTD was effective in improving the reproduction of PCOS subjects (notably pregnancy and ovulation rates), and the results were consistent. Regrettably, live birth rates were not reported in the included studies.

Moreover, the present study evaluated the effects of CFDTD on the levels of reproductive hormones, glucose metabolism, lipid metabolism, and BMI. With regard to the investigation of reproductive hormones, CFDTD exhibited no significant effect on FSH and LH levels, whereas it significantly increased E_2 levels and decreased T levels. However, the results indicated significant heterogeneity. By using sensitivity analysis, it was found that CFDTD exhibited consistent results on FSH, E_2, and T levels. However, the effect size of CFDTD on LH levels was altered following the removal of specific literature studies, and the results were not consistent. Therefore, this conclusion should be treated with caution. Modern medicine has also confirmed that the improvement in the concentration levels of the reproductive hormones alleviates ovulation disorders in PCOS. Hyperandrogenemia is an endocrine characteristic of PCOS. High androgen levels can inhibit E_2 levels, and low E_2 levels can lead to excessive secretion of LH by the pituitary gland. Combined with the negative feedback produced by estrogen, FSH secretion levels are significantly reduced [44]. Due to high levels of androgens and reduced FSH levels, the follicles in PCOS patients stop developing to a certain state, which leads to disorders in follicle maturation and ovulation [45]. Therefore, CFDTD improves abnormal levels of reproductive hormones in PCOS patients by increasing E_2 levels and decreasing T levels, which ultimately improves ovulation disorders.

In the present study, it was also found that CFDTD could improve abnormal glucose tolerance and reduce blood lipids and weight gain. However, the CFDTD group exhibited large heterogeneity in HOMA-IR and BMI. It can also be deduced from the figure that the results of each study reflected the superiority of the experimental group compared with the control group. Since the effect size of each study was different, certain studies were decreased to a higher and a lower extent, which led to the emergence of heterogeneity. Therefore, a sensitivity analysis was not performed on the study group. CFDTD has been applied to patients with phlegm-dampness syndrome and obesity as reported by early studies [15]. Recent studies have confirmed the effects of both obesity and glucose and lipid metabolism on fertility [21, 46]. It has been shown [47, 48] that a 5% decrease in body weight of PCOS patients can reduce fasting blood glucose and serum insulin levels, thereby improving IR. Concomitantly, the reduction in the body weight of the PCOS patients can reduce abdominal fat, decrease serum TG and LDL levels, and increase HDL levels [2]. In addition, weight loss can reduce leptin levels in the body, which inhibits the secretion of ovarian steroids regulated by the neuroendocrine system, ultimately improving the menstrual cycle, promoting ovulation, and increasing the chance of pregnancy. However, in the present meta-analysis, only one study was included that evaluated the effects of CFDTD on blood lipid levels. Therefore, the conclusions regarding the effects of CFDTD on blood lipids should be drawn with caution.

In addition, nine studies mentioned adverse effects. Although these adverse reactions were mild and resolved quickly, the results of the present study indicated that CFDTD significantly reduced the incidence of adverse reactions. This indicates that the safety margin of CFDTD was optimal.

The present study contains certain limitations as follows: (1) a certain risk of bias was noted in the included literature, and the majority of the studies were not fully described in terms of allocation hiding, blind implementation, and selectivity of the research results. Certain risks of selectivity and implementation bias were evident. (2) The sample size of the included literature was small, and the effectiveness of the test was limited. (3) Certain intervention measures were noted in different studies resulting in various confounding factors. Heterogeneity was apparent among studies. (4) All studies were conducted in China and published in domestic journals, which may lead to bias. The aforementioned limitations may reduce the reliability of the meta-analysis. Finally, a larger sample size is required and a multicenter study design to further validate the effectiveness of CFDTD in the treatment of PCOS.

5. Conclusion

In conclusion, the systematic evaluation of CFDTD for the treatment of PCOS indicated that the CFDTD was beneficial to the pregnancy and ovulation rates, while its significance was not clear on the reproductive hormones, glucose metabolism, lipid metabolism, and BMI. In addition, the results of the present study suggested that CFDTD was generally safe. However, due to the low quality of the studies included, we recommend caution in generalizing these results. In the future, additional large-sample, multicenter prospective studies are required to obtain scientific, objective, and reliable conclusions.

Data Availability

The data in this study can be obtained from the corresponding author.

Disclosure

Han Zhang is the co-first author.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

Linling Wu and Han Zhang contributed equally to this work.

Acknowledgments

This work was funded by the Young Scientists Fund of the National Natural Science Foundation of China (82104912), the Tianjin Hashi Gynecology School Inheritance Studio (LPGZS2012-04), the Scientific Research Project of Tianjin Education Commission (2018KJ019), and the Scientific Research Topics of Traditional Chinese Medicine and Integrated Traditional Chinese and Western Medicine (2019058).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7220011, 7 pages
https://doi.org/10.1155/2022/7220011

Research Article

Characteristics of Pulse Parameters in Patients with Polycystic Ovary Syndrome Varied at Different Body Mass Index Levels

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Received 6 January 2022; Accepted 25 March 2022; Published 2 May 2022

Academic Editor: Min Hu

Objective. To analyze the characteristics of pulse graph parameters in patients with polycystic ovary syndrome (PCOS) varied at different body mass index (BMI) levels and to provide pulse diagnosis basis for syndrome differentiation and treatment of PCOS. *Methods*. Pulse graph parameters of 152 patients with PCOS (26 lean patients, 63 patients with moderate weight, and 63 overweight patients) were measured by a Z-BOX pulse meter, and the pulse graph parameters of patients with PCOS varied at different BMI levels were analyzed. *Results*. Fine pulse, slippery pulse, and string-like pulse were the most common pulse conditions in patients with PCOS. The common pulse conditions of patients with PCOS varied at different BMI levels. The order of pulse conditions was as follows: lean group: fine pulse > string-like pulse > slippery pulse; moderate group: fine pulse > slippery pulse > string-like pulse; and overweight group: slippery pulse > fine pulse > sunken pulse. Compared to the overweight group, the pulse graph parameters $h1$, $h3$, $h4$, $h5$, $h4/h1$, As, and Ad increased in the moderate group ($P < 0.05$), and the parameters $h1$, $h3$, and Ad increased ($P < 0.05$) and the parameter t1 decreased ($P < 0.05$) in the lean group. *Conclusion*. Pulse graph parameters among patients with PCOS varied at different BMI levels, which can probably provide pulse diagnosis basis for syndrome differentiation and treatment of PCOS by traditional Chinese medicine (TCM).

1. Introduction

Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disorder, and it is common in females with long-term anovulation, hyperandrogenemia, rare menstruation or even amenorrhea or irregular uterine bleeding, infertility, hirsutism, acne, and other clinical symptoms. Obesity and insulin resistance are also common in patients with PCOS.

Studies have shown that more than 60% of patients with PCOS are overweight or obese [1]. High body mass index (BMI) is not only a risk factor for the occurrence and development of PCOS, but it is also an influencing factor for insulin resistance, spontaneous abortion, pregnancy-related hypertension, low ovarian response, and reduced rate of frozen-thawed embryo implantation in patients with PCOS [2–7]. The relationship between PCOS and BMI has mostly been reported in clinical studies on the association of PCOS with physical and chemical indices, risk factors, clinical phenotypes, and ethnic gene polymorphisms [8–10].

With the development and application of pulse diagnosis in objective clinical assessments, the discussion on the characteristics of pulse graph for PCOS is gradually gaining interest among researchers. According to "Pulse Confirmation (mai que, 《脉确》)," obese people are considered to have a deeply located pulse, while thin people have a superficially located pulse, indicating that obesity and leanness affect the pulse of the human body. In the present study, the Z-BOX pulse meter jointly developed by Shanghai University of Traditional Chinese Medicine (SHUTCM) and Shanghai Asia and Pacific Computer Information System Co., Ltd. was used to obtain pulse graph, and the parameters in the time-domain of pulse graph of patients with PCOS varied at different BMI levels were analyzed to provide an objective basis of pulse diagnosis for clinical syndrome differentiation and treatment of PCOS. A study flowchart is shown in Figure 1.

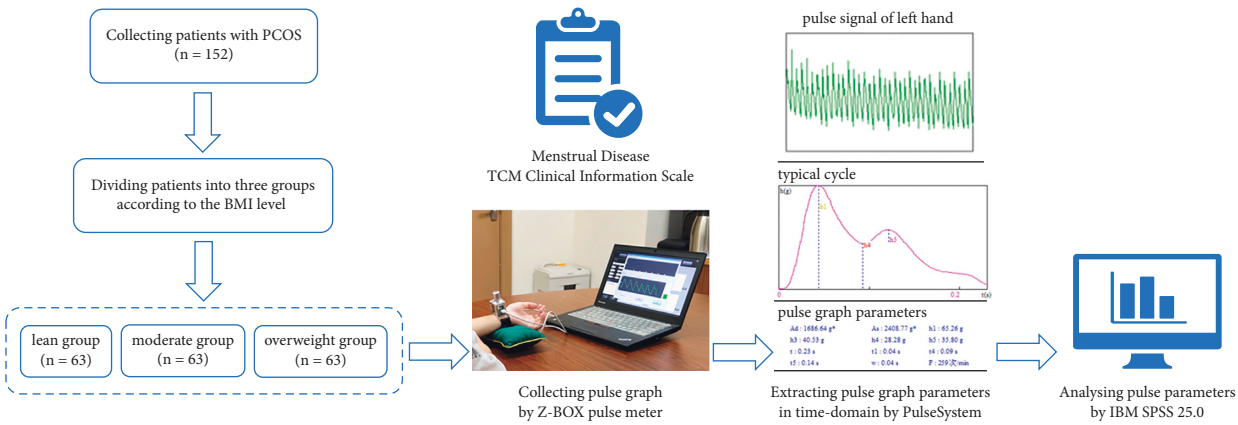

Figure 1: Study flowchart.

2. Methods

2.1. Participants

2.1.1. General Condition of Patients. The study included 152 patients with PCOS who were admitted to SHUTCM and Shuguang Hospital affiliated to SHUTCM from August 2018 to March 2021. According to the guidelines for prevention and control of overweight and obesity in Chinese adults, the patients with PCOS were divided into the lean group (BMI $<18.5\,kg/m^2$, 26 patients), moderate group ($18.5 \le$ BMI $<24\,kg/m^2$, 63 patients), and overweight group (BMI $\ge 24\,kg/m^2$, 63 patients) [11]. The blood pressure of these patients was normal.

2.1.2. Inclusion Criteria. Inclusion criteria for patients with PCOS were as follows: (1) patients were diagnosed to have PCOS according to the Chinese guidelines for diagnosis and treatment of polycystic ovary syndrome (2018) [12], where other diseases that may cause hyperandrogenism and abnormal ovulation were excluded, and the patients presented with sparse menstruation, amenorrhea, or irregular uterine bleeding and one of the following two findings: clinical and/or biochemical hyperandrogenism (HA) or polycystic ovarian morphology (PCOM); (2) the age of the included patients ranged between 18 and 40 years; (3) patients had no other apparent gynecological diseases and organic diseases such as liver or kidney disorder; and (4) patient provided informed consent to participate in this clinical study.

2.1.3. Exclusion Criteria. Exclusion criteria were also based on the Chinese guidelines for diagnosis and treatment of polycystic ovary syndrome (2018) [12]. Patients were excluded from the study if they presented any of the following conditions: patients with adenomyosis, Cushing's syndrome, chromosomal abnormalities, congenital adrenal cortical hyperplasia, and chocolate cyst of the ovary; patients with organic lesions of other organs; patients with significant incomplete clinical data; and patients who refused to cooperate.

2.2. Pulse Collection. The pulse graph information was collected from the guan pulse of the patient's left hand because it pulsates clearly and can be easily detected. The Z-BOX pulse meter was used to collect the pulse graph information of patients with PCOS from 9 AM to 11 AM or from 1 PM to 4 : 30 PM. The patients remained relatively calm and were prohibited to eat, drink, or have violent emotional fluctuations 30 min before the test. The patients assumed the sitting position, and the forearm was naturally spread forward and placed at the same level as the heart. The wrist was kept straight, the palm was maintained upward, the fingers were slightly bent, and a soft pulse pillow was placed under the wrist joint. A series of pulse graphs of three pressure sections within the pulse pressure range of 25–250 g were recorded continuously. The pulse graph for each pressure section was collected for 10 s and recorded continuously for 60 s. The pulse graph with the highest main amplitude, apparent fluctuation of three peaks, and a steep ascending branch without incisure was selected for time-domain parameter analysis. The physiological significance of the pulse graph parameters was determined by referring to "Pulse Diagnosis of Modern Traditional Chinese Medicine" [13].

The pulse graph parameters are shown in Figure 2. Each parameter of pulse graph has its corresponding physiological significance. Parameters $h1$–$h5$ are amplitude parameters and mainly represent the amplitude height. Parameter $h1$ is the main wave amplitude, $h3$ is the tidal wave amplitude, $h4$ is the dicrotic notch amplitude, and $h5$ is the dicrotic wave amplitude. Parameters t–$t5$ are the time value parameters and t represents a complete pulse cycle. On the sphygmogram, $t1$ is the time value from the start point to the crest point of the main wave, $t4$ is the time value from the start point to the dicrotic notch, and $t5$ is the time value from the dicrotic notch to the end point. Parameter w is the width at 1/3 of the main wave. As and Ad are the area parameters: As represents the systolic area and Ad represents the diastolic area. The $h3/h1$, $h4/h1$, $h5/h1$, and w/t are the ratio parameters. Parameter $h3/h1$ mainly reflects vascular wall compliance and peripheral resistance, $h4/h1$ reflects the level of peripheral resistance, $h5/h1$ mainly reflects aortic compliance and aortic valve function, and w/t corresponds to the

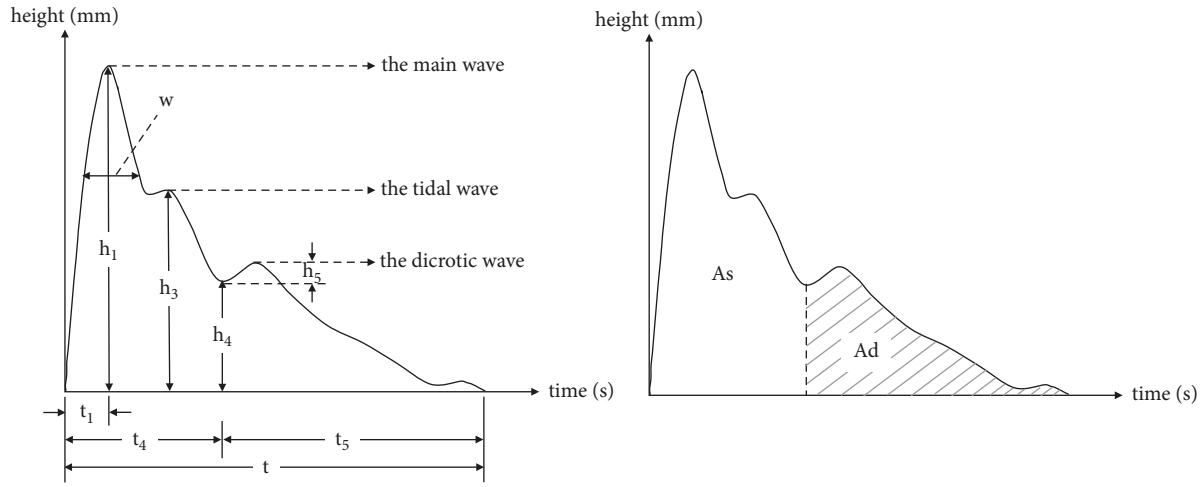

Figure 2: The amplitude, time, and area of the pulse graph.

duration of elevated aortic pressure and is related to peripheral resistance.

2.3. Statistical Analysis. Statistical analysis was performed using IBM SPSS version 25.0. One-way analysis of variance (ANOVA) was used to compare the measurement data that conformed to normal distribution or had an equal variance between groups, while the Bonferroni correction was used for multiple comparison between groups. An independent sample Wilcoxon rank sum test was used to compare the measurement data that did not conform to normal distribution or had an unequal variance. The chi-square test was used to analyze count data between groups. The level of significance was set at $P < 0.05$ for all the analyses.

3. Results

3.1. Age. The age of patients with PCOS is given in Table 1 and Figure 3. The average age of the patients from the lean, moderate, and overweight groups was 25.423 ± 4.12, 27.603 ± 4.563, and 27.635 ± 4.787 years, respectively. One-way ANOVA showed no significant difference in age among the three groups ($P > 0.05$).

3.2. Distribution of Pulse Conditions of Patients with PCOS in the Lean, Moderate, and Overweight Groups. Figure 4 and Table 2 show the pulse conditions of patients with PCOS in the lean, moderate, and overweight groups. Among the 152 patients with PCOS, fine pulse (74.34%) accounted for the highest proportion of pulse condition, followed by slippery pulse (55.92%) and string-like pulse (37.50%). The first three most common pulse conditions were not identical in the different groups of patients with PCOS. According to the order of frequency from high to low, the following trend was noted: lean group: fine pulse > string-like pulse > slippery pulse; moderate group: fine pulse > slippery pulse > string-like pulse; overweight group: slippery pulse > fine pulse > sunken pulse.

3.3. Comparison of the Time-Domain Parameters of Pulse Graph among the Lean, Moderate, and Overweight Groups. Tables 3 and 4 provide comparisons of the time-domain parameters of pulse graph among the lean, moderate, and overweight groups. The pulse graph parameters $t4$, $t5$, t, w, $h4/h1$, and w/t conformed to normal distribution and had an equal variance, and these parameters were analyzed by one-way ANOVA and Bonferroni correction. The pulse graph parameters $h1$, $h3$, $h4$, $h5$, $t1$, As, Ad, $h3/h1$, and $h5/h1$ did not conform to normal distribution or had an unequal variance, and these parameters were analyzed by the Wilcoxon rank sum test. The results showed that compared to the overweight group, the moderate group showed a significant increase ($P < 0.05$) in the pulse graph parameters $h1$, $h3$, $h4$, $h5$, $h4/h1$, As, and Ad, while the lean group showed a significant increase ($P < 0.05$) in the parameters $h1$, $h3$, and Ad and a significant decrease ($P < 0.05$) in the parameter $t1$. No significant differences were observed in the residual pulse graph parameters between the groups ($P > 0.05$).

4. Discussion

Obesity is not only the clinical manifestation of patients with PCOS but also the influencing factor of the PCOS disease process. An increase in BMI can directly or indirectly have a negative influence on the endocrine level, pregnancy complications, and outcomes of assisted reproduction in patients with PCOS [14–17]. A correlation has been found between female BMI and pulse graph parameters [18]. However, to date, no clinical studies have analyzed the characteristics of pulse graph parameters in patients with PCOS varied at different BMI levels.

The results of the present study showed that fine pulse, slippery pulse, and string-like pulse were the most common pulse conditions in patients with PCOS. Fine pulse is mostly related to the deficiency of qi and blood that leads to inadequate filling and inflation of the artery. The formation mechanism of string-like pulse is related to stressful pulse and pulse path, which is caused by liver dysfunction of dredging and deficiency of qi or blood [19]. According to

Table 1: The age of patients with PCOS ($n = 152$).

Variables	Lean group ($n = 26$)	Moderate group ($n = 63$)	Overweight group ($n = 63$)	P value
Average age	25.423 ± 4.12	27.603 ± 4.563	27.635 ± 4.787	0.088
Minimum age	18	19	18	-
Maximum age	32	39	40	-

The data are represented as the mean ± standard deviations.

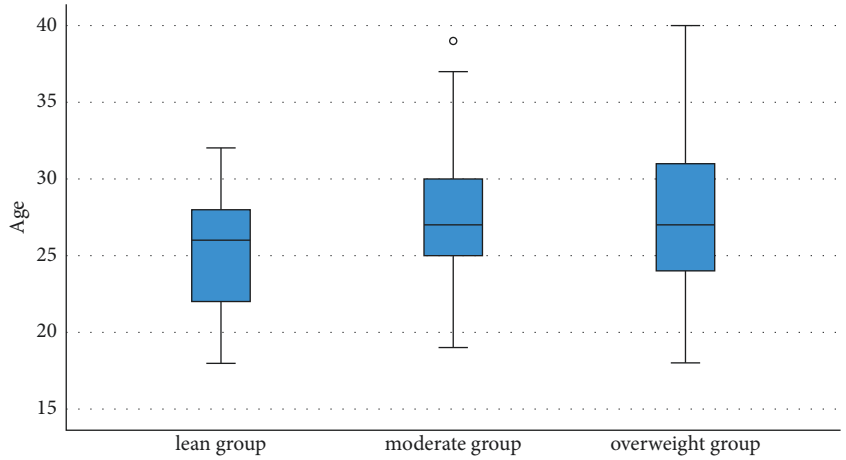

Figure 3: Box plot of age distribution of 152 patients with PCOS.

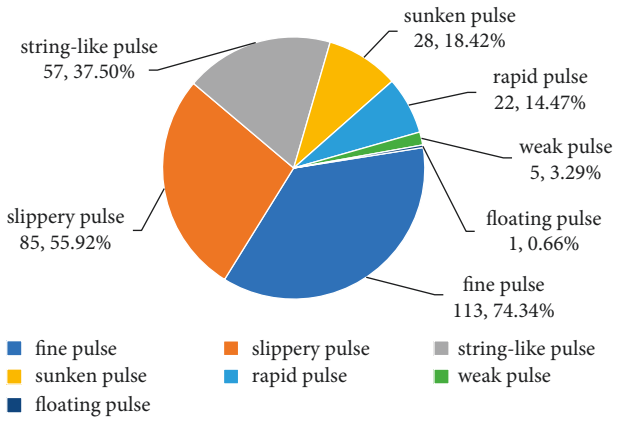

Figure 4: The distribution of pulse condition in 152 patients with PCOS.

TCM, liver dysfunction is common in women with constant depressive mood and deficiency of qi and blood. The cause and mechanism of PCOS are related to the dysfunction of the kidney, liver, and spleen. Kidney heavenly tenth or blood deficiency, depressed liver qi running transversely impairs the spleen function in transportation and transformation, and formation of phlegm-dampness can lead to the development of fine pulse, slippery pulse, and string-like pulse.

This study revealed that the pulse conditions of patients with PCOS varied at different BMI levels. At the same BMI level, the top three pulse conditions were not exactly the same. According to the order of frequency from high to low, the lean group showed fine pulse, string-like pulse, and slippery pulse; the moderate group showed fine pulse, slippery pulse, and string-like pulse; and the overweight group showed slippery pulse, fine pulse, and sunken pulse. The distribution of the same pulse condition in patients with PCOS varied at different BMI levels was also different. According to the proportion of pulse conditions from high to low in different groups, the following trend was observed: string-like pulse: moderate group > lean group > overweight group; fine pulse: moderate group > overweight group > lean group; sunken pulse: overweight group > moderate group- > lean group.

According to TCM, obese people have more phlegm-dampness and deeply located pulse. Slippery pulse and sunken pulse were more common in the overweight group of patients with PCOS and were related to obesity and phlegm-dampness, while string-like pulse was more common in the lean group and was related to stagnation of qi in the liver resulting from impairment of free coursing. However, patients with the proportion of string-like pulse were more likely to have a moderate BMI than a low BMI. It was speculated that this is related to physiological variation. A study found that the formation of physiological string-like pulse was related to the increase in BMI [20]. In the present study, the distribution of fine pulse was not negatively correlated with the BMI level, which may be related to the severity of phlegm-dampness and deficiency of qi and blood in patients with PCOS.

The present study showed that the pulse graph parameters of patients with PCOS varied at different BMI levels. Compared to the overweight group, the moderate group showed a significant increase in the pulse graph parameters $h1$, $h3$, $h4$, $h5$, $h4/h1$, As, and Ad, while the lean group

Table 2: Frequencies of patients with PCOS in lean, moderate, and overweight groups ($n = 152$).

Variables	Lean group ($n = 26$)	Moderate group ($n = 63$)	Overweight group ($n = 63$)
Floating pulse	0 (0.00)	0 (0.00)	1 (1.59)
Sunken pulse	0 (0.00)	3 (4.76)	25 (39.68)
String-like pulse	17 (65.38)	27 (42.86)	13 (20.63)
Slippery pulse	10 (38.46)	30 (47.62)	45 (71.43)
Fine pulse	22 (84.62)	56 (88.89)	35 (55.56)
Rapid pulse	3 (11.54)	7 (11.11)	12 (19.05)
Weak pulse	0 (0.00)	5 (7.93)	0 (0.00)

The data are represented as the frequency (percentage).

Table 3: Comparison of pulse graph parameters among lean, moderate, and overweight groups ($n = 152$).

Variables	Lean group ($n = 26$)	Moderate group ($n = 63$)	Overweight group ($n = 63$)	P value
$t4$	0.072 ± 0.022	0.076 ± 0.020	0.073 ± 0.022	0.520
$t5$	0.151 ± 0.027	0.150 ± 0.036	0.145 ± 0.035	0.637
w	0.061 ± 0.014	0.061 ± 0.016	0.060 ± 0.015	0.861
t	0.219 ± 0.022	0.226 ± 0.035	0.217 ± 0.032	0.276
$h4/h1$	0.299 ± 0.142	$0.332 \pm 0.139^{\Delta}$	0.265 ± 0.150	0.036
w/t	0.280 ± 0.054	0.270 ± 0.051	0.274 ± 0.049	0.651

The data are represented as the mean ± standard deviations. Compared with the overweight group, $^{\Delta}P < 0.05$.

Table 4: Comparison of pulse graph parameters among lean, moderate, and overweight groups ($n = 152$).

Variables	Lean group ($n = 26$)	Moderate group ($n = 63$)	Overweight group ($n = 63$)	P value
$h1$	55.926 (37.068, 66.888)$^{\Delta}$	56.888 (37.202, 65.226)$^{\Delta\Delta}$	40.254 (25.843, 50.723)	<0.01
$h3$	42.753 (32.424, 56.366)$^{\Delta\Delta}$	43.126 (29.512, 52.820)$^{\Delta\Delta}$	30.698 (20.296, 42.191)	<0.01
$h4$	14.412 (8.861, 25.130)	16.843 (11.115, 25.017)$^{\Delta\Delta}$	8.961 (4.894, 17.058)	<0.01
$h5$	21.105 (10.346, 31.799)	25.909 (18.258, 32.548)$^{\Delta}$	18.589 (11.460, 28.332)	0.020
$t1$	0.041 (0.039, 0.044)$^{\Delta}$	0.043 (0.041, 0.046)	0.045 (0.041, 0.049)	0.110
As	1366.125 (921.447, 2114.489)	1736.219 (956.143, 2200.505)$^{\Delta\Delta}$	978.471 (558.252, 1746.957)	0.002
Ad	2097.540 (1335.111, 2787.269)$^{\Delta\Delta}$	2043.218 (1358.543, 2604.711)$^{\Delta\Delta}$	1346.452 (800.123, 2096.211)	0.001
$h3/h1$	0.848 (0.737, 0.901)	0.826 (0.751, 0.869)	0.823 (0.742, 0.862)	0.451
$h5/h1$	0.439 (0.247, 0.545)	0.479 (0.407, 0.550)	0.493 (0.370, 0.624)	0.313

The data are represented as the median (quartile). Compared with the overweight group, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$.

showed a significant increase in the parameters $h1$, $h3$, and Ad and a significant decrease in the parameter $t1$. These findings suggested that the left ventricular pumping function and arterial compliance in obese patients with PCOS were worse than those in nonobese patients, and the peripheral resistance of obese patients was also lower than that of nonobese patients; this may be related to the decline in myocardial contractility and cardiac function compensation in obese patients. Previous studies have shown an independent negative correlation between BMI and myocardial contractility [21], and a decrease in myocardial contractility led to a decrease in left ventricular ejection function. Because obesity can lead to cardiac hypertrophy, the heart plays a compensatory role by increasing physiological circulating blood volume and reducing systemic vascular resistance [22].

Recent studies have revealed a chronic inflammatory state in patients with PCOS. BMI and expression levels of serum inflammatory markers, namely, hypersensitive C-reactive protein, procalcitonin, and interleukin-6, were found to be significantly positively correlated, and obesity may increase the correlation between PCOS and inflammation [23, 24]. The decrease in arterial compliance in obese patients with PCOS may be related to arteriosclerosis caused by chronic inflammation.

In conclusion, the results of this study showed differences in the distribution of pulse conditions and pulse graph time-domain parameters in patients with PCOS varied at different BMI levels. These differences were related to stagnation of qi in the liver resulting from impairment of free coursing, degree of phlegm-dampness, deficiency of qi and blood, myocardial dysfunction, and aggravation of chronic inflammation caused by obesity. The present study had three limitations. First, the differences in pulse conditions between patients with PCOS and normal women under the same BMI level were not compared. Second, the age range of the patients was relatively narrow, and the changes in pulse conditions at different age stages were not analyzed. Third, the sample size was small, and patient distribution among groups with different BMI levels was uneven. In the follow-up study, we will increase the sample size for in-depth research in order to provide an objective basis for the use of pulse graph to guide syndrome differentiation and treatment of PCOS by TCM.

Data Availability

The Ethics Committee of Shanghai University of Traditional Chinese Medicine limited the measurement data used to support the results of this study in order to protect the privacy of patients. For researchers who meet the criteria for obtaining confidential data, the data of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

ZXX and XF provided the research idea and designed the study, LF and XF acquired and analyzed the data, and XF drafted the manuscript. All authors edited and revised the manuscript and approved the final version.

Acknowledgments

This study was supported by the Shanghai Key Laboratory of Health Identification and Assessment (21DZ2271000).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 2112359, 11 pages
https://doi.org/10.1155/2022/2112359

Research Article

Baicalin Attenuates Continuous Activation of β-Catenin Induced by Lipopolysaccharide (LPS) and Depression Complicated by Infertility in Male Rats

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Received 29 December 2021; Accepted 8 March 2022; Published 7 April 2022

Academic Editor: Yong Wang

Background. Baicalin (BA) is a potential candidate drug to inhibit depressive behavior. However, the mechanism of BA's role on depression complicated with male infertility (DCMI) is still unclear. This study aimed to investigate the role of BA in alleviating inflammatory factor-induced DCMI by regulating β-catenin. *Methods*. Firstly, we performed sucrose preference test (SPT), open field test (OFT), tail suspension test (TST), and forced swim test (FST) in the chronic unpredictable mild stress (CUMS) + lipopolysaccharide (LPS) model rats to study the effect of BA on depressive behavior. The levels of neuropeptide Y (NPY), testosterone (*T*), and IL-1β, IL-6, TNF-α, IL-10, and IL-4 in the peripheral blood plasma of normal people, patients with depression, and patients with DCMI were measured. Then, the levels of IL-1β, IL-6, TNF-α, IL-10, IL-4, β-catenin in rat testis and peripheral blood and ANXA2, APP, SEMG1, and SEMG2 in seminal plasma proteins were examined. Moreover, the level of β-catenin in the testicular tissue was detected. LPS was used to treat Sertoli cells, and the level of β-catenin was detected. Finally, we evaluated the reproductive phenotype and sperm motility of rats. *Results*. BA (especially 100 mg/kg) could notably ameliorate depression-like behavior induced by CUMS + LPS. The levels of IL-4, IL-10, *T*, and NPY in depression patients, DCMI patients, and CUMS + LPS model rats elevated, while the levels of IL-1β, IL-6, and TNF-α were reduced. However, BA alleviated the changes in these factors. Moreover, BA alleviated male rat depression induced by CUMS + LPS. LPS upregulated β-catenin (NP) but could not adjust β-catenin (TP) level in rat Sertoli cells. BA relieved the symptoms of DCMI by regulating β-catenin. Furthermore, BA ameliorated the reproductive ability of depressed rats. *Conclusion*. BA modulated β-catenin in the relief of inflammatory factor-induced DCMI.

1. Introduction

Depression is the most common comorbid psychiatric disorder in the world population and in the primary health care [1]. The clinical characteristics of depression are low mood and anxiety, accompanied by internal and mental restlessness [2]. Long-term depression will affect the normal metabolism of the human body. To some extent, it will cause endocrine disorders, affect androgen secretion, hinder the normal movement of sperm, and lead to sterility [3], which is known as depression complicated with male infertility (DCMI). Recently, the relationship between depression and infertility treatment has been in the spotlight. Severe depression can reduce pregnancy rates during infertility treatment with assisted reproductive technology [4]. DCMI is a difficult problem in clinical medicine. Therefore, researchers in this field should continuously address this problem.

Sertoli cells were closely related to the differentiation of germ cells and the targets of follicle-stimulating hormone and testosterone (T), which regulate spermatogenesis [5]. Wnt signaling pathway is indispensable for normal testicular

function [6]. β-catenin is a protein with dual functions in cells, which plays a role in intercellular adhesion and gene transcription through the canonical Wnt/β-catenin signaling pathway [7]. Li et al. reported that the Wnt/β-catenin signaling pathway was related to the proliferation of spermatogonia. Abnormal expression of inflammatory factors plays a role in the pathophysiology of depression [8–10]. At the same time, the Wnt/β-catenin signaling pathway is closely related to inflammation [11, 12]. Therefore, we speculate that the stimulation of Sertoli cells by inflammatory factors leads to the activation of the Wnt/β-catenin signaling pathway, which promotes the incomplete differentiation of Sertoli cells and the exhaustion of germ cells, leading to infertility.

Baicalin (BA) is a flavonoid compound extracted from the root of *Scutellaria baicalensis* without obvious side effects [13]. Previous studies have shown that BA has anti-inflammatory, antioxidant, and antidepressant effects. Dai et al. found that BA protected neurons from cell death and enhanced neural function after cerebral ischemia [14]. Furthermore, BA has been found to attenuate learning and memory impairments induced by global cerebral ischemia/reperfusion, abrogate depressive-like behaviors caused by chronic mild stress, and prevent neuronal apoptosis in chronic unpredictable mild stress (CUMS) rats [15, 16]. Therefore, BA may represent a potential drug candidate for patients with depression [17]. More and more research reveals that BA has a role in treating depression. Studies have shown that BA could inhibit the neurogenesis of the hippocampus in mice through the Wnt/β-catenin signaling pathway, thereby slowing down the depressive behavior of mice [18]. However, the effect of BA on male infertility has not been studied. Therefore, we hypothesized that BA could affect DCMI by modulating the Wnt/β-catenin signaling pathway.

Based on the above studies, to better understand the pathophysiology of depression complicated by infertility, we investigated the mechanism of DCMI in rodents exposed to CUMS and lipopolysaccharide (LPS) at different concentrations of BA.

2. Materials and Methods

2.1. Clinical Sample Collection and Processing. Sixty male patients were admitted from Xiangya Hospital of Central South University from October 2019 to June 2021, aged 20 to 42 years, including 30 cases of depression and 30 cases of DCMI. Thirty normal people came from social health volunteers. Peripheral blood of all subjects was collected. All subjects agreed to provide the information required for the experiment. The experiment was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University (202108383).

2.2. Cell Culture and Processing. Rat Sertoli cells were purchased from Wuhan Procell Co., Ltd (Wuhan, China, CP-R160). Cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin in a constant temperature incubator at 37°C and 5% CO_2. Cells were seeded in two Petri dishes. In one Petri dish, cells were cultured normally (control group), and in other Petri dish, cells were added with 10 μg/mL LPS for 24 h (10 μg/mL LPS group) [19]. After treatment, cells were stored at −20°C for later experiment.

2.3. Animal Modeling and Grouping. Forty adult C57BL/6 male rats (180–220 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. The rats were randomly housed in the cages under a 12 h light/dark cycle (lights on at 7: 00 a.m.), 60% humidity, and temperature at 24 ± 1°C with free access to the water and food. All of the procedures were strictly performed according to the Provision and General Recommendation of the Chinese Experimental Animals Administration Legislation and were approved by Xiangya Hospital of Central South University (Approval No. 202108011).

We performed CUMS on all rats except for the control group [20, 21]. Nine different pressures (water shortage for 20 h, water shortage for 18 h, shroud tilted at 45°C for 17 h, overnight lighting, wet cage feeding for 21 h, swimming in 4°C water for 5 min, stay on a swing bed at 160 Hz for 30 min, tail kneading for 1 min, and fixing for 2 h) were randomly assigned to rats during 42 consecutive days of the day and night. On the 8th day after CUMS, rats were injected intraperitoneally with LPS (500 μg/kg) every other day for two weeks. In the CUMS + LPS + BA (25, 50, and 100 mg/kg) group on the 22nd day after CUMS, rats received different concentrations of BA (25, 50, and 100 mg/kg) every other day for 21 days [22, 23]. The control and CUMS + LPS groups were injected with the same amount of saline as BA. The behavioral test was performed on the 43rd day. After the experiment, the rats were euthanized. The testes, epididymis, peripheral blood, and semen of all rats were collected.

2.4. Depressive Behavior Test. All rats were tested for depressive behavior at the same time. We used the sucrose preference test (SPT), open field test (OFT), tail suspension test (TST), and forced swim test (FST) to assess the degree of depression in rats. First, we performed SPT on rats [24]. Before the test, the rats were housed in the cage with sucrose water (1% (w/v)) and tap water (regular water) to acclimate for 24 h. After acclimatization, the rats were deprived of water and food for 12 h. Subsequently, we reared the rats individually and gave two bottles of sucrose water (1% (w/v)) and tap water for 12 h, alternating the positions of two bottles every 6 h alternately to eliminate the possibility of side or position preference. All SPT measurements were performed at night due to the influence of circadian rhythm on the drinking water of rats. The formula for calculating the sucrose preference (SP) value was as follows: SP (%) = sucrose intake (g)/(sucrose intake (g) + water intake (g)) × 100%.

Then, we performed OFT on the rats [25, 26]. To assess anxiety, we placed the rats individually in the center of an open device, which included a square wooden arena (50 cm × 50 cm × 40 cm), with the bottom divided into 25

black and white square lattices. The number of times each rat crossed the border of the small grid and entered the next grid within 6 min was recorded. The equipment was cleaned with ethanol after each test.

Next, we performed TST on the rats [27, 28]. The rear 1/3 of the tail of the rat was fixed with tape and hung on the bracket, with the head 15 cm away from the table. After acclimatization for 2 min, the immobility time of rats was counted in the next 4 min.

Finally, we performed FST on the rats [29, 30]. Briefly, each rat was placed in a glass cylinder (20 cm high, 14 cm diameter) filled with water ($25 \pm 2^{\circ}$C) to a height of 10 cm. The immobility time of rats after forced swimming for 6 min was recorded after 4 min.

2.5. Sterility Test. First, we evaluated the reproductive phenotype of rats. When the rats were grown to 6 weeks old, one male rat and four female rats with proven fertility were placed in the same cage for one week (T0). The mating plug (coagulated semen) of female rats was monitored daily as a sign of mating behavior. After one week, male rats proved to be fertile were grouped and fed separately according to the above animal experiments. The investigation was also performed according to the abovementioned experimental methods. In the third week (T3) of the experiment, each rat was kept in the same cage with four female rats for one week, and the mating plug of female rats was monitored daily. Similarly, in the 6th week (T6) of the experiment, each rat was caged with four female rats for one week, and the mating plug of female rats was monitored every day.

2.6. Sperm Motility Test. We put an epididymal tail into the 35 mm dishes containing 1.5 mL of bicarbonate buffered human fallopian tube fluid medium, which was supplemented with 3 mg/mL BSA and covered with liquid paraffin. After that, we used a tuberculin syringe with a 26G needle to quickly rupture the epididymis, and then, we gently squeezed the epididymal sperm out of the small tube. Next, we cultured the epididymis in the Petri dish, released sperm in an incubator at 37°C and 5% CO_2 for 30 min, and then we removed the epididymal tissue immediately. The sperm suspension was diluted at 1 : 10 in a pre-balanced medium (37°C). A small amount of suspension was immediately absorbed, added to a preheated slide (37°C), and allowed to stand for 20 s. Then, we immediately used a microscope to evaluate sperm motility at 37°C blindly. At least 200 spermatozoa were counted in each sperm sample. Sperm motility data were recorded as the number of progressive motility sperm of the total number of sperm in the grid × 100.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA). Fresh peripheral blood of humans and rats were centrifuged, the plasma was taken, and the plasma samples were stored at −20°C or −80°C for later experiments. The thawed samples were centrifuged again and then tested. According to the manufacturer's instructions, we used Neuropeptide Y (NPY) ELISA Kit (Cusabio, CSB-E08168h/CSB-E08170m), Serum *T* ELISA Kit (Cusabio, CSB-E05099h/CSB-E05101m), IL-1β ELISA Kit (Cusabio, CSB-E08053h/CSB-E08054m), IL-6 ELISA Kit (Cusabio, CSB-E04638h/CSB-E04639m), TNF-α ELISA Kit (Cusabio, CSB-E04740h/CSB-E04639m), IL-10 ELISA Kit (Cusabio, CSB-E04593h/CSB-E04594m), and IL-4 ELISA Kit (Cusabio, CSB-E04633h/CSB-E04634m) to detect the levels of NPY, *T*, IL-1β, IL-6, TNF-α, IL-10, and IL-4 in human and rat peripheral blood plasma.

2.8. Western Blot (WB). RIPA lysis buffer was applied to extract total protein and nucleoprotein from rat testis tissue. According to the BCA Protein Determination Kit for protein quantification of each group, the protein was separated on a 10% SDS-PAGE gel and then transferred to the PVDF membranes. The membranes were sealed with a 5% skimmed milk solution at room temperature for 2 h, combined with the primary antibodies IL-1β (ProteinTech, 16806-1-AP), IL-6 (Abcam, ab229381), TNF-α (ProteinTech, 17590-1-AP), IL-10 (Abcam, ab271261), IL-4 (ProteinTech, 66142-1-Ig), MIS (Abcam, ab229212), ANXA2 (ProteinTech, 11256-1-AP), APP (ProteinTech, 25524-1-AP), SEMG1 (Abcam, ab139405), SEMG2 (ThermoFisher, PA5-88785), and β-actin (ProteinTech, 51067-2-AP/60008-1-Ig), and incubated overnight at 4°C. β-actin was used as internal reference *l*. TBST was used to wash the membranes three times. Then, the membranes were incubated with the secondary antibodies HRP goat anti-rat IgG (ProteinTech, SA00001-1) or HRP goat anti-rabbit IgG (ProteinTech, SA00001-2) 90 min at room temperature. After using ECL to develop color exposure, the Odyssey Infrared Imaging System was performed to detect protein bands.

2.9. Immunohistochemistry (IHC). The testicular tissue of rats was fixed with paraformaldehyde, then embedded in paraffin, and cut into 4 μm thin slices. The slices were baked at 60°C for 12 h, dewaxed with xylene, and then rehydrated. The slices were immersed in 0.01 M citrate buffer (pH = 6.0) and heated to boiling in a microwave oven. After boiling for 23 min, the slices were cooled to room temperature. To inactivate the endogenous enzyme, 1% periodate was added into the slices for 10 min at room temperature. The slices and primary antibody β-catenin (BIOSS, 51067-2-ap) were incubated overnight at 4°C. Then, the slices were added (50–100μL HRP goat F (ab) anti-rabbit IgG (Abcam, ab7171)) and incubated at 37°C for 30 min. Then, DAB developer working solution of 50–100 μL was added to the slices and incubated at room temperature for 1–5 min. Hematoxylin was used to counterstain the sections for 5–10 min, and PBS was used to return to blue. We dehydrated the slices with various alcohol levels (60–100%) for 5 min. After removal, the slices were placed in xylene for 10 min twice and sealed with neutral gum. Finally, we obtained fluorescence images under a confocal microscope.

2.10. TUNEL. The preparation and hydration process of the slices were the same as before. 100 μL proteinase K working solution was dropped on each slice and reacted at 37°C for

20 min. Then, each slice was dropped with 100 μL 1 × equilibration buffer and incubated at room temperature for 10–30 min. Around the equilibrated area, an absorbent paper was used to wash off most of the 100 μL 1 × equilibration buffer. Then, 50 μL TdT incubation buffer was added to the slices. The slices were incubated at 37°C for 60 min and kept away from the light. Under the dark condition, DAPI working solution was used to stain cell nuclei at 37°C. After 10 min, we used buffered glycerol to seal the slices. The fluorescence microscope was applied to obtain fluorescence pictures.

2.11. Statistical Analysis. SPSS 17.0 software was applied for statistical analysis. Quantitative data were expressed as mean ± standard deviation (±***SD***). *t*-Test was used for comparison between two groups. After the one-way ANOVA, the Student–Newman–Keuls test was performed for comparison between multiple groups, and the rank-sum test was used for comparison between groups of data that did not conform to normal distribution. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. BA Affected Depressive Behavior in Rats. The depressive behavior of rats in the control, CUMS + LPS, and CUMS + LPS + BA (25, 50, and 100 mg/kg) groups was tested, including SPT, OFT, TST, and FST. The results showed that the levels of SPT (Figure 1(a)) and OFT (Figure 1(b)) in the CUMS + LPS group were notably reduced compared with the control group. After the BA treatment, the levels of SPT and OFT in the CUMS + LPS and CUMS + LPS + BA (25, 50, and 100 mg/kg) groups showed significant regression, and the effect of treatment was more obvious with the increase in BA concentration. However, compared with the control group, the levels of TST (Figure 1(c)) and FST (Figure 1(d)) in the CUMS + LPS group were significantly elevated. After the BA treatment, the levels of TST and FST in the CUMS + LPS + BA (25, 50, and 100 mg/kg) groups reduced notably, and the therapeutic effect of 100 mg/kg BA on rats was the most obvious. It could be seen that BA improved the depressive behavior of rats induced by CUMS + LPS. The optimal therapeutic concentration of BA for CUMS + LPS rats was 100 mg/kg, and the therapeutic concentration was dependent.

3.2. BA Reduced Inflammation in DCMI. To investigate the differences in the expression of inflammatory factors in the serum of normal person, patients with depression, and patients with DCMI, we performed ELISA to detect the levels of NPY, *T*, IL-1β, IL-6, TNF-α, IL-10, and IL-4 in the peripheral blood plasma. The results showed that the levels of NPY, *T*, IL-10, and IL-4 in the normal group, depression group, and DCMI group were from high to low (Figure 2(a)). However, the levels of IL-1β, IL-6, and TNF-α were just opposite to the expression trends of NPY, *T*, IL-10, and IL-4. It could be seen that there was inflammation in the patients with depression, and the inflammation was more serious in patients with DCMI. In addition, depression had led to lower NPY and *T* levels in the peripheral blood and caused endocrine disorders and immune disorders.

Next, we treated CUMS + LPS rats with different concentrations of BA and then detected the levels of inflammatory factors in the testis tissue. The WB results are shown in Figure 2(b). Compared with the CUMS + LPS group, BA treatment significantly inhibited the levels of IL-1β, IL-6, and TNF-α in the rat plasma and promoted the levels of IL-4 and IL-10. Finally, we used WB to detect the changes in inflammatory factors, NPY, and *T* in rat plasma. The results are shown in Figure 2(c). The levels of IL-1β, IL-6, and TNF-α were decreased in BA-treated rats. The levels of NPY, IL-10, IL-4, and *T* were increased. It could be seen that BA had a very obvious improvement on DCMI, which was in a concentration-dependent manner.

3.3. BA Reduced the Continuous Activation of β-Catenin Induced by Inflammation. To explore the effects of inflammatory factors on Sertoli cells, we treated Sertoli cells with LPS to induce cell inflammation. Subsequently, we used WB to detect the levels of IL-1β, IL-6, TNF-α, IL-10, and IL-4 in each group. The results showed that compared with the control group, the levels of IL-1β, IL-6, and TNF-α in the 10 μg/mL LPS group were significantly upregulated, while the levels of IL-10 and IL-4 were downregulated (Figure 3(a)). Further, we explored the effects of LPS on β-catenin (nuclear protein, NP; total protein, TP) in Sertoli cells. The results showed that compared with the control group, the β-catenin (NP) level in the 10 μg/mL LPS group was significantly upregulated, and the β-catenin (TP) level was not significantly different (Figure 3(b)). Immediately afterward, we continued to perform WB detection on the testicular tissues of rats. Compared with the control group, the level of β-catenin (NP) was increased in the CUMS + LPS group. After the BA treatment, the level of β-catenin (NP) decreased. However, β-catenin (TP) level was not significantly different in each group (Figure 3(c)). The IHC results are shown in Figure 3(d). Compared with the control group, the level of β-catenin (NP) was significantly inhibited after CUMS + LPS rats were treated with different concentrations of BA. The results indicated that the inflammatory response stimulated the level of β-catenin in the nucleus of the Sertoli cells but could not regulate the total protein level of β-catenin.

3.4. BA Alleviated the Decline of Reproductive Capacity in Depressed Rats Induced by Inflammation. First, we detected the content of MIS (Müllerian) in rat testis tissue (Figure 4(a)). In the CUMS + LPS group, the content of MIS was increased compared with the control group, but after the BA treatment, the content of MIS decreased. Then, we used WB to detect the expression of ANXA2, APP, SEMG1, and SEMG2 in rat seminal plasma protein. The results showed that the expression of ANXA2 and APP in CUMS + LPS rats treated with BA was significantly inhibited, while the expression of SEMG1 and SEMG2 was promoted (Figure 4(b)). Next, we used TUNEL Apoptosis Detection Kit to detect cell

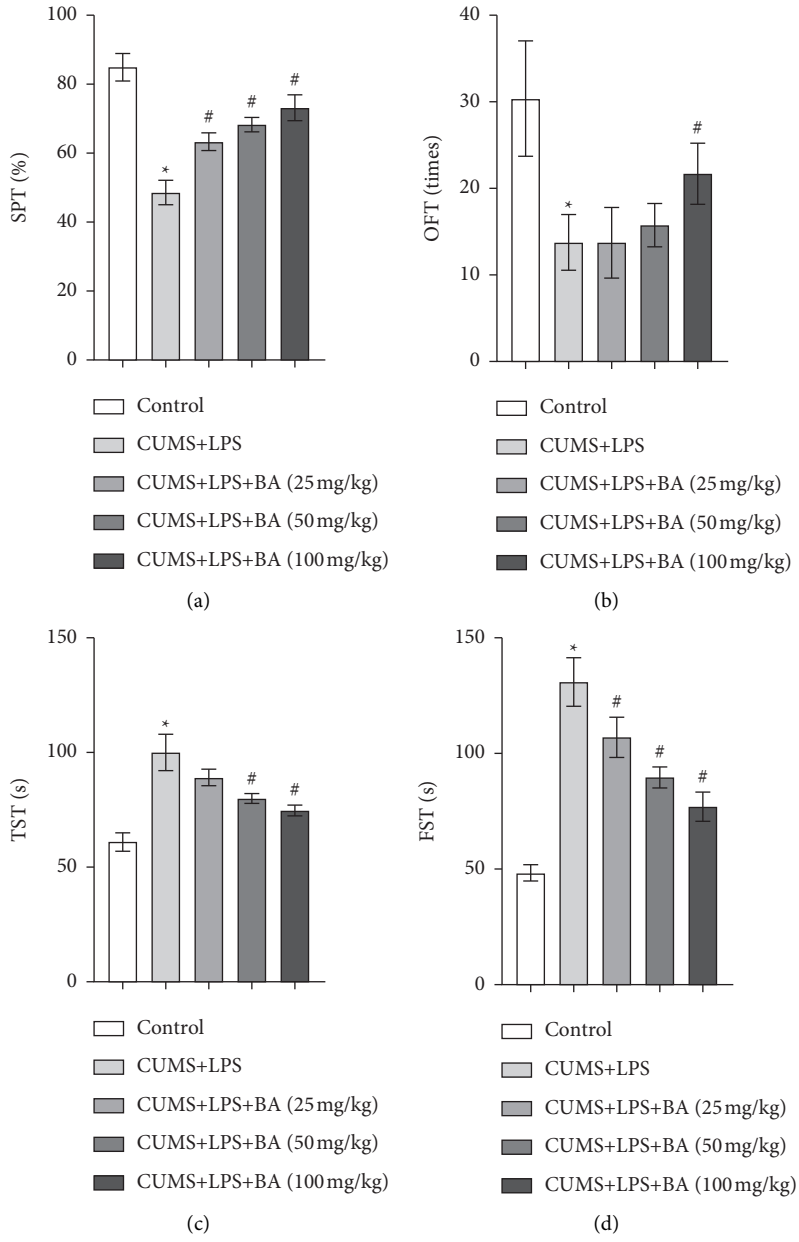


Figure 1: Effects of BA on depressive behavior in rats. (a) Sucrose preference test (SPT) in rats. (b) Open field test (OFT) in rats. (c) Tail suspension test (TST) in rats. (d) Forced swim test (FST) in rats. * $P < 0.05$ vs control group; # $P < 0.05$ vs. CUMS + LPS group.

apoptosis in rat testes. As shown in Figure 4(c), the number of apoptosis in the control group was the least. After treatment with BA, the apoptosis of testicular tissue cells in CUMS + LPS rats was reversed. To detect the effect of BA on the reproductive ability of each group, we evaluated the reproductive phenotype and sperm motility of the rats. The results showed that the number of mating plugs was increased after the BA treatment (Figure 4(d)). Moreover, the pregnancy rate of CUMS + LPS rats was decreased notably. After BA treatment, the pregnancy rate of rats was also increased (Figure 4(e)). There was no significant difference in the pregnancy time of each group (Figure 4(f)). After the BA treatment, the sperm motility of rats was also significantly improved (Figure 4(g)).

4. Discussion

In recent years, the anti-inflammatory effects of BA on the inflammatory diseases have been frequently studied. Many studies have shown that neuroinflammation plays a vital role in depression [31–34]. This research showed that BA showed the antidepressive and infertility effects by reducing neuroinflammation in CUMS + LPS model rats. The underlying mechanism was related to the regulation of β-catenin expression.

Previous clinical studies have revealed that elevated pro-inflammatory cytokines in the central and peripheral nerves might cause depressive behavior [35]. Anti-inflammatory cytokines IL-10 and IL-4 and pro-inflammatory cytokines

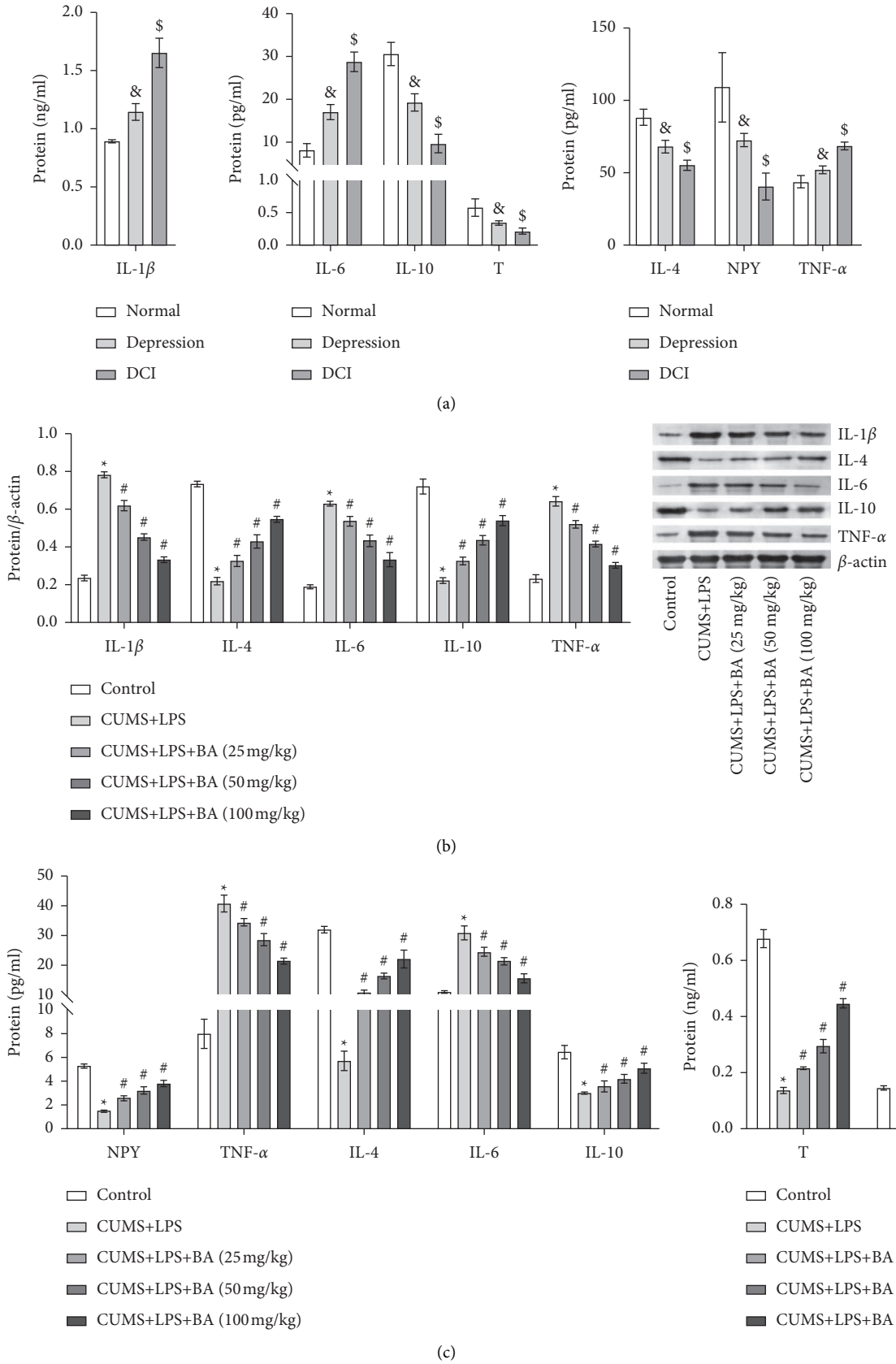


Figure 2: Expression of inflammatory factors in patients with DCMI. (a) The levels of plasma NPY, (t) IL-1β, IL-6, TNF-α, IL-10, and IL-4 in peripheral plasma were determined by ELISA. (b) WB was used to detect the levels of IL-1β, IL-4, IL-6, IL-10, and TNF-α in rat testicular tissue. (c) The serum levels of (T) IL-1β, IL-6, TNF-α, NPY, IL-10, and IL-4 were determined by ELISA. & $P < 0.05$ vs normal group; \$ $P < 0.05$ vs. depression group; * $P < 0.05$ vs control group; #$P < 0.05$ vs. CUMS + LPS group.

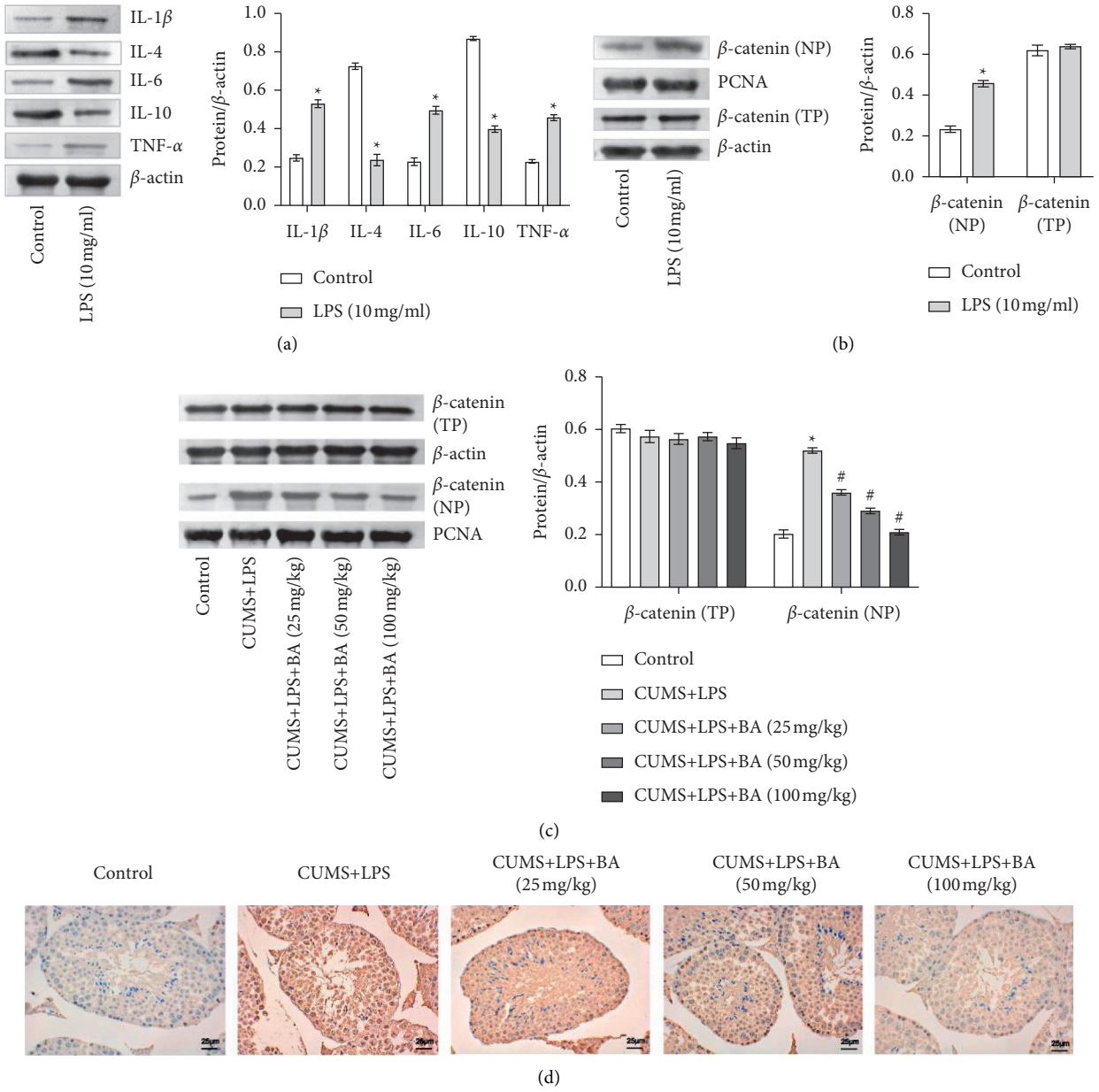
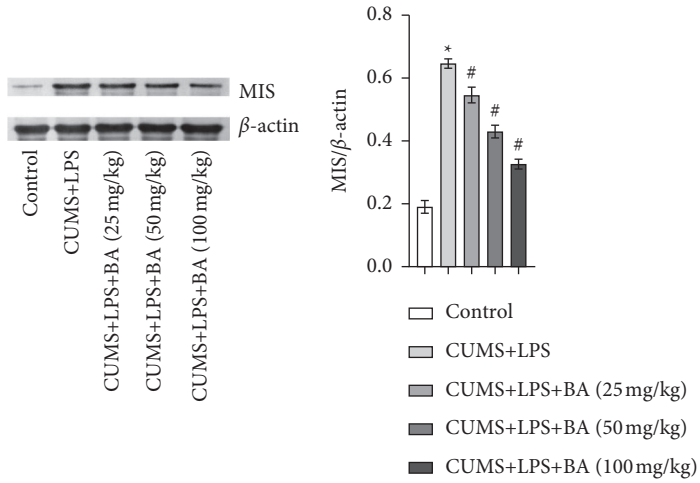
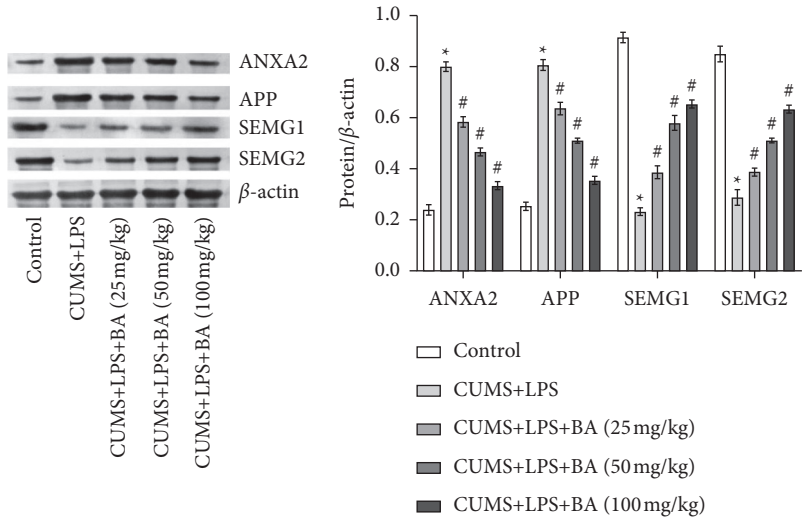


Figure 3: Inflammatory factors affected the expression of β-catenin in Sertoli cells. (a) The expression of IL-1β, IL-6, TNF-α, IL-10, and IL-4 in Sertoli cells was measured by WB. (b) The expression of β-catenin (TP) and β-catenin (NP) in Sertoli cells was detected by WB. (c) WB was applied to detect the expression of β-catenin (TP) and β-catenin (NP) in rat testicular tissue. (d) IHC was performed to detect the expression of β-catenin (NP) in rat testicular tissue. Scale bar = 25 μm. $^{*}P < 0.05$ vs control group.

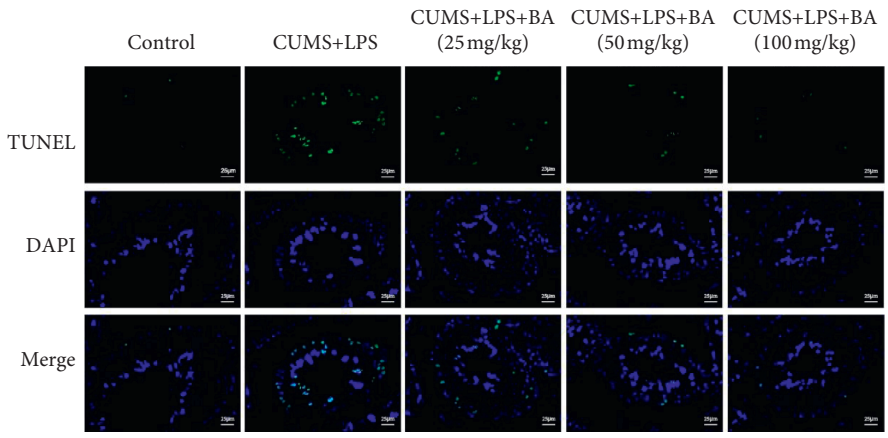
IL-1β, IL-6, and TNF-α play a vital role in the occurrence and development of inflammation diseases [36, 37]. A survey showed that the level of *T* in patients with depression was lower than that in normal people [38]. Furthermore, both preclinical evidence and clinical evidence showed that the low level of NPY directly led to a variety of mental illnesses, including depression and related diseases [39–41]. Our study detected the levels of NPY, *T*, IL-1β, IL-6, TNF-α, IL-10, and IL-4 in the peripheral blood plasma of patients with DCMI. The results showed that the levels of IL-1β, IL-6, and TNF-α were notably elevated in the patients with DCMI, while the levels of NPY, *T*, IL-10, and IL-4 were notably reduced. Therefore, we speculated that patients with DCMI would suffer from endocrine disorders and immune disorders due to the long-term irresistible stress. This led to the low expression of anti-inflammatory factors IL-10 and IL-4 and the high expression of pro-inflammatory factors IL-1β, IL-6, and TNF-α. At the same time, the levels of serum *T* and NPY were higher than those of patients with depression. NPY and *T* might be the effective targets for treating depression and infertility. After that, we treated CUMS + LPS rats with different concentrations of BA. The results indicated that the inflammation in the rat serum and testis tissue has been relieved. At the same time, the levels of NPY and *T* have also



(a)



(b)



(c)

Figure 4: Continued.

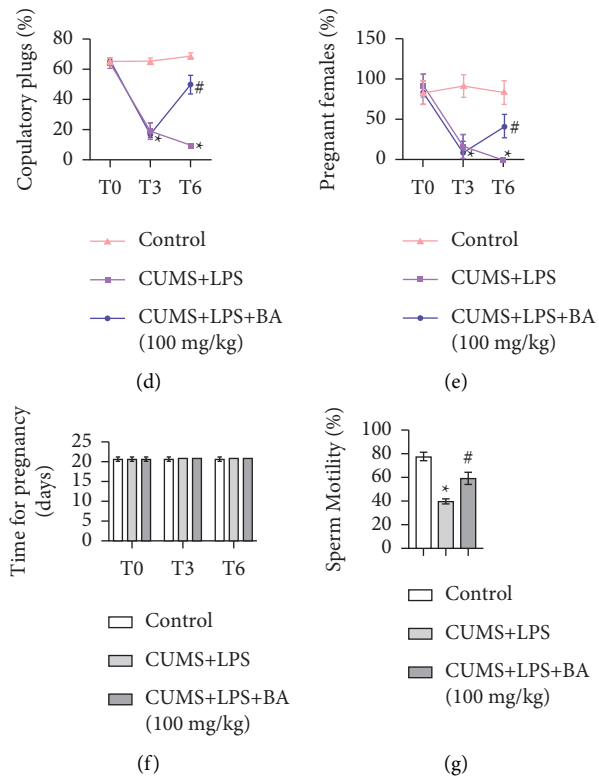


Figure 4: BA alleviated sterility by regulating β-catenin. (a) WB was used to measure the levels of MIS in rat testicular tissue. (b) WB was performed to detect the expression of ANXA2, APP, SEMG1, and SEMG2 in rat seminal plasma proteins. (c) TUNEL was applied to measure the apoptosis rate of germ cells. Scale bar = 25 μm. (d) The number of mating plugs in each group. (e) The pregnancy rate of female rats mated with rats in each group. (f) Pregnancy time of rats in each group. (g) Sperm motility of rats in each group. $^{*}P < 0.05$ vs control group; $\#P < 0.05$ vs. CUMS + LPS group.

been restored to a certain extent. It suggested that BA effectively alleviated the inflammation and immune disorders in depressed and infertile rats.

Studies have shown that BA could slow down neuroinflammatory cells apoptosis by regulating the Wnt/β-catenin signaling pathway [42–44]. Our results indicated that the expression trends of IL-1β, IL-6, TNF-α, IL-10, and IL-4 in Sertoli cells induced by LPS were consistent with the results of our clinical studies. The level of β-catenin (NP) was highly expressed, while the level of β-catenin (TP) was not significantly different. It was known that the entry of β-catenin into the nucleus was its activation symbol, which could enhance β-catenin transcription and increase the level of β-catenin. This indicated that LPS promoted the activation of β-catenin in Sertoli cells. At the same time, we used BA to treat CUMS + LPS rats. The results showed that after BA treatment, the level of β-catenin (NP) was decreased. This showed that BA alleviated depression in rats. Previous report showed that the seminal plasma proteins ANXA2 and APP were overexpressed in secondary infertility, and both SEMG1 and SEMG2 were underexpressed [45]. Pradeep S. Tanwar et al. showed that the expression of AMH/MIS (Müllerian) was a marker of immature Sertoli cells and was regulated by the transcription of β-catenin [46]. In our experiments, BA indeed repressed the expression of ANXA2 and APP in CUMS + LPS rats and promoted the expression of SEMG1 and SEMG2. This indicated that BA improved the infertility of rats. In general, BA might alleviate depression and infertility in CUMS + LPS rats through the β-catenin signaling pathway.

The previous study has shown that BA could alleviate depressive behavior in CUMS-induced mice and protect nerves [44]. Therefore, we also tested the depressive behavior of CUMS + LPS rats. Our results indicated that BA significantly increased the levels of SPT and OFT in rats and reduced the levels of TST and FST. The above results revealed that BA reduced the depression-like behavior of rats. The higher the concentration of BA, the more obvious the effect.

To explore the effect of BA on the infertility symptoms of CUMS + LPS rats, we tested the fertility of rats. The results showed that the fertility of CUMS + LPS rats was significantly reduced. After the BA treatment, the reproductive ability of rats was improved. A semen sample with poor sperm motility was called asthenospermia, which was considered to be one of the main factors leading to male infertility [47]. In this study, the sperm motility of CUMS + LPS rats was decreased notably. After BA treatment, the sperm motility has been improved. All the results showed that CUMS + LPS caused the infertility of rats, while BA treated the infertility symptoms.

5. Conclusion

BA attenuated LPS-induced DCMI by regulating β-catenin expression. In addition, our results for the first time demonstrated the potential mechanism by which BA regulated the expression of β-catenin in CUMS rats.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

All subjects agreed to provide the information needed for the experiment. The experiment was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University (202108383). All of the procedures were strictly performed in accordance with the Provision and General Recommendation of the Chinese Experimental Animals Administration Legislation and were approved by Xiangya Hospital of Central South University (Approval No. 202108011).

Conflicts of Interest

None of the authors have any conflicts of interest to declare.

Authors' Contributions

Rong Fan and Feng Dai designed the experiment, performed the experiments, and wrote the original draft. Chunhu Zhang, Lu Zhou, and Xinjian Qiu analyzed and validated the data. Yunhui Li wrote, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the Laboratory Animal of Xiangya Hospital, Central South University for their technical assistance. This work was supported by the Science Foundation of Hunan Province (2019JJ40511).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 9053930, 15 pages
https://doi.org/10.1155/2022/9053930

Review Article

Complementary and Alternative Medicine for Premature Ovarian Insufficiency: A Review of Utilization and Mechanisms

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Received 30 January 2022; Accepted 18 March 2022; Published 1 April 2022

Academic Editor: Tian-Bo Liu

Premature ovarian insufficiency (POI) is defined as a decline in ovarian function before the age of 40 and is one of the leading causes of infertility in women. The etiology is complex, and the pathogenesis is not clear. The main treatment is hormone replacement therapy, but a growing body of data confirms that such treatment can increase the risk of endometrial disease and cardiovascular disease. Complementary and alternative medicine (CAM) has been widely used in patients with POI due to its limited adverse reactions and high efficiency. According to literature reports, CAM therapy for POI mainly includes traditional Chinese medicine, acupuncture, psychotherapy, dietary supplements, and exercise therapy. This article reviews the application of CAM in the treatment of POI and attempts to determine the therapeutic effects and the mechanisms behind these effects based on existing clinical and experimental studies in order to provide theoretical support for the treatment of POI.

1. Introduction

Premature ovarian insufficiency (POI) refers to a decline of ovarian function before the age of 40 and is one of the major causes of female infertility [1]. It is biochemically characterized by elevated gonadotropin levels and low estradiol (E_2) levels, which lead to menstrual disturbance, induce menopausal symptoms such as hot flashes, night sweats, and insomnia, and increase the risk of decreased bone mineral density and cardiovascular disease [2]. The incidence of POI is approximately 1%–5% [3, 4], and its diagnosis should be based on the presence of menstrual disturbances with biochemical confirmation. According to the guidelines for POI developed by the European Society of Human Reproduction and Embryology (ESHRE), the diagnostic criteria are as follows: age <40 years, oligo/amenorrhea for at least 4 months, and elevated follicle-stimulating hormone (FSH) levels >25 mIU/ml on two occasions >4 weeks apart [5]. The exact etiologies of POI are still unclear, but they appear to include gene factors, iatrogenic causes, autoimmunity, unhealthy lifestyle, and psychological stress [6, 7] (Figure 1). Currently, the main way to treat POI is hormone replacement therapy (HRT), which can ameliorate the clinical complications caused by low estrogen but has no obvious effect on improving ovarian function or fertility [2, 4]. In addition, long-term HRT may increase the risk of endometrial disease, breast disease, and thrombotic disorders [8–11]. Therefore, there is an urgent need to develop better strategies for treating POI.

Complementary and alternative medicine (CAM) refers to various health care and treatment systems that are independent of western medicine. It has the advantages of being natural, convenient, and affordable and is widely used in many countries [12]. A large number of studies have shown that the use of CAM, including Chinese herbal medicine, acupuncture, moxibustion, psychotherapy, dietary supplements, and exercise therapy, can effectively treat POI (Table 1) with fewer adverse reactions. This review summarizes the research progress of CAM in the treatment of POI and analyzes the potential

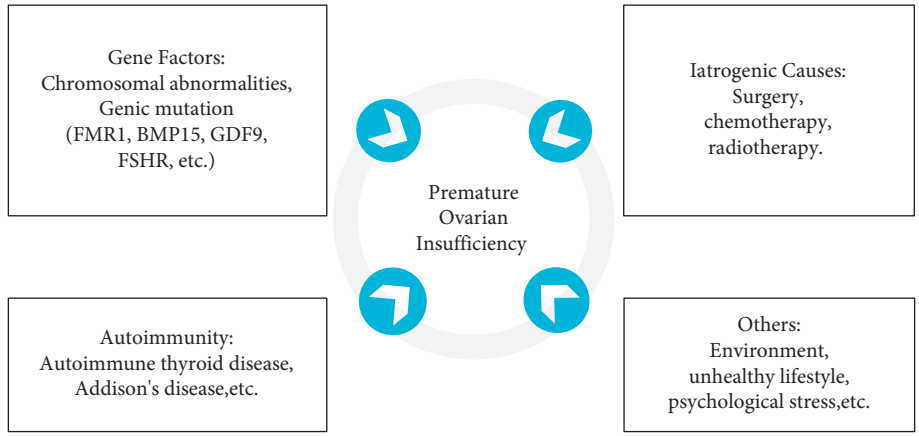

Figure 1: Etiologies of POI.

Table 1: General view of all therapeutic approaches.

Therapeutic approaches	Specifications	Efficacy	Precautions	Refs.
Herbal products	Appropriate TCM prescriptions are proposed according to TCM doctors' judgment.	Improves hormone levels and relieves clinical symptoms in patients with POI.	Allergies to drugs or contraindications.	[13–68]
Acupuncture and moxibustion	The appropriate acupoints or moxa-moxibustion therapy are chosen based on the disease status of the patient. A typical course of acupuncture is about 30 min, a course of electroacupuncture is about 5–20 min, and a course of treatment with moxibustion is about 40–50 min.	Improves hormone levels and relieves clinical symptoms in patients with POI.	Be careful of fainting conditions.	[69–109]
Psychotherapy	Physicians train, educate, and treat patients to reduce or eliminate their physical symptoms, either verbally or nonverbally.	Relieves clinical symptoms and improves hormone levels in patients with POI.	Be alert for the development of psychological resistance.	[110–116]
Dietary supplements	Different dietary interventions are used to regulate bodily functions and promote health.	Improves hormone levels and relieves clinical symptoms in patients with POI.	Adherence to use should be monitored, but this is not to be an alternative to the usual diet.	[117–133]
Exercise therapy	Planned, structured, and repetitive limb activities are used to improve physical health as well as mental health.	Relieves clinical symptoms in patients with POI.	Avoid excessive muscle fatigue.	[134–142]

mechanism in order to provide more accurate evidence for its clinical application.

2. Search Strategy and Selection Criteria

A review of the literature was conducted to investigate the use of CAM to treat POI. We searched the PubMed, Web of Science, Embase, CNKI, and VIP databases using the keywords "Premature ovarian insufficiency," "Traditional Chinese Medicine," "Complementary and alternative medicine," "Herbal Extracts," and "Acupuncture and moxibustion". References from relevant articles published between 1990 and 2021 were analyzed, and references in the identified studies were also searched. The search yielded 2,591 articles, of which 143 articles were deemed potentially relevant. Among them, there were 56 articles on Chinese herbal products, 42 articles on acupuncture and moxibustion, 7 articles on psychotherapy, 17 articles on dietary supplements, and 9 articles on exercise therapy. Articles were excluded due to various reasons, including selection bias, detection bias, reporting bias, and other possible sources of bias.

3. Herbal Products

Herbal products refer to compound preparations composed of two or more herbs that are processed into boiled decoctions, herbal extracts, medicinal gels/jellies, pills, or capsules (patent medicines). Tables 2 and 3 list the most commonly used herbs for treating POI. Their potential mechanisms include (1) inhibiting the secretion of the gonadotropin-releasing hormone through negative feedback

Table 2: Herbal mixtures for POI treatment in the literature.

Herbal mixture; number of patients (n)	Ingredients	Control treatment; number of patients (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Bushen Culuan decoction (BCD); $n = 45$	Tu Si Zi, Yin Yang Huo, Xian Mao, Xu Duan, Gou Qi Zi, Nv Zhen Zi, Ze Lan, Pu Huang, Xiang Fu, Chuan Shan Long	E_2 valerate, clomiphene, and progesterone; $n = 45$	T: 95.35% vs. 88.37%; O: 59.69% vs. 71.32%; P: 27.91% vs. 23.26%	Human study	FSH↓ LH↓ E_2↑ AMH↑ AFC↑ reduced TCM syndrome scores	[13]
Bushen Huoxue Decoction (BHD); $n = 20$	Shu Di Huang, Shan Yao, Shan Yu Rou, Tu Si Zi, Gou Qi Zi, Dang Gui, Bai Shao, Chuan Xiong, Dan Shen, Xiang Fu, Chuan Niu Xi, Gan Cao	HRT; $n = 20$	T: 100% vs. 70%	Human study	FSH↓ LH↓ E_2↑ FSH/LH↓ reduced TCM syndrome scores	[14]
Huyang Yangkun Recipe (HYR); $n = 55$	Huang Qi, Dang Gui, Shan Yao, Shu Di Huang, Xian Ling Pi, Tu Si Zi, Sha Shen	Dehydroepi androsterone; $n = 55$	T: 94.55% vs. 85.45%	Human study	Promoted menstruation recovery. FSH↓ E_2↑ AMH↑	[15]
Erxian Decoction (EXD); $n = 40$	Ba Ji Tian, Xian Mao, Dang Gui, Lu Jiao Shuang, Mu Dan Pi, Zhi Mu, Niu Xi, Huang Bai, Gan Cao, Yin Yang Huo, Chuan Xiong, Shu Di Huang, Nv Zhen Zi, Yi Mu Cao	HRT; $n = 40$	T:95.00% vs. 77.50%	Human study	FSH↓ LH↓ E_2↑ reduced TCM syndrome scores increased quality of life scores	[16]
Huluan Decoction (HLD); $n = 30$	Du Zhong, Tu Si Zi, Fu Pen Zi, Gou Qi Zi, Dang Shen, Huang Qi, Huang Jing, Bai Zhu, Ju Ye, San Qi Hua, Shi Hu, Yu Zhu, Bai He, Shan Yao, Lian Zi, Hei Dou, Ge Gen, Zi He Che, E Jiao, Gan Cao	Femoston; $n = 30$	T: 83.3% vs. 66.7%	Human study	FSH↓ LH↓ E_2↓ FSH/LH↓ AFC↑ ovarian volume↑ reduced TCM syndrome scores	[17]

Note. T (total effect rate) = number of effective cases/total number of cases, where effective case refers to the patients or animal models whose signs and symptoms were improved after treatment; O: ovulation rate; P: pregnancy rate; E_2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AFC: antral follicle counts; AMH: anti-Müllerian hormone; TCM: traditional Chinese medicine; HRT: hormone replacement therapy.

Table 3: Chinese traditional patent medicines for treating POI.

Chinese traditional patent medicine; number of patients (n)	Ingredients	Control sample; number of patients (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Kuntai Capsules (KTC); $N = 50$	Shu Di Huang, Huang Lian, Bai Shao, Huang Qin, E Jiao, Fu Ling	E_2 valerate; $n = 50$		Human study	FSH↓ LH↓ E_2↑ TC↓ TG↓ LDL-C↓ HDL-C↑	[18]
Yulin pill (YLP); $n = 30$	Shu Di Huang, Tu Si Zi, Dang Gui, Chuan Xiong, Bai Shao, Ren Shen, Bai Zhu, Fu Ling, Lu Jiao Shuang, Du Zhong, Gan Cao, Chuan Jiao	HRT; $n = 30$	T: 83.3% vs. 70.0%	Human study	FSH↓ LH↓ E_2↑ EM↑ AFC↑ Reduced TCM syndrome scores	[19]
Bushen Yangjing Granules (BYG)	Shu Di Huang, Dang Gui, Bai Shao, Chuan Xiong, Tu Si Zi, Gou Qi Zi, Che Qian Zi, Wu Wei Zi, Fu Pen Zi, Chuan Niu Xi, Xiang Fu, Zhi Qiao, Dang Shen, Yin Yang Huo, Zhi Mu, Yi Mu Cao	Progynova		Sprague–Dawley rat model	NF-κB↑ MMP-2↑ MMP-9↑ VEGF↑ Caspase-3↓ Caspase-9↓	[20]
Yangyin Shugan Capsules (YSC); $n = 30$	Chai Hu, Yu Jin, Bai Shao, Shan Yao, Di Huang, Fu Ling, Xiang Fu	Placebo; $n = 30$	T: 93.3% vs. 63.3%	Human study	FSH↓ LH↓ E_2↑ reduced TCM syndrome scores	[21]

Note. T (total effect rate) = number of effective cases/total number of cases, where effective case refers to the patients or animal models whose signs and symptoms were improved after treatment; E_2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AFC: antral follicle count; TCM: traditional Chinese medicine; HRT: hormone replacement therapy; VEGF: vascular endothelial growth factor; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: low-density lipoprotein cholesterol; MMP: matrix metalloproteinase; EM: endometrium.

regulation in order to maintain the normal menstrual cycle, (2) increasing the number of primordial follicles, reducing the number of atretic follicles, and promoting the growth of granulosa cells, (3) inhibiting or promoting the expression of related pathways and proteins and changing the apoptotic state of ovarian cells, and (4) reducing the level of proinflammatory cytokines and enhancing immune responses.

3.1. Herbal Decoction Therapies. There are several common decoctions used to treat POI in China, including Bushen Culuan Decoction (BCD), Bushen Huoxue Decoction (BHD), Huyang Yangkun Recipe (HYR), Erxian Decoction (EXD), and Huluan Decoction (HLD). Many clinical and experimental studies have confirmed that Chinese herbal decoctions play an important role in the treatment of POI.

3.1.1. BCD. BCD is an empirical decoction that can restore normal serum sex hormone levels in mouse models of POI, increase the antral follicle count (AFC) and developed follicle count, and decrease the atretic follicle count. It can also downregulate caspase-3 protein expression and upregulate bone morphogenetic protein-7 (BMP-7) protein expression, thereby decreasing the granulosa cell apoptosis rate and maintaining ovarian function. The possible regulatory signaling pathways include the Smad pathway and NF-κB pathway [22]. Two randomized controlled trials (RCTs) showed that after three months of treatment the total effective rate and pregnancy rate of BCD were better than the combination of E_2 valerate + clomiphene + progesterone or the combination of climen + clomiphene [13, 23]. BCD has proven to be safe and effective for clinical use in treating infertility due to POI.

3.1.2. BHD. BHD has been widely used to treat various disorders in China since the Qing dynasty. In traditional Chinese medicine (TCM), most doctors agree that the main pathogenesis of POI is kidney deficiency and blood stasis, so BHD is also extensively applied in treating POI [24]. More and more studies have shown that chronic stress is an important cause of decreased ovarian function and reproductive endocrine disorders [25–27], and BHD can restore serum levels of follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in POI rats induced by the stress hormone corticosterone and can reduce follicular atresia and improve ovarian function. A mechanistic study also showed that BHD might regulate the expression of Np4 and Angptl 4 to improve corticosterone-induced POI [28]. In addition, BHD can also reduce the infiltration of inflammatory cells and the formation of zona pellucida in the ovaries of POI mice, reverse the abnormal sex hormone state, downregulate serum AzpAb levels, activate the Keap1/Nrf2/ARE signaling pathway, and increase the activity of the downstream antioxidant enzymes superoxide dismutase (SOD), heme oxygenase-1 (HO-1), and NAD(P)H: quinone oxidoreductase 1. Therefore, BHD may improve ovarian function in POI mice, reduce oxidative stress, and regulate the immune system through the Keap1/Nrf2/ARE signaling pathway [29,30]. In a clinical study, Zhong et al. [14] selected 40 patients who were randomly divided equally into the treatment group (BHD) and control group (HRT with E_2 valerate and progesterone) for 3 months. The total effective rate of the treatment group was 100.0%, which was significantly higher than 70.0% in the control group.

3.1.3. HYR. HYR was developed based on the theory of TCM as well as clinical experience. According to TCM theory, HYR is mainly used to treat POI due to spleen and kidney deficiency. HYR may slow down the atresia of follicles and enable more follicles to mature by decreasing the overexpression of aquaporins caused by 4-vinylcyclohexene diepoxide in order to regulate the expression of the apoptosis signaling pathway, especially the Bcl-2 family of proteins, in the ovary [31, 32]. Yang [15] treated 110 patients with POI with HYR or dehydroepiandrosterone, and the total effective rates were 94.55% and 85.45%, respectively. At the same time, the menstrual recovery rate of patients with mild and severe menses in the experimental group (HYR) was higher than that in the control group (dehydroepiandrosterone) ($P < 0.05$). Furthermore, transplantation of embryonic stem cells (ESCs) has great potential for improving POI, and studies have confirmed that HYR promotes the treatment effect of ESCs. The combination of HYR and ESCs might promote follicle development by inhibiting the activity of the TGF-β1/TAK1 pathway [33].

3.1.4. EXD. EXD contains curculigo, radix morindae, angelica, and cornu cervi and is a common decoction for treating POI. The beneficial effects of EXD in POI are probably exerted via regulation of the immune system, modulation of estrogen levels, and antioxidative activities, and EXD may act in a synergistic or cooperative manner with other therapeutic agents [34]. Wu and Liu [35] found that EXD enhanced the expression of forkhead protein 3 (FoxP3) in POI model mice and activated $CD4^+CD25^+$ regulatory T cells (Tregs), thereby regulating immune function and preventing the occurrence of POI. After 3 months of treatment with EXD, the total effective rate in the 40 POI patients was 95% compared to 77.5% in the HRT control group, and the EXD group had significantly reduced TCM syndrome scores and significantly increased quality of life scores [16]. Yuan et al. [36] treated POI patients with EXD and reported an overall efficiency of 93.33%.

3.1.5. HLD. HLD is an empirical decoction, including *Eucommia ulmoides*, *Cuscuta*, and other kidney-tonifying Chinese medicines. Eucommia ulmoides can inhibit the expression of T lymphocytes and reduce the level of proinflammatory cytokines so as to regulate immune homeostasis, improve immunity, and treat POI [37]. Shang [38] used HLD to treat immune-induced POI mice and found that it could regulate immunity by restoring the balance of Th17 and Treg cells, thereby promoting follicle formation and improving ovarian function. Deng [39] found that HLD could boost the activity of the SIRT1/NF-κB/p53/

p21 pathway in ovarian cells, thus changing the apoptotic state, increasing the numbers of growing and mature follicles, and reducing the number of atretic follicles. It could also significantly reverse the aging process and improve ovarian function. A random single-blind clinical observation of 60 POI patients showed that the symptoms of 83.3% and 66.7% patients were alleviated in the HLD treatment and the Femoston treatment groups, respectively [17]. HLD thus appears to be effective in treating POI and should be considered for clinical applications.

3.1.6. Other Herbal Decoction Therapies. In China, several herbal mixtures are used to treat POI and have demonstrated positive effects. For example, Yishen Yangluan Decoction can regulate the PI3K/AKT signaling pathway, upregulate the expression of Bcl-2, and inhibit the expression of Bax protein in order to reduce oocyte apoptosis, alleviate the clinical symptoms of POI patients, and improve the ovarian reserve [40]. The results of a Danggui Buxue Decoction intervention in a rat model of POI showed that it might inhibit Foxo3a by upregulating Jak2, thereby mediating Bcl-2 family activities and inhibiting apoptosis in ovarian cells [41]. Moreover, after 6 months of oral treatment with Wumei Pill in 73 POI patients, their ovulation rate and positive pregnancy rate were significantly higher than those in the HRT group (47.3% vs. 39.1% and 29.5% vs. 20.1%, respectively) [42]. Yukun Decoction combined with E_2 valerate tablets in the treatment of kidney deficiency and liver depression associated with POI can reduce FSH and LH levels and increase the level of E_2, the AFC, the thickness of the endometrium, the diameter of the ovary, and the peak systolic velocity [43, 44]. Yijing Decoction has also been shown to be better than western medicine at reducing POI symptoms, and the total effective rate was up to 93.33% in one study compared with 73.33% for Femoston capsules [45]. Because various herbal mixtures are used, only the most commonly used mixtures are discussed in this section.

3.2. Herbal Extract Therapies. Several herbal extracts are commonly used to treat POI in China, including resveratrol, hyperin, and icariin. All of these may affect ovarian cell proliferation, apoptosis, expression of related signal transduction pathways, and local ovarian regulatory factors through multiple pathways, thus playing important roles in the treatment of POI.

3.2.1. Resveratrol. Resveratrol is a natural nonflavonoid polyphenol compound mainly derived from peanut, grape, knotweed, cassia, veratrum, mulberry, and other plants [46]. Studies have confirmed that resveratrol can prevent and treat dementia, osteoporosis, cardiovascular disease, and radiation damage and that it has anti-inflammatory, antitumor, antioxidant, antiaging, and neuroprotective effects [47]. Zeng and Li [48] found that resveratrol can significantly improve triptolide-induced ovarian damage, and Kong et al. [49] found that resveratrol is an effective regulator of ovarian development and oocyte apoptosis. These results indicate that in rat ovaries resveratrol increases the number of resting follicles and total oocytes and reduces the number of developing oocytes and primary follicles. In recent years, studies have shown that SIRT1 can prolong the lifespan of ovaries by inhibiting the apoptosis of oocytes, improving the reserve of primordial follicles, and participating in the regulation of the cell aging process [50], and resveratrol downregulates the expression of Bax, upregulates the activity of SIRT1 and Ku70, and promotes the expression of SIRT1 mRNA to inhibit cell apoptosis [51].

3.2.2. Hyperin. Hyperin is the main flavonoid compound in the kidney-tonifying TCM Cuscutae Semen and is considered to be its main bioactive ingredient [52]. Pharmacological studies have shown that it has antioxidant, antidepressant, and anti-inflammatory effects and has certain therapeutic effects on liver injury, cardio-cerebral ischemia, hypertension, coronary heart disease, and other diseases [53]. Ma and Tan [54] found that after using hyperin gavage treatment for 4 weeks, the body mass and gonadal index of POI mice increased, the pathological damage to the ovary decreased, and the number of follicles at all levels as well as the number of corpora lutea increased. E_2, AMH, SOD, and catalase levels also increased, and FSH levels decreased. At the molecular level, it is suggested that hyperin improves ovarian reserve in tripterygium glycoside-induced POI mice through the Nrf-2/HO-1 antioxidant stress response and the antiapoptotic effect of the PI3K/Akt pathways.

3.2.3. Icariin. Epimedium, also known as Xianling spleen and which was first published in Shennong's *Herbal Classic of Materia Medica*, is a perennial herb in the Berberidaceae family. Flavonoids are the most important active ingredients of Epimedium and have a variety of biological activities, and icariin is one of the most important [55]. Epimedium is often used to treat female infertility, irregular menstruation, POI, and other diseases, and many studies have shown that icariin can increase alkaline phosphatase activity, reduce tartrate-resistant acid phosphatase activity, and stimulate E_2 production [55]. The secretion of E_2 increases, which initiates a feedback loop that inhibits FSH secretion thereby regulating the function of the hypothalamus-pituitary-ovarian axis and promoting the improvement and recovery of ovarian function. In addition, icariin has an estrogen-like effect and can upregulate the expression of estrogen receptors, and it also has a positive effect on the development of rat ovarian follicles [56]. Recent studies have shown that the apoptotic effect of cyclophosphamide on human ovarian granulosa cells depends on the activation of the SMAD/TGF-β pathway, while icariin can effectively block the activation of the SMAD/TGF-β pathway to reduce the apoptosis of these cells. At the same time, icariin can effectively promote the secretion of E_2-β, a key cytokine, by ovarian granulosa cells [57]. Dong et al. [58] found that icariin can increase SOD levels, increase antioxidant capacity, reduce malondialdehyde production, and reduce lipid peroxide content, suggesting that icariin has antioxidant activity. Its activity in

treating POI might be through activation of the PI3K/Akt/mTOR signaling pathway through phosphorylation so as to inhibit oxidative stress-induced ovarian cell apoptosis.

3.2.4. Other Herbal Extracts. Herbal extracts play an irreplaceable role in basic research into POI. For example, quercetin is a flavonoid that promotes follicular development and ovarian granulosa cell proliferation, promotes estrogen secretion, and improves ovarian function in POI mice [59]. Lobetyolin promotes ovarian granulose cells to secrete E_2, and the possible mechanism is through regulation of the CAMP and P38 MAPK signaling pathways, but lobetyolin does not affect granulosa cell proliferation or differentiation [60]. Moreover, black cohosh extract can relieve the perimenopausal symptoms of POI patients such as hot flashes and night sweats [61]. Herbal extracts are a hot topic in the development of Chinese medicine, but more large-scale clinical studies are needed to clarify its more detailed molecular mechanisms and to apply the research results to the clinic so as to benefit a large proportion of POI patients.

3.3. Herbal Patent Medicine Therapies. Herbal patent medicines are herbal products that are processed into various dosage forms based on TCM theory, using Chinese herbal medicines as the raw materials and according to prescribed prescriptions and preparation techniques. Herbal patent medicines have the advantages of stable properties, ease of ingestion, ease of transport, etc., and the most commonly used include Kuntai Capsules (KTC), Yulin Pill (YLP), Bushen Yangjing Granules (BYG), and Yangyin Shugan Capsules (YSC).

3.3.1. KTC. KTC is a commonly used herbal patent medicine for the treatment of POI. It may increase the expression of Smad2/3 and FSH receptors in rat ovarian tissue, thereby increasing ovarian responsiveness to FSH, promoting follicular development, and downregulating the expression of Smad7 in rat ovarian tissue to improve ovarian function [62]. Cui [63] showed that KTC can repair damaged rat ovarian tissue structures, improve ovarian blood supply, promote follicular development, reduce follicular atresia, and increase the number of mature follicles. At the same time, it improved ovarian reserve by increasing the expression of GDF-9 and EGR-1 proteins. Clinical studies have also confirmed that KTC can effectively improve the serum sex hormone levels of patients with POI and can reduce blood lipid levels [18]. A meta-analysis on the effectiveness and safety of KTC in the treatment of POI, including 21 relevant studies covering 1,777 patients with POI, showed that the effective rate and the improvement of serum sex hormone levels were similar to those of western medicine alone. When KTC was combined with western medicine, the effective rate, the Kupperman score, and the improvement in serum sex hormones were better than those of western medicine alone. However, the specific mechanism behind this effect needs further study [64].

3.3.2. YLP. YLP comes from JB Zhang's *Jing Yue's Complete Work* and is often used to treat female infertility and irregular menstruation. In recent years, many studies have shown that YLP has a positive effect on POI patients, and the mechanism might be related to the regulation of mTOR signaling pathway-related genes and their protein expression levels [65]. Yang et al. [66] used a YLP solution to gavage the POI mouse model once a day for six weeks and found that YLP could improve ovarian function by upregulating antioxidant factors and downregulating hypoxia-related factors to improve the ovarian microenvironment and promote follicular development. Another study by their team confirmed that YLP improved oocyte quality by affecting oocytes' mitochondria in POI mice, thus improving ovarian function [67]. An RCT showed that YLP can treat POI more effectively than HRT with a total effective rate of 83.3% vs. 70.0% [19].

3.3.3. BYG. BYG can increase the expression of angiogenesis-related factors (NF-κB, MMP-2, MMP-9, and VEGF), reduce the expression of apoptosis-related factors (caspase-3 and caspase-9), and improve the level of serum E_2 and AMH, thus promoting the formation of ovarian blood vessels and the development of follicles in the ovarian tissue of a POI rat model [20]. BYG treats POI through multiple functions such as participating in angiogenesis, inhibiting granular cell apoptosis, promoting follicular development, and preventing ovarian interstitial fibrosis [68, 69].

3.3.4. YSC. The pharmacodynamics and pharmacokinetics studies of YSC showed that all compounds displayed strong estrogen-like effects and increased endocrine and antioxidant functions through activation of the aromatase and catalase detoxifying pathways. Furthermore, YSC is involved in prolonging antiosteoporotic effects and delaying the aging of the hypothalamus-pituitary-target gland axis [70]. In a clinical study, Cao and Wang [21] used YSC to treat POI with an effective rate of 93.3%. However, the specific mechanism of YSC remains unclear.

4. Acupuncture and Moxibustion Treatment

Acupuncture and moxibustion are methods of preventing and treating diseases and are two of the most ancient practices in TCM. Acupuncture and moxibustion treatments focus on correcting imbalances in Yin Yang and on reinforcing qi and blood to strengthen the body's resistance to and elimination of pathogenic factors. Due to its superiorities, namely, its ease of operation, its low cost, and its satisfactory effect, acupuncture has been used more and more in the treatment of POI. Acupuncture has evolved from the initial forms of acupuncture to now include electroacupuncture, moxibustion, acupoint catgut embedding, acupoint application, auricular point application therapy, transcutaneous electrical acupoint stimulation (TEAS), and acupuncture combined with other therapies [71]. All of these have a positive effect on POI (Table 4). Based on a large number of clinical and animal experiments,

Table 4: Acupuncture for POI.

Treatment; sample number (n)	Control; sample number (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Acupuncture; $n = 30$	E_2 and dydrogesterone tablets; $n = 30$	T: 93.3% vs. 60.0%	Human study	TCM symptom score↓ FSH↓ LH↓ E_2↑ AMH↑ AFC↑	[77]
Moxibustion; $n = 31$	Complex packing E_2; $n = 31$	T: 87.1% vs. 80.65%	Human study	FSH↓ LH↓ E_2↑	[78]
Acupoint sticking treatment; $n = 35$	Vitamin E; $n = 35$	T: 94.29% vs. 71.43%	Human study	FSH↓ E_2↑	[79]
Auricular acupoint treatment; $n = 33$	TCM decoction; $n = 33$	T: 93.75% vs. 81.25%	Human study	TCM symptom score↓ FSH↓ LH↓ E_2↑	[80]
Acupuncture combined with TCM; $n = 86$	Artificial menstrual cycle; $n = 82$	T: 86.05% vs. 71.95%	Human study	TCM symptom score↓	[81]
Acupuncture combined with ginger moxibustion; $n = 20$	Acupuncture combined with uterus-warming moxibustion; $n = 20$	T: 75% vs. 77.78%	Human study	TCM symptom score↓ FSH↓ LH↓ E_2↑	[82]

Note. T (total effect rate) = number of effective cases/total number of cases; effective case refers to the patients or animal models whose signs and symptoms were improved after treatment; E_2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AFC: antral follicle count; AMH: anti-Müllerian hormone; TCM: traditional Chinese medicine.

the different mechanisms of acupuncture treatment for POI include relaxation of the meridians and promotion of blood circulation, regulation of the hypothalamus-pituitary-ovarian axis, improvement of hormone levels, increasing the production of the antiapoptotic factor Bcl-2, and reducing the production of the proapoptotic factor Bax in ovarian granulosa cells [72–76].

4.1. Acupuncture Treatment. Acupuncture refers to using a metal needle to penetrate specific acupoints on the surface of the body and using different techniques to dredge the meridians and collaterals to achieve the purpose of adjusting ying, wei, qi, and blood. Studies have shown the effectiveness of acupuncture and moxibustion in the treatment of POI, and they exert their effects by improving clinical symptoms, slowing down female genital atrophy, regulating hormone levels, and improving pregnancy rates [72, 83]. Wang et al. [84] used acupuncture at different stages in the menstrual cycle to treat POI and showed that it could not only improve the levels of FSH, LH, and E_2 but could also increase the levels of AMH and increase the AFC in patients with POI. Hui et al. [85] randomized 60 POI patients into a treatment group and a control group. The treatment group was treated with E_2 and dydrogesterone tablets combined with acupuncture at Guanyuan (RN 4), Zhongji (RN 3), Mingmen (DU 4), Zigong (CA 1), Shenshu (BL 23), Ciliao (BL 32), Guilai (ST 29), Xuehai (SP 10), Sanyinjiao (SP 6), and Taixi (KI 3), while the control group was only given E_2 and dydrogesterone tablets. After 3 months, they found that acupuncture could increase the efficacy of the medication on improving ovarian function by regulating FSH, AMH, and AFC. Zhuo et al. [77] treated POI with Fang's acupuncture method for regulating menstruation and promoting pregnancy. After three menstrual cycles, the level of FSH was significantly reduced, E_2 was increased, the endometrium became thicker, and the menstrual symptoms were improved, suggesting that the acupuncture therapy was effective in improving ovarian function and low estrogen symptoms in POI patients.

Acupuncture belongs to the field of TCM and thus follows the principle of syndrome differentiation and treatment. Therefore, the selection of acupoints is extremely important when using acupuncture to treat POI. TCM holds that the main pathogenesis of POI is kidney deficiency and liver depression, and the treatment is mainly based on tonifying the kidney and essence, soothing the liver, and relieving depression [86–88]. We summarized and listed the rules for acupoint selection for POI (Table 5).

4.2. Electroacupuncture Treatment. Electroacupuncture therapy is a method to prevent and treat diseases by passing a small amount of current close to the bioelectric currents in the human body through the acupuncture needle, thus adding electric stimulation to the manual stimulation of the needle. Electroacupuncture can adjust human physiological functions, relieve pain, provide sedation, promote blood circulation, reduce muscle tension, and so on [97]. The protective effect of electroacupuncture on the ovary might be by upregulating the expression of the antiapoptotic factor Bcl-2 and downregulating the proapoptotic factor Bax in ovarian granulosa cells [98, 99]. Wang et al. [98] found that electroacupuncture at the Zhongliao (BL 33) and Tianshu (ST 25) acupoints improved ovarian function in POI rats, which may be mediated by regulating insulin-like growth factor 1 receptor (IGF-1R) mRNA expression in ovarian tissue and thus intervening in the IGF-1/IGF-1R axis.

4.3. Moxibustion Treatment. Moxibustion involves burning strips or pillars of wormwood over acupoints on the surface of the skin to warm the meridians and reconcile qi and blood. Xiao [78] sought evidence to confirm moxibustion's effect and randomly assigned 66 POI patients to either the moxibustion treatment group or the Femoston control group. The TCM symptom scores were decreased in the treatment group and were less than those in the control group ($P < 0.05$), and the total effective rate of the moxibustion group was 87.1% compared to 80.6% for the Femoston group. Due to the acupoint effect of acupoints, the

Table 5: The rules of acupoint selection of acupuncture for POI.

Meridian	Acupoints	Refs.
Ren Channel (RN)	Guanyuan (RN 4), Zhongwan (RN 12)	[89, 90]
Du Channel (DU)	Baihui (DU 20), Shenting (DU 24)	[91, 92]
Stomach Meridian of Foot Yangming (ST)	Zusanli (ST 36), Tianshu (ST 25), Guilai (ST 29)	[84, 93]
Shaoyin Kidney Meridian of Foot (KI)	Taixi (KI 3)	[94, 95]
The Spleen Meridian of Foot Taiyin (SP)	Sanyinjiao (SP 6)	[75, 94]
Taiyang Bladder Meridian of Foot (BL)	Shenshu (BL 23), Ciliao (BL 32)	[91, 96]

physical effect of moxibustion, the chemical effect of drugs, and the comprehensive effect of time, it can achieve the effects of tonifying the kidney, warming Yang, regulating menstruation, dispersing cold, and promoting the harmony between Chong and Ren thus attaining a certain clinical effect.

4.4. Acupuncture Combined with TCM. Acupuncture combined with TCM in the treatment of POI is a simple and effective treatment method. Compared with the treatments on their own, this combination can improve the clinical efficacy of POI. Li et al. [81, 100] found that acupuncture combined with TCM can effectively improve sex hormone levels and relieve clinical symptoms better than sequential treatment with estrogen and progesterone alone.

4.5. Acupuncture Combined with Moxibustion. Acupuncture combined with moxibustion can effectively improve the clinical state of POI patients, adjust sex hormone levels, increase the thickness of the endometrium, and increase the number of sinus follicles to promote the recovery of ovarian function [101–103]. Li [82] found that the clinical symptoms were effectively alleviated and the levels of FSH, LH, and E_2 were improved through acupuncture combined with ginger-separated moxibustion at the lumbosacral area and acupuncture combined with warm palace moxibustion in the treatment of POI patients.

4.6. Acupoint Catgut Embedding. Acupoint catgut embedding is a product of modern technology combined with traditional acupuncture methods. This technique exerts long-lasting stimulatory effects on acupoints to prevent diseases by incorporating absorbable surgical sutures into the acupoints, and it has the advantages of easy manipulation, long-lasting effects, and few toxic side effects. It is widely applicable to various diseases [104], and in recent years, the effectiveness of acupoint catgut embedding in the treatment of POI has become increasingly prominent [105, 106]. Li [107] randomly divided 74 POI patients into an acupoint catgut embedding group and an HRT group. The acupoint catgut embedding group was treated with catgut embedding at Ganshu (BL18), Pishu (BL20), Shenshu (BL23), Guanyuan (CV4), Zhongji (CV3), Sanyinjiao (SP6), and Zigong (EX-CA1) once every 15 days for 6 consecutive treatments, while the HRT group was treated with continuous oral estrogen and progestin for 3 months. The final results showed that acupoint embedding had a more sustained effect on the improvement of hot flashes, sweating, sexual dysfunction, menstrual cycle irregularities, menstrual color, and sex hormone levels in POI patients and that the long-term effects of improving hot flashes, sweating, and menstrual color and increasing E_2 levels were superior to those of HRT. Lin's clinical study on the treatment of POI by combining TCM for tonifying the kidney and soothing the liver with acupoint catgut embedding with a treatment period of 3 months had a total effective rate of 95%. After treatment, the endometrial thickness and FSH and E_2 levels were significantly improved. In addition, the results also showed that this combination therapy had few adverse reactions and had a good safety profile [108]. Yang et al. conducted a network meta-analysis of the published literature and found that the efficacy of acupoint catgut embedding is the best among acupoint stimulation therapies, but more randomized controlled trials are needed to verify its efficacy and to promote its clinical application [106].

4.7. Acupoint Sticking Treatment. Acupoint sticking treatment is a noninvasive acupoint therapy in which therapeutic drugs ground into a powder are mixed into a paste or a cake and applied on the skin surface at acupoints to treat diseases. Bao [79] divided the patients into a treatment group receiving acupoint application at Qihai (RN 6), Sanyinjiao (SP 6), and Zusanli (ST 36) and a control group treated with oral vitamin E. Both groups were treated continuously for 6 months and stopped treatment during the menstrual period. The results showed that acupoint application could improve hormone levels, and the total effective rate was higher than that of the control group.

4.8. Auricular Acupoint Treatment. Auricular acupoints are the acupoints distributed on the auricle, also known as reaction points and stimulation points. When the human viscera or body is sick, there will be local reactions in certain parts of the auricle, such as tenderness, nodules, and discoloration, and these reaction points (ear points) can be stimulated to prevent and treat disease [109]. Qi [80] randomly divided 66 patients into a treatment group treated with auricular acupoints based on TCM at the points for the uterus, ovary, kidney, liver, pituitary, hypothalamus, endocrine, and gonadotropin and a control group treated with pure TCM decoction. The patient massaged the ear points with the paste and pressed the ear points 3–5 times a day for more than 20 s each time until the auricle turned red and hot. The continuous stimulation for 3 months was a course of treatment. The results showed that the total effective rate of auricular plaster therapy was higher than that of TCM alone,

and the improvement in FSH and LH in the treatment group was significantly higher than that in the control group.

4.9. TEAS. TEAS is a method that combines transcutaneous electrical nerve stimulation therapy with acupuncture points, and it applies pulsed currents to the acupoints through an electrode sheet placed on the skin surface. Compared with traditional acupuncture, it has the advantages of no trauma, less pain, and easy operation. TEAS can increase the number of antral follicles, improve basic endocrine indicators, and improve ovarian reserve function in POI patients, and it has a similar treatment effect as artificial cycle treatment [110]. The mechanism may be through regulation of the expression of FSH receptors on ovarian granulosa cells to promote oocyte development. More clinical trials are needed to verify its efficacy in the future [111].

5. Psychotherapy

Psychological stress is one of the main causative factors of POI [112], while, in turn, POI patients suffer from tidal fever and night sweating, irregular menstruation, and infertility, which affect their mental health to varying degrees. Psychotherapy is an important auxiliary method in the treatment of POI, and it can have a positive impact on the treatment of POI [113, 114]. From the perspective of modern medicine, psychotherapy might play a role by affecting the brain center that regulates the hypothalamus-pituitary-ovary axis [115]. Li [116] studied the correlation and distribution between TCM constitution, syndrome types, and anxiety and depression in 120 patients with POI. The results showed that anxiety and depression were common in POI patients and that spleen deficiency and liver depression syndrome were the most closely related to depression. TCM treatments focusing on soothing the liver can effectively alleviate anxiety and depression in POI patients [117]. Xiao et al.'s clinical study showed a significantly higher pregnancy rate in POI patients when supplemented with emotional behavior therapy compared with the control group (73.49% vs. 27.71%) [118]. Because most of the current trials on psychotherapy for POI have been small-scale studies, more large-scale trials are needed to prove its efficacy on POI.

6. Dietary Supplements

6.1. Medicated Diet. A Chinese medicated diet is a rational combination of different Chinese drugs taken with food under the guidance of TCM theory and is devised using traditional and modern scientific processing technology. The theory of "medicine and food homology" has been known since ancient times in China, and the medicated diet embodies medicine in food, and it increases not only the efficiency of the medicine but also the nutritive value of food, and it can be used to prevent and cure diseases, build up health, and prolong life [119, 120]. Chinese medicine scholar Professor Han followed the ancient admonition and repeated clinical practice to formulate three kinds of medicated diet prescriptions for patients with POI. The prescriptions are (1) swim bladder, pork, medlar, *Pseudostellaria*, and *Rehmannia glutinosa*, (2) *Curculigo orchioides* Gaertn, *Epimedium brevicornu* Maxim, ginger, and mutton, and (3) medlar, green beans, and *Rana dybowskii*. All three diets can tonify the liver and kidneys, improve ovarian function, and relieve the clinical symptoms of POI [121].

6.2. Vitamins

6.2.1. Vitamin D. Vitamin D, a precursor of several lipid-soluble steroidal hormones, is involved in the regulation of the female reproductive system in addition to its well-known role in regulating the calcium-phosphorus balance of the blood and bone tissues. Its receptors are widely distributed in the reproductive tissues, including the ovary [122, 123]. Studies have shown that the serum vitamin D concentration has a correlation with antral follicle number, and vitamin D deficiency plays an important role in POI patients [123, 124]. Vitamin D may inhibit the premature activation of primordial follicles by upregulating PTEN, thereby maintaining the balance of the follicular pool and avoiding the occurrence of POI. In addition, it may also inhibit the TLR4/NF-κB signaling pathway to inhibit immune responses and reduce the risk of immune-induced POI [122]. It has been shown that women with POI have decreased bone mineral density due to estrogen deficiency; thus, adequate supplementation of calcium and vitamin D is important to avoid the decline in bone mineral density [125]. In addition, lifestyle changes such as regular exercise, cessation of smoking, and avoidance of excessive alcohol intake are recommended [126].

6.2.2. Vitamin E. Vitamin E is a lipid-soluble antioxidant with α-tocopherol as the major form, and it can effectively reverse the adverse effects of oxidative stress on the reproductive system [127]. Increased accumulation of reactive oxygen species during oogenesis is one of the most well-known causes of ovarian insufficiency and decreased ovarian reserve [127–129], and vitamin E, as a cofactor of glutathione peroxidase, plays an important role in scavenging reactive oxygen species in the ovary [130]. Ma et al. found that vitamin E levels are significantly lower in women with POI than in women with normal menstrual cycles [131], and a recent study showed that supplementation with selenium and vitamin E can increase the level of AMH in women with occult POI and can increase the number of antral follicles and the ovarian volume [132].

6.2.3. Vitamin C. Vitamin C (ascorbic acid) is an essential nutrient with an antioxidant effect and can reduce the risk of chronic diseases, and it must be consumed regularly to prevent deficiency [133]. An experiment conducted by Hou et al. showed that vitamin C promoted the proliferation, migration, self-renewal, and paracrine functions of human amniotic epithelial cells in vitro, thus demonstrating the therapeutic potential of these cells in treating POI [134]. Nowadays, many regulatory authorities have increased the recommended intake of vitamin C in their respective countries [135].

7. Exercise Therapy

Exercise is a type of physical activity that uses planned, structured, and repetitive limb activities to improve the function of the body as well as the mental health of women with POI. The application of exercise and psychotherapy in POI patients is of great value and can improve the level of sex hormones and clinical symptoms [136, 137]. Women with POI experience significant deterioration in musculoskeletal health due to loss of the protective effects of estrogen, and this deterioration can be delayed or prevented through exercise and resistance training [138, 139]. Additionally, studies have shown that elevated levels of inflammatory cytokines play a critical role in POI [140, 141], and physical activity can reduce the markers of a proinflammatory state [142]. Thus, regular exercise is beneficial for patients with POI. By searching the literature, we also found that some other training methods may also be beneficial to POI patients. For example, Tai Chi, a unique Chinese exercise, can prevent osteoporosis by strengthening the muscles of the lower extremities and can help maintain a sense of balance [143]. Yoga, as a popular physical and mental training method, can alleviate mild vasomotor symptoms and improve sleep [144].

8. Conclusions

POI refers to a decline in ovarian function before the age of 40 and is characterized by menstrual disturbances, infertility, and varying degrees of menopausal symptoms. CAM for POI mainly includes herbal products, acupuncture and moxibustion, psychotherapy, dietary supplements, and exercise therapy. Through these therapies, the levels of sex hormones in POI patients can be improved, menstrual cycles can be resumed, menopausal symptoms can be alleviated, pregnancy rates can be improved, and anxiety and depression can be relieved, thereby improving the quality of life of these patients. In conclusion, the active principle of CAM therapies has a strong scientific foundation, and researchers have shown increased interest in this area of medical treatment. Due to the small sample sizes of most studies, however, it is currently difficult to make clear recommendations for clinical guidance. Larger samples and RCTs are needed in the future to verify the efficacy and safety of CAM for the treatment of POI and to provide new strategies for treating POI.

Disclosure

Yang Fu and Dan-Ni Ding are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Yang Fu and Dan-Ni Ding contributed equally to this work.

Acknowledgments

This study was funded by Luo Yuankai Zishen Yutai Pill Young and the Middle-Aged Scientific Research Fund Project of China (20190801).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3616036, 15 pages
https://doi.org/10.1155/2022/3616036

Review Article

Effects of Acupuncture Combined with Moxibustion on Reproductive and Metabolic Outcomes in Patients with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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Received 2 December 2021; Accepted 19 March 2022; Published 31 March 2022

Academic Editor: Yue Hui Zhang

Objectives. In this systematic review, the effects of acupuncture combined with moxibustion on reproductive and metabolic outcomes in patients with polycystic ovary syndrome (PCOS) were evaluated. *Methods*. Randomized controlled trials (RCTs) assessing acupuncture combined with moxibustion + basic treatment (experimental group) versus basic treatment alone (control group) for treating PCOS were identified from English and Chinese databases up to November 3, 2021. Outcomes related to pregnancy, ovulation, miscarriage, sex hormones, and metabolic disorders were of interest. In the meta-analysis, risk ratios (RRs) and mean differences (MDs) and their 95% confidence intervals (CIs) were used as effect measures. *Results*. Twenty-five RCTs ($n = 1991$) were included. The pooled results showed that the experimental group had significant increases in the pregnancy rate (RR 1.81, 95% CI 1.58 to 2.08) and ovulation rate (RR 1.31, 95% CI 1.22 to 1.40) and decreases in the miscarriage rate (RR 0.45, 95% CI 0.28 to 0.73), and ovarian volume (MD −0.75 cm^3, 95% CI −1.30 to −0.20). In the experimental group, improvements in the luteinizing hormone (LH) level, the LH-to-follicle-stimulating hormone (FSH) ratio, total testosterone level, fasting insulin level, and body mass index, but not in FSH, oestradiol, or dehydroepiandrosterone sulfate levels, were significantly greater. All reported adverse events were mild. Based on the limitations of risk of bias, inconsistency, imprecision, and/or publication bias, the level of evidence was judged to be moderate for the pregnancy rate, ovulation rate, miscarriage rate, LH level, and LH/FSH ratio and very low for the other outcomes. *Conclusion*. Among patients with PCOS, using acupuncture combined with moxibustion as a complementary therapy to basic treatments can improve pregnancy, ovulation, and miscarriage rates, the levels some sex hormones, and metabolic indicators, with good safety. Additionally, this combination therapy may have no effect on the FSH, oestradiol, or dehydroepiandrosterone sulfate level.

1. Introduction

Polycystic ovary syndrome (PCOS), an endocrinopathy, is the most common cause of reproductive impairment in women of reproductive age. In their consensus conference in 2003, the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) defined the PCOS as meeting at least two of the following three criteria, namely, hyperandrogenism, hypo-ovulation/anovulation, and polycystic ovaries, and these are currently the most universally accepted diagnostic criteria [1]. Globally, in 2017, the age-standardized incidence of PCOS globally was 82.4 per 100,000, representing a 1.45% increase compared to 10 years prior [2], and the total prevalence of PCOS was estimated to be 5–15% [3]. The predominant negative effect of PCOS is that it causes reproductive disorders; 70–80% of patients with PCOS develop anovulatory infertility [4], and patients with PCOS are also more likely to experience miscarriages (16 versus 5.3% among healthy controls) [5]. PCOS also causes amenorrhea, acne, and hirsutism and is associated with metabolic diseases related to insulin resistance (e.g., diabetes and central obesity) [6, 7].

PCOS is not curable to date, and there are no specific drugs approved for PCOS. Therefore, clinical treatment for PCOS is symptom-oriented. For anovulatory infertility,

treatment options include drugs, surgery, and in vitro fertilization. Unfortunately, these treatments are limited by uncertain efficacy, side-effect concerns, and high costs [6]. A 6-month course of clomiphene, a first-line drug for ovulation induction, is associated with a pregnancy rate of only 67% and may cause side effects such as flushing, headache, visual disturbances, and abdominal discomfort [8]. The natural pregnancy rate after ovarian drilling is 54–70%, whereas surgery is invasive and may cause premature ovarian insufficiency [9]. In vitro fertilization is expensive, and patients undergoing this treatment carry a risk of ovarian hyperstimulation syndrome [10]. In addition, the long-term use of cyproterone, a commonly used androgen receptor blocker against hyperandrogenemia, may even cause fatal pulmonary embolism [11]. Therefore, there is a need to seek more complementary and alternative therapies for the treatment of PCOS, particularly with regard to both efficacy and safety.

In China, acupuncture is prevalent as a complementary and alternative therapy. It is performed by inserting filiform needles into the skin to stimulate specific acupoints; additionally, pairs of needles can be connected with continuous electrical pulses to enhance stimulation, a procedure known as electroacupuncture. Acupuncture has been used to treat endocrine and metabolic diseases such as obesity and diabetes (and its consequent neuropathy), and its efficacy for treating these diseases is established [12–14]. Notably, acupuncture is widely used to treat PCOS, but a recent large factorial trial (the PCOS Acupuncture and Clomiphene Trial, PCOSAct) negated both the reproductive and metabolic benefits of acupuncture for patients with PCOS [15]. The study generated considerable controversy after publication, especially because many acupuncturists believed that the reason why acupuncture was ineffective was that the regimen was not optimal [16, 17].

For instance, in clinical practice, specifically in the acupuncture and moxibustion departments of Chinese hospitals, acupuncture is often administered in combination with moxibustion to achieve better efficacy. Moxibustion is also an acupoint stimulation therapy that is performed by igniting a moxa stick and placing it on the acupoints to deliver thermal stimulation. There are two strategies for combining acupuncture and moxibustion: (1) warm-needle acupuncture, namely, attaching a burning moxa cone to the needle after insertion; and (2) the separate application of acupuncture and moxibustion, wherein different forms of moxibustion, such as suspended moxibustion, herb-separated moxibustion, and heat-sensitive moxibustion, can be performed. The combined use of acupuncture and moxibustion has been shown to have advantages over acupuncture alone in treating multiple diseases, such as knee osteoarthritis [18], low back pain [19], and stroke management [20].

To date, there is no lack of animal experiments or systematic review evidence on the efficacy of acupuncture or moxibustion for PCOS [21–23]. A number of randomized controlled trials (RCTs) of acupuncture combined with moxibustion for treating PCOS in humans have also been published, but their conclusions have been inconsistent. Considering that these RCTs were predominantly small studies, some of the negative results may be due to type 2 error caused by an insufficient sample size. Therefore, we conducted a systematic review of currently available RCTs to draw more precise conclusions via meta-analysis techniques to inform clinical practice regarding the use of acupuncture combined with moxibustion to treat PCOS.

2. Materials and Methods

This systematic review was developed with the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement [24], and its protocol was prospectively registered in the PROSPERO database with the number CRD42021284400.

2.1. Literature Search. We searched seven literature databases (PubMed, EMBASE, Cochrane Controlled Register of Trials, CNKI, Wanfang, VIP, and Chinese Biomedical Literature), two preprint platforms (MedRxiv and bioRxiv), and two clinical trial registration databases (Clinicaltrials.gov and Chinese Clinical Trial Registry) to identify RCTs published from database inception to November 3, 2021. Keywords related to PCOS, acupuncture, and moxibustion were used, without limitations on language or publication status (see details in Table S1 in Supplementary Materials). Relevant narrative reviews and systematic reviews were examined to identify additional studies.

2.2. Eligibility Criteria. Parallel-group RCTs were included if they investigated the association of the combined use of acupuncture and moxibustion with PCOS. The diagnosis of PCOS could be based on any criteria consistent with the ESHRE/ASRM 2003 consensus [1]. Eligible interventions were manual acupuncture or electroacupuncture combined with moxibustion, regardless of how these two interventions were performed. Studies of thermotherapy not involving moxa materials, such as infrared laser moxibustion, were excluded. The comparison had to be between (1) acupuncture and moxibustion + medications for PCOS and the same medications; or (2) acupuncture and moxibustion and lifestyle/no intervention. Acupuncture and moxibustion were not allowed in the control group. Studies involving other acupoint stimulation therapies in either group, such as acupressure, cupping, and acupoint catgut embedding, were excluded. Conference abstracts, repeated reports, and literature lacking the complete data required for meta-analysis were also excluded.

2.3. Outcomes. The primary outcomes were defined as the pregnancy rate after the treatments, counting both biochemical and clinical pregnancies and using the number of patients with a need for pregnancy as the denominator. The secondary outcomes included ovulation rate, miscarriage rate, change in ovarian volume, change in hormones (luteinizing hormone [LH] level, follicle-stimulating hormone [FSH] level, LH/FSH ratio, total testosterone level,

oestradiol level, and dehydroepiandrosterone sulfate [DHEAS] level), change in fasting insulin level, change in body mass index (BMI), and incidence of adverse events.

2.4. Literature Screening and Data Extraction. Literature screening and data extraction were independently performed by two reviewers. They first checked titles and abstracts to discard irrelevant records and then read the full texts of the remaining studies to determine which would be included. Baseline characteristics (sample size, sex proportion, age, and duration of disease), treatment details (type, dose, and course), and outcome data were extracted from each included RCT. If there were outcome data for multiple time points, we retained only the data from the last follow-up visit. For crossover trials, we utilized the data only before its wash-out period. A third reviewer resolved disagreements during these processes.

2.5. Risk-of-Bias Assessment. We used a modified version of the Cochrane risk-of-bias assessment tool for RCTs [25]. The domains assessed were the same as the original scale, including random sequence generation, allocation concealment, blinding of patients and physicians, blinding of assessors and analysts, data completeness, selective reporting, and other potential for risk of bias. In addition to making "yes (low risk)" and "no (high risk)" judgments for each domain, the tool also requires a reasonable inference of "probably yes" or "probably no" for "unclear" items based on relevant information in the reporting. The overall risk of bias in the included RCTs was graded as low, moderate, or high based on the following criteria: (1) overall low risk indicated that all items were judged as yes or probably yes, in which the unblinding of patients and physicians was allowed because it is impossible in this topic; (2) overall high risk indicated that more than three items were judged as no or probably no; and (3) overall moderate risk included the cases that did not fit the above two sets of criteria. The assessment was independently performed by two reviewers, and disagreements were resolved by a third reviewer.

2.6. Statistical Analysis. We performed random-effects meta-analyses to incorporate data from individual RCTs. Effects on binary outcomes were measured by means of risk ratios (RRs) and pooled by the Mantel–Haenszel method, in which 0.5 was added to the zero events. Effects on continuous outcomes were measured by mean differences (MDs) and pooled by the inverse variance method, in which missing standard deviations in the change from baseline assessment were imputed by the Cochrane handbook method with a correlation coefficient of 0.5. Confidence intervals (CIs) and prediction intervals were also calculated to assess the imprecision of effect estimates.

The heterogeneity between summary data was tested using Cochran's Q test and the I^2 statistic. A P value in the Q test of less than 0.10 or an I^2 of greater than 50% represented statistically significant heterogeneity [26]. To identify the source of heterogeneity, we planned the following subgroup analyses with an anticipated effect direction [27]: (1) type of cointervention: Western medicine + Chinese herbal medicine (anticipated to be the best option) versus Western medicine versus Chinese herbal medicine versus lifestyle intervention; (2) type of acupuncture: electroacupuncture (anticipated to be the better option) versus manual acupuncture; (3) type of moxibustion: warm-needle moxibustion (anticipated to be the better option) versus other forms; and (4) course of treatment: ≥3 months (anticipated to be the better option) versus <3 months.

Sensitivity analyses were performed to test the robustness of the estimates, including the exclusion of studies with a high overall risk of bias, studies that counted the number of menstrual cycles rather than the number of patients in the analysis of ovulation rate, and studies using a fixed-effect model for meta-analyses with nonsignificant heterogeneity. If there were 10 or more studies included in a meta-analysis, we detected publication bias by drawing funnel plots and conducting the Thompson test (for binary outcomes) or Egger's test (for continuous outcomes). The "meta" package in *R* version 4.0.2 (Ross Ihaka, Robert Gentlemen, New Zealand) was used for the statistics and plotting.

Finally, we evaluated the level of evidence for each outcome using the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach and the Confidence in Network Meta-Analysis (CINeMA) guidelines [28]. As the meta-analyses were conducted based on RCTs, the initial level of evidence was high. If the meta-analytic results suffered from within-study bias, inconsistency, imprecision, indirectness, or publication bias, the level of evidence was downgraded to moderate, low, or very low according to the guidelines.

3. Results

3.1. Study Screening. The search yielded 2768 records, and 25 RCTs [29–53] were ultimately included in the systematic review after screening (Figure 1). One of the RCTs [31] had two independent pairs of comparisons, which we treated as two studies in the data analysis.

3.2. Characteristics of the Included Studies. The basic characteristics of the included RCTs are compiled in Table 1. In summary, a total of 1991 patients were enrolled in the RCTs, with a sample size ranging from 60 to 200 and a mean age of 25.3 to 34.5 years. The mean course of PCOS ranged from 1.43 to 4.94 years. Electroacupuncture was applied in 5 trials, and the remaining 21 trials used only manual acupuncture. The type of moxibustion was warm-needle moxibustion in 16 trials, suspended moxibustion in five trials, moxibustion box in three trials, and ginger-separated and thunder-fire moxibustion in one trial each. The cointervention in both groups was Western medicine in 16 trials, Chinese herbal medicine in 6 trials, Western medicine + Chinese herbal medicine in 2 trials, and lifestyle intervention in 1 trial. The course of treatment was ≤3 months in 17 trials >3 months in five trials and unclear in the rest. The mean baseline LH and total testosterone levels were higher than the normal range

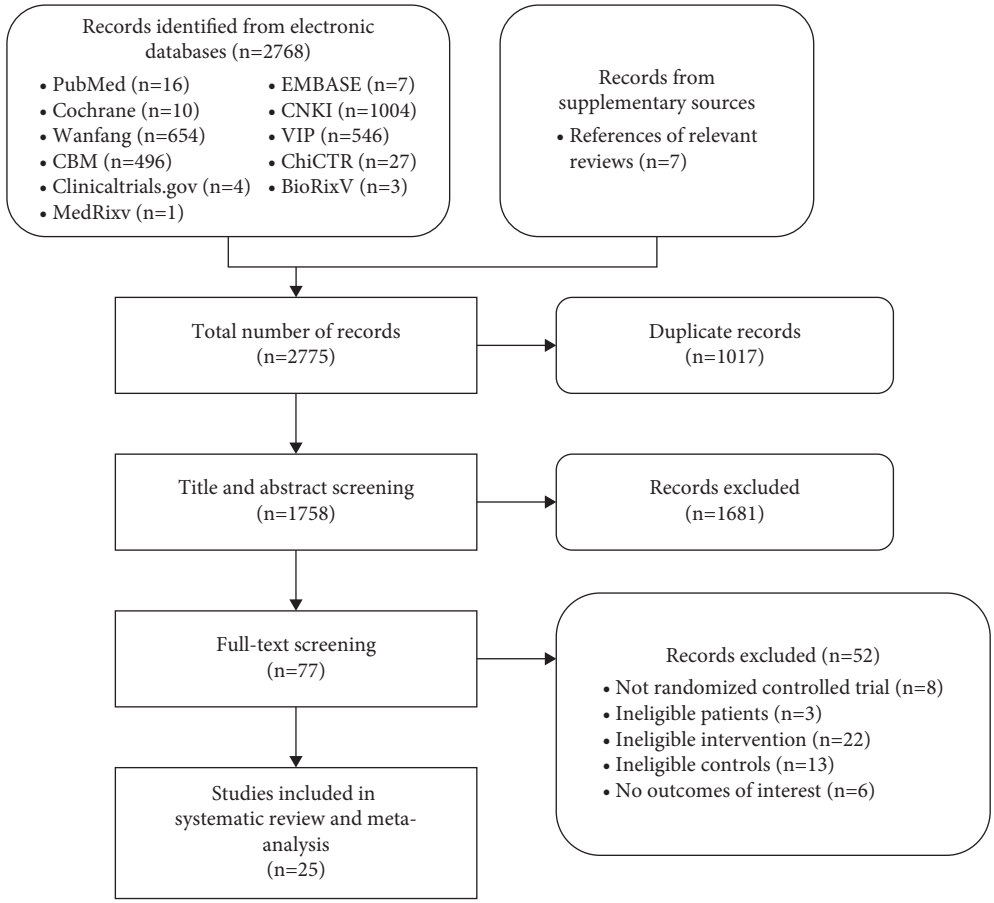

Figure 1: Flowchart of study screening.

in four [32, 41, 42, 48] and seven trials [32, 34, 39, 41, 42, 46, 51], respectively, and the mean baseline FSH level was normal in all trials. Detailed information regarding the acupoint selection and Chinese medicine formulas can be found in Table S2 and Table S3 in the Supplementary Materials.

3.3. Results of the Risk-of-Bias Assessment. Sixteen trials used a correct method to generate random numbers (12 used a random number table [29, 31, 35, 36, 40, 42, 44, 47, 50–53], three used computer software [30, 45, 46], and one used a lottery method [38]). Sealed envelopes [47] and central randomization [45] were used in one trial each to conceal the allocation. The assessors and analysts were blinded in one trial [45]. Two trials reported zero attrition [40, 50], and five trials reported an attrition rate of less than 10% [30, 36, 37, 42, 46]. The remaining trials did not report the attrition rate, but we inferred that they probably completed the follow-up for all patients. All the trials were probably free from selective reporting and other biases. Overall, only one trial was considered to have a low risk of bias [45], 15 had a moderate risk of bias [29–31, 35, 36, 38, 40, 42, 44, 46, 47, 50–53], and nine had a high risk of bias [32–34, 37, 39, 41, 43, 48, 49] (Figure 2).

3.4. Outcomes of Pregnancy and Ovulation

3.4.1. Pregnancy Rate. With regard to the pregnancy rate, pooled results from 18 RCTs involving 1386 patients with PCOS [30, 31, 33, 34, 36–41, 44, 45, 48–50, 52, 53] showed that an increase in the pregnancy rate was associated with the additional use of acupuncture and moxibustion compared with medication treatment alone (50.9% vs. 27.5%; RR 1.81, 95% CI 1.58 to 2.08, $P < 0.01$; Figure 3), without statistical heterogeneity ($I^2 = 0\%$).

3.4.2. Ovulation Rate. As shown in Figure 4, a significant increase in the ovulation rate with low heterogeneity ($I^2 = 19\%$) was also found in the pooled result of 14 RCTs [30, 31, 33, 37–40, 44, 46, 48, 49, 52, 53] that compared the additional use of acupuncture plus moxibustion with medication alone (79.7% vs. 58.3%; RR 1.31, 95% CI 1.22 to 1.40, $P < 0.01$).

3.4.3. Miscarriage Rate. In all, 13.1% (25/191) and 27.7% (28/101) of patients experienced miscarriage in the experimental and control groups, respectively, as reported in nine RCTs [31, 36, 40, 44, 45, 48, 50, 53]. The between-group difference was statistically significant (RR 0.45, 95% CI 0.28 to 0.73, $P < 0.01$; Figure 5), without heterogeneity ($I^2 = 0\%$).

Table 1: Characteristics of the included studies.

Author	Sample size (E/C)	Mean age (E/C, year)	Course of PCOS (E/C, year)	Type and dose of acupuncture and moxibustion	Cointerventions	Course of treatment	Baseline LH (E/C, mIU/mL)	Baseline FSH (E/C, mIU/mL)	Baseline TT (E/C, ng/dL)
Chen 2020	30/30	29.55/29.47	3.10/3.06	MA + WNM; 30 min, q.o.d.	CHM	3 months	NR	NR	NR
Cui 2015	33/33	33.45/31.38	1.43/1.54 *	MA + suspended moxibustion; 25 min/session, q.w.	CHM	6 cycles	9.44/9.42	5.42/5.37	51.3/57.6
Gao 2019a	30/30	28.1/27.6	3.9/3.6	MA + WNM; 30 min/session, q.o.d.	EEC + HCG	3 cycles	15.97/16.24	4.83/4.79	49.6/48.7
Gao 2019b	30/30	28.3/27.2	4.2/3.5	MA + WNM; 30 min/session, q.o.d.	EEC + HCG + CHM	3 cycles	16.09/15.45	4.97/4.94	50.1/47.6
He 2020	35/35	31.97/32.05	3.48/3.52	EA 20 min/session + WNM 30 min/session	Clomiphene	NR	37.53/37.13	9.98/9.83	100.0/101.4
Jiang 2020	44/44	31.22/30.12	NR	MA + suspended moxibustion; 20 min/session, q.o.d.	EEC + letrozole + HCG	NR	14.11/13.45	5.99/6.15	35.2/34.9
Li 2019	50/50	27.78/28.03	2.97/2.56	MA + WNM; 20 min/session, q.o.d.	Clomiphene	3 cycles	16.12/16.08	6.82/6.87	NR
Li 2018	30/30	25.70/26.00	3.91/4.30	MA 30 min/session + moxibustion box 15–30 min/session; t.i.w.	CHM	6 cycles	14.15/14.25	3.86/3.45	79/79.1
Lin 2021	35/35	27.74/26.71	3.13/3.18	EA + ginger-separated moxibustion; 30 min/session, q.o.d.	Letrozole	3 cycles	NR	NR	NR
Lv 2016	100/100	32.00/33.00	NR	EA + WNM; dose was not reported	Clomiphene + HCG	NR	14.19/13.96	5.85/6.13	33.1/35.2
Peng 2020	34/34	28.42/28.31	NR	MA + WNM; 30 min/session	Clomiphene + HCG	NR	NR	NR	NR
Qiao 2012	30/30	20–25 y: 7/8 26–30 y: 16/16 31–35 y: 7/6	2–3 y: 17/19 * 3–4 y: 8/9 >4 y: 4/3	MA + suspended moxibustion; 30 min/session, q.d.	CHM	6 cycles	14.55/14.46	7.09/7.04	81.1/80.9
Qiu 2019	38/37	29.07/28.84	4.61/4.42*	MA + WNM; 30 min/session, q.o.d.	Letrozole + HMG	3 cycles	8.61/8.32	6.35/6.29	38.3/36.8
Shangguan 2017	35/35	NR	NR	EA, 15–25 min/session + WNM, 2 cones/session; q.o.d.	Clomiphene	9 months	37.35/35.31	8.89/9.92	107.8/111.0
Wang 2021	31/31	28.40/28.52	3.43/3.37	MA, 30 min/session + thunder-fire moxibustion, 1 cone/session; q.o.d.	Clomiphene + progesterone for amenorrhea	3 months	11.74/11.71	5.69/5.65	62.8/62.2
Wang 2020	30/30	30.36/29.81	NR	MA + WNM; 20 min/session, 10 sessions per month	CHM	3 cycles	31.91/34.85	8.70/9.01	102.9/106.9
Xie 2019	50/50	25.34/25.32	NR	MA 30 min/session + WNM 2 cone/session	Clomiphene + progesterone[#]	3 cycles	14.07/13.98	5.89/6.14	35.7/35.4
Xing 2020	36/36	34.85/34.24	4.64/4.94 *	EA + WNM; 30 min/session, t.i.w.	Triptorelin + rhFSH + HCG + IVF	3 cycles	14.97/14.90	5.61/5.44	40.1/40.6
Xu C 2019	30/30	28.87/29.571	2.53/2.83	MA + WNM; 40 min/session, q.o.d.	Clomiphene + progesterone[#]	3 cycles	15.6/14.73	5.83/5.77	91.6/91.9
Xu 2020	41/41	27.73/28.16	4.01/3.71 *	MA + WNM; 30 min/session, q.o.d.	Clomiphene + progesterone[#]	9 months	25.6/25.7	9.4/9.1	62.8/63.2
Xu J 2019	30/30	27.83/26.17	1.94/1.97	MA + WNM; 30 min/session, q.o.d.	Lifestyle intervention	3 cycles	13.52/13.53	7.95/7.53	23.9/25.4
Yue 2019	30/30	25.17/26.23	2.97/3.20	MA + moxibustion box; 30 min/session, q.o.d.	Letrozole + CHM	3 cycles	14.76/14.54	5.52/5.76	66.6/66.0
Zheng 2019	30/30	28.50/28.63	NR	MA + WNM, 3 cones/session, q.d.	CHM	3 cycles	NR	NR	NR
Zhong H 2019	30/30	26.77/26.57	3.64/3.21	MA + moxibustion box; 30 min/session, b.i.w.	EEC	3 months	14.48/14.60	8.28/8.11	73.9/72.6
Zhong Q 2019	63/63	33 ± 5/33 ± 5	1.50/1.53 *	MA 25 min/session, q.w. + suspended moxibustion 15 min/session, q.d.	CHM	6 cycles	9.43/9.46	5.40/5.41	55.3/55.9
Zhu 2015	42/40	28.86/29.02	4.55/4.33 *	MA + suspended moxibustion, 20 min/session, q.o.d.	EEC + letrozole + HMG	3 cycles	8.50/8.21	6.24/6.31	39.5/37.9

C/E = experimental group/control group; CHM = Chinese herbal medicine; DEE = desogestrel—ethinyl oestradiol; EA = electroacupuncture; EEC = ethinyl oestradiol—cyproterone; FSH = follicle-stimulating hormone; HCG = human chorionic gonadotropin; HMG = human menopausal gonadotropin; IVF = in vitro fertilization; LH = luteinizing hormone; MA = manual acupuncture; NR = not reported; PCOS = polycystic ovary syndrome; rhFSH = recombinant human follicle-stimulating hormone; TT = total testosterone; WNM = needle warming moxibustion; q.d. = once a day; q.o.d. = once every other day; t.i.w. = three times a week; b.i.w. = twice a week; q.w. = once a week. * Data are the course of infertility; [#]progesterone was only for patients with amenorrhea.

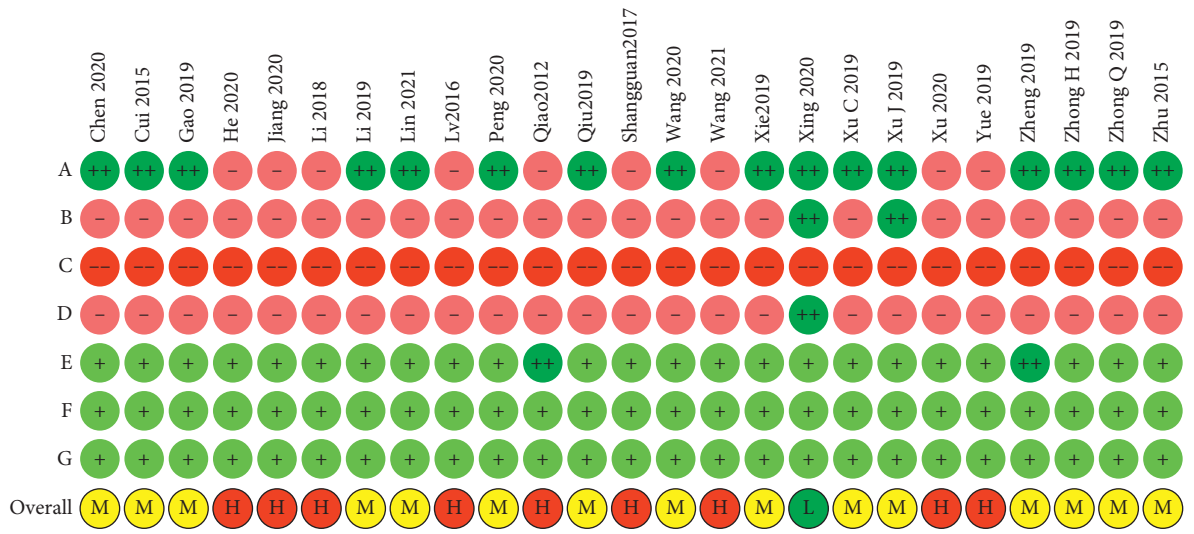

Figure 2: Risk-of-bias assessment (a) random number generation; (b) allocation concealment; (c) blinding of patients and clinicians; (d) blinding of assessors and analysts; (e) data completeness; (f) selective reporting; (g) other sources of bias; "++": low risk; "+": probably low risk; "−": probably high risk; (h) overall high risk; (m) overall moderate risk; (l); overall low risk.

Study	Experimental group		Control group		Risk Ratio	RR	95% -CI	Weight (%)
	Events	Total	Events	Total				
Cui 2015	12	32	9	31		1.29	[0.64; 2.63]	3.7
Gao 2019a	11	30	7	30		1.57	[0.71; 3.50]	2.9
Gao 2019b	15	30	12	30		1.25	[0.71; 2.20]	5.8
Jiang 2020	19	44	10	44		1.90	[1.00; 3.61]	4.5
Li 2018	8	17	3	19		2.98	[0.94; 9.45]	1.4
Lin 2021	19	32	10	33		1.96	[1.08; 3.54]	5.3
Lv 2016	47	100	21	100		2.24	[1.45; 3.45]	10.0
Peng 2020	20	34	6	34		3.33	[1.53; 7.26]	3.1
Qiao 2012	8	30	3	30		2.67	[0.78; 9.09]	1.2
Qiu 2019	23	38	10	37		2.24	[1.24; 4.03]	5.4
Shangguan 2017	13	15	10	18		1.56	[0.99; 2.47]	8.9
Xie 2019	30	50	14	50		2.14	[1.30; 3.53]	7.5
Xing 2020	28	34	15	34		1.87	[1.24; 2.81]	11.2
Xu 2020	21	41	11	41		1.91	[1.06; 3.43]	5.4
Yue 2019	10	30	8	30		1.25	[0.57; 2.73]	3.1
Zheng 2019	19	30	11	30		1.73	[1.00; 2.97]	6.3
Zhong Q 2019	24	63	20	63		1.20	[0.74; 1.94]	8.1
Zhu 2015	25	42	11	40		2.16	[1.23; 3.80]	5.9
Random effects model		**692**		**694**		**1.81**	**[1.58; 2.08]**	**100.0**

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $P = 0.76$
Test for overall effect: $Z = 8.50$ ($P < 0.01$)

Figure 3: Forest plot of meta-analysis of pregnancy rate.

3.4.4. Ovarian Volume. Four RCTs [29, 34, 43, 48] reported data on changes in ovarian volume before and after treatment. The pooled results (Figure 6) showed that a significant reduction in ovarian volume was associated with the additional use of acupuncture and moxibustion compared with the basic treatment alone (MD −0.75 cm^3, 95% CI −1.30 to −0.20, $P < 0.01$; $I^2 = 20.4\%$).

3.5. Sex Hormones

3.5.1. LH and FSH. Twenty-two RCTs ($n = 1720$) [30–35, 37, 39–49, 51–53] reported on changes in the levels of LH and FSH, and 9 of these RCTs ($n = 705$) [31, 32, 34, 37, 41, 45, 47, 49] had data on the LH/FSH ratio. As shown in Figures 7–9, compared to those receiving basic

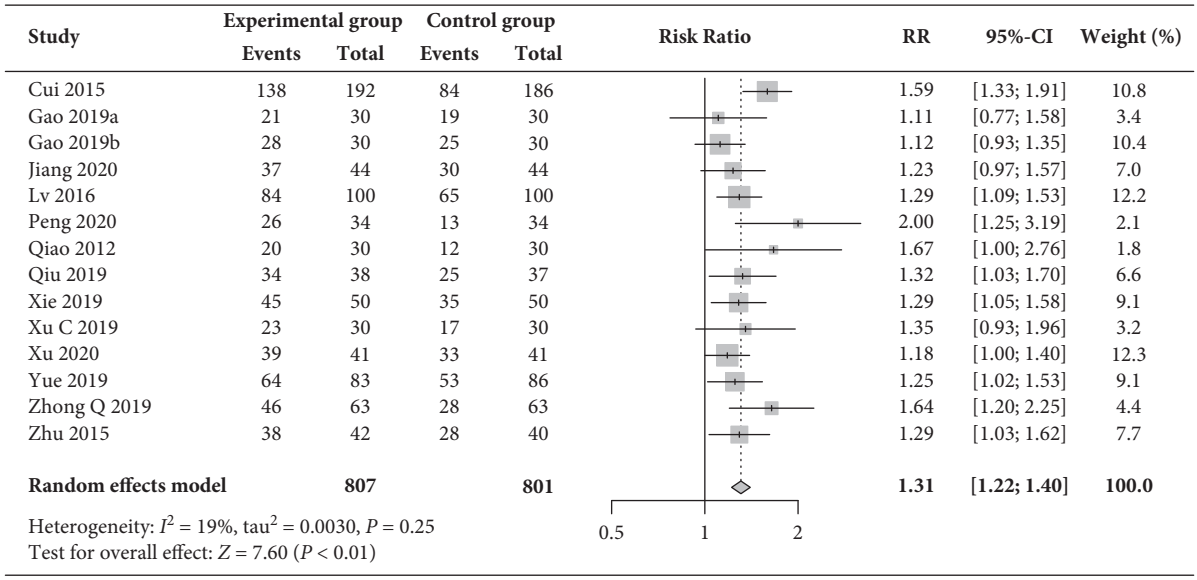

Figure 4: Forest plot of the meta-analysis of ovulation rate.

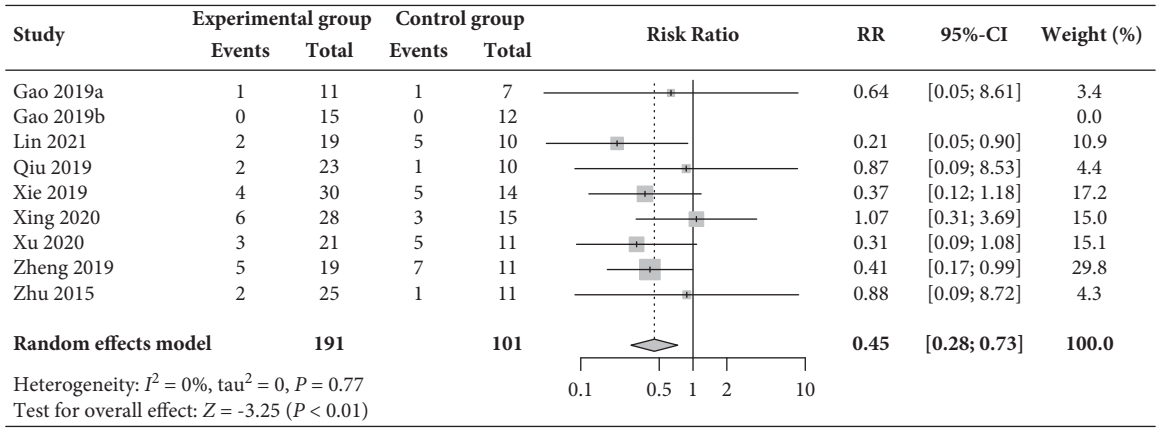

Figure 5: Forest plot of the meta-analysis of miscarriage rate.

treatment alone, patients receiving acupuncture combined with moxibustion had a significantly reduced LH level (MD −2.31 mIU/mL, 95% CI −2.86 to −1.77, $P < 0.01$; $I^2 = 76\%$) and LH/FSH ratio (MD −0.47, 95% CI −0.64 to −0.30, $P < 0.01$; $I^2 = 67\%$). However, the combined use of acupuncture and moxibustion did not significantly impact the level of FSH (MD −0.08 mIU/mL, 95% CI −0.36 to 0.21, $P = 0.60$; $I^2 = 81\%$).

3.5.2. Total Testosterone and DHEAS. Data pooled from 21 RCTs ($n = 1620$) [30–34, 37, 39–49, 51–53] revealed that compared to basic treatment alone, acupuncture plus moxibustion significantly reduced the level of total testosterone (MD −7.04 ng/dL, 95% CI −9.38 to −4.70, $I^2 = 89\%$; Figure 9). However, pooled data from three RCTs ($n = 145$) [30, 44, 52] indicated no clear benefit of acupuncture plus moxibustion on the DHEAS level (MD −0.56 μmol/L, 95% CI −1.64 to 0.52; $I^2 = 97\%$; Figure 10).

3.5.3. Oestradiol. Pooling of data for oestradiol was possible for 15 RCTs ($n = 1281$) [30, 31, 33–35, 37, 39, 40, 44, 45, 48, 49, 52, 53] and showed that there was no significant change in oestradiol when acupuncture plus moxibustion was administered (MD 2.94 pg/mL, 95% CI −1.05 to 6.93, $I^2 = 86\%$; Figure 11).

3.6. Outcomes of Metabolism. Ten [29–31, 34, 40, 43, 49, 52, 53] and four trials [30, 43, 44, 52] contributed data regarding BMI and fasting insulin level, involving 742 and 348 patients, respectively. As shown in Figure 12, the

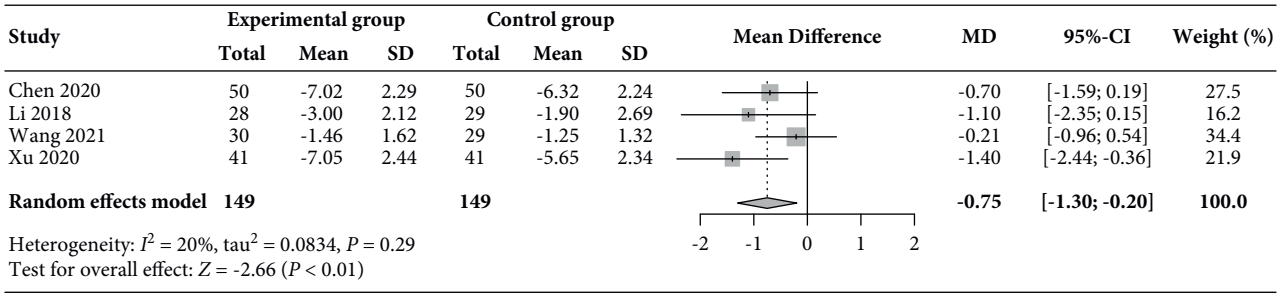

Figure 6: Forest plot of meta-analysis of changes in ovarian volume (cm^3).

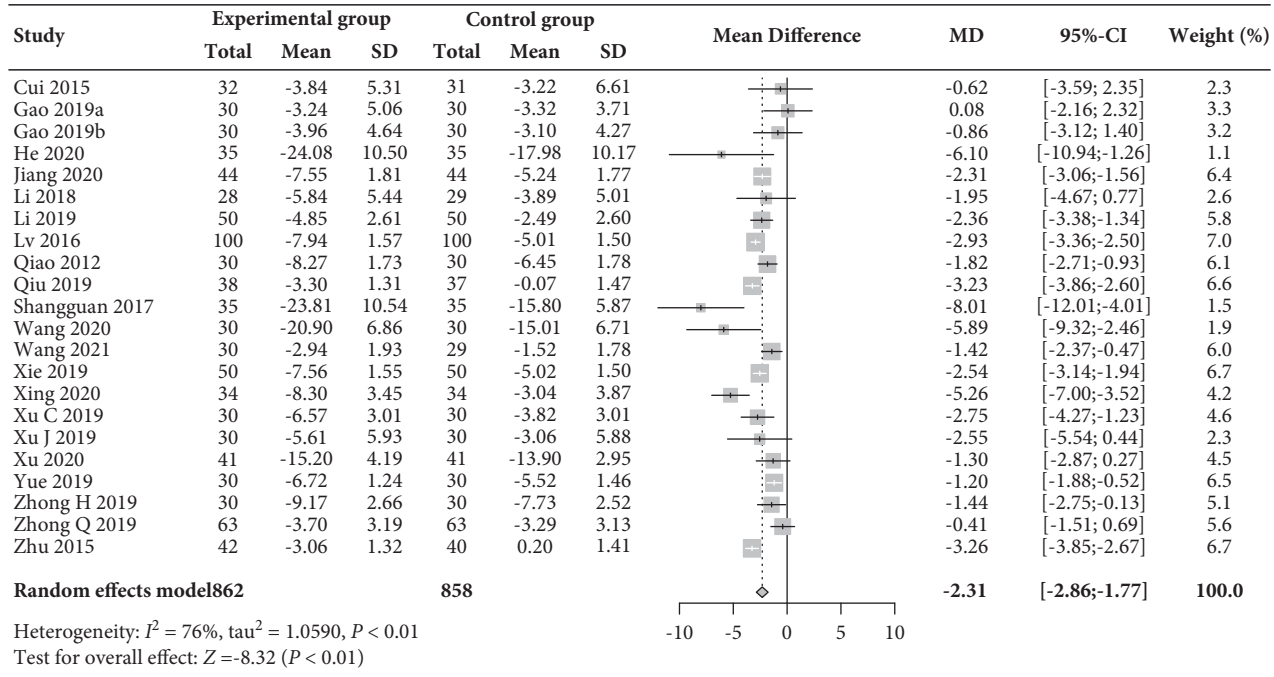

Figure 7: Forest plot of meta-analysis of changes in LH (mIU/mL).

experimental group had greater reductions in both BMI (MD −1.78 kg/m^2, 95% CI −2.53 to −1.03, I^2 = 71%) and fasting insulin level (MD −2.48 mIU/L, 95% CI −3.85 to 1.12, P < 0.01; I^2 = 68%) than the control group.

3.7. Additional Analysis

3.7.1. Subgroup Analysis. A significant subgroup difference (indicated by an interaction P value < 0.05) was found in the subgroup analyses stratified by the basic treatments for the ovulation rate (Western medicine was the best, interaction P = 0.01) and for the level of FSH (Western medicine combined with Chinese herbal medicine was the best, P = 0.01); in the analyses stratified by the basic treatments (Chinese herbal medicine was the best, P = 0.01), type of acupuncture (manual acupuncture was better, P = 0.01), and type of moxibustion (nonwarm-needle moxibustion was better, P = 0.03) for the LH level; in the analyses stratified by the basic treatments (Western medicine was the best, P < 0.01), type of acupuncture (manual acupuncture was better, P < 0.01), type of moxibustion (warm-needle moxibustion was better, P < 0.01), and course of treatment (≤3 months was better, P = 0.04) for the LH/FSH ratio; and in the analyses stratified by the course of treatment (>3 months was better, P < 0.01) for the total testosterone level. Details of the subgroup analyses are shown in Table S4 in the Supplementary Materials.

3.7.2. Sensitivity Analysis. A change in the effect direction was found for the ovarian volume after excluding studies with a high risk of bias (MD −0.70 [95% CI −1.59 to 0.19] vs. main analysis: MD −0.75 [95% CI −1.30 to −0.20]). No other important changes were found. Details of the sensitivity analyses are presented in Table S5 in the Supplementary Materials.

3.7.3. Publication Bias. As shown in Figure 13, a significant publication bias was found for only the analysis of the total testosterone level, indicated by an asymmetric funnel plot and a P value of 0.007 by Egger's test.

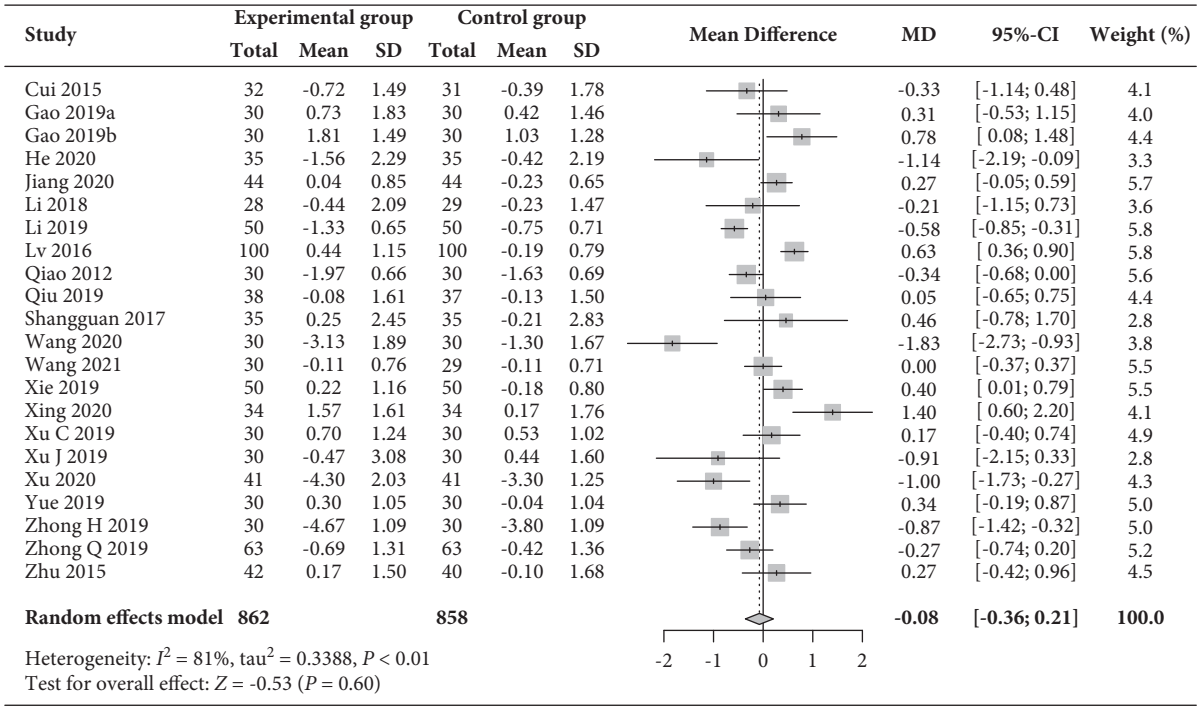

Figure 8: Forest plot of meta-analysis of changes in FSH (mIU/mL).

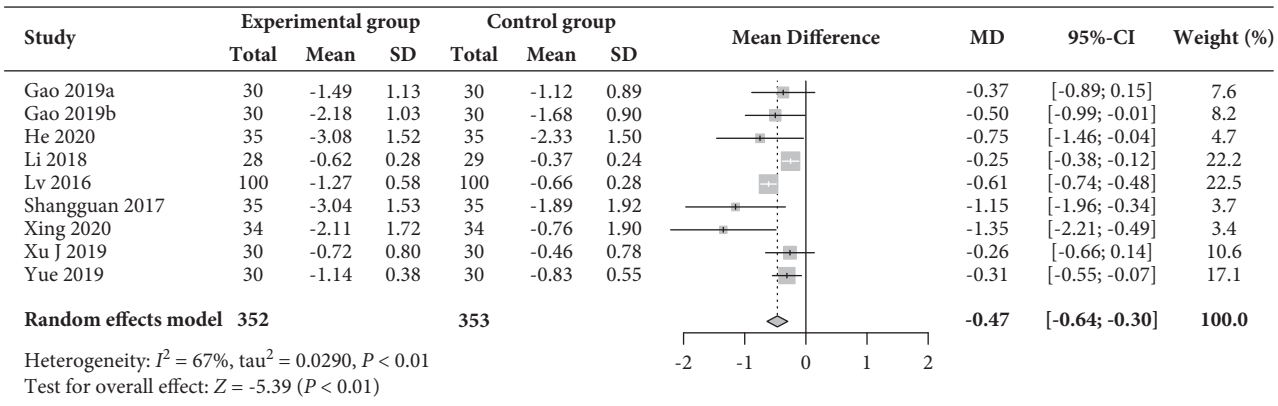

Figure 9: Forest plot of meta-analysis of changes in the LH/FSH ratio.

3.8. Level of Evidence. The meta-analytic results for the pregnancy rate, ovulation rate, miscarriage rate, and LH level were associated with a serious risk of bias and were downgraded to a moderate level of evidence. The levels of evidence of all remaining outcomes were judged to be very low because of serious or very serious limitations regarding risk of bias, inconsistency, imprecision, and/or publication bias. The GRADE evidence profiles are compiled in Table S6 in the Supplementary Materials.

3.9. Safety. Seven trials [31, 34, 39, 45, 46, 49, 50] reported safety data. There were two cases of subcutaneous bleeding and two cases of pain at the acupuncture site in Xing et al.'s study [45], one case of pelvic pain in Xu's study [46], and two cases of needle sickness in Yue et al.'s study [49]. These events were mild, and remission was achieved without specific treatment. The remaining trials claimed that no adverse events occurred.

4. Discussion

The systematic review included 25 RCTs assessing the efficacy and safety of acupuncture combined with moxibustion in treating PCOS, and all of them contributed data to the meta-analysis. Evidence of an association between acupuncture and moxibustion therapy and greater increases in the pregnancy rate and ovulation rate and greater reductions

Study	Experimental group Total	Mean	SD	Control group Total	Mean	SD	MD	95%-CI	Weight (%)
Total testosterone									
Cui 2015	32	-16.14	28.10	31	-17.58	22.65	1.44	[-11.14; 14.02]	2.3
Gao 2019a	30	-29.11	11.71	30	-27.38	13.25	-1.73	[-8.06; 4.60]	4.4
Gao 2019b	30	-32.28	14.35	30	-27.67	9.99	-4.61	[-10.87; 1.64]	4.5
He 2020	35	-55.04	8.79	35	-45.53	16.28	-9.51	[-15.64; -3.38]	4.5
Jiang 2020	44	-20.75	4.07	44	-12.39	5.26	-8.36	[-10.32; -6.39]	6.3
Li 2018	28	-33.70	10.41	29	-13.13	10.34	-20.57	[-25.96; -15.18]	4.9
Lv 2016	100	-18.73	6.00	100	-12.97	5.96	-5.76	[-7.42; -4.11]	6.3
Qiao 2012	30	-29.27	8.79	30	-28.72	7.62	-0.55	[-4.71; 3.61]	5.4
Qiu 2019	38	-11.02	5.60	37	-2.63	7.38	-8.39	[-11.36; -5.42]	5.9
Shangguan 2017	35	-59.37	13.27	35	-55.62	24.67	-3.75	[-13.03; 5.53]	3.2
Wang 2020	30	-42.07	12.60	30	-29.39	13.69	-12.68	[-19.34; -6.02]	4.3
Wang 2021	30	-12.39	8.24	29	-11.82	7.74	-0.58	[-4.65; 3.50]	5.5
Xie 2019	50	-21.61	6.25	50	-12.97	6.42	-8.65	[-11.13; -6.16]	6.1
Xing 2020	34	-13.26	7.80	34	-0.29	8.95	-12.97	[-16.96; -8.98]	5.5
Xu C 2019	30	-54.76	31.01	30	-29.97	30.51	-24.78	[-40.35; -9.21]	1.7
Xu J 2019	30	-5.48	5.20	30	-2.88	5.96	-2.59	[-5.42; 0.23]	6.0
Xu 2020	41	-54.50	1.65	41	-53.10	1.71	-1.40	[-2.13; -0.67]	6.5
Yue 2019	30	-21.33	15.38	30	-12.97	16.18	-8.36	[-16.35; -0.37]	3.7
Zhong H 2019	30	-39.88	7.35	30	-29.86	8.20	-10.02	[-13.96; -6.08]	5.5
Zhong Q 2019	63	-19.88	14.56	63	-15.56	15.57	-4.32	[-9.59; 0.94]	4.9
Zhu 2015	42	-11.02	31.56	40	-0.71	18.41	-10.31	[-21.43; 0.81]	2.6
Random effects model	**812**			**808**			**-7.04**	**[-9.38; -4.70]**	**100.0**
Heterogeneity: I^2 = 89%, tau^2 = 21.7571, P < 0.01									
Test for overall effect: Z = -5.90 (P < 0.01)									
Dehydroepiandrosterone sulfate									
Cui 2015	32	0.54	3.55	31	0.48	2.59	0.06	[-1.47; 1.59]	22.1
Xie 2019	50	-5.07	0.52	50	-3.59	0.61	-1.48	[-1.70; -1.26]	39.0
Zhong Q 2019	63	0.34	0.70	63	0.33	0.69	0.01	[-0.23; 0.25]	38.9
Random effects model	**145**			**144**			**-0.56**	**[-1.64; 0.52]**	**100.0**
Heterogeneity: I^2 = 97%, tau^2 = 0.7676, P < 0.01									
Test for overall effect: Z = -1.01 (P = 0.31)									

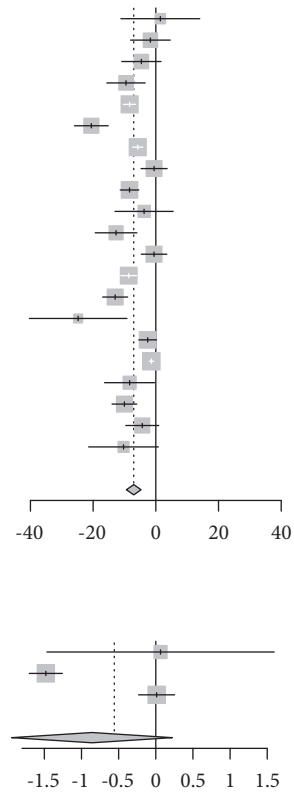

Figure 10: Forest plot of meta-analysis of changes in total testosterone (ng/dL) and dehydroepiandrosterone sulfate (μmol/L).

Study	Experimental group Total	Mean	SD	Control group Total	Mean	SD	MD	95%-CI	Weight (%)
Cui 2015	32	6.39	29.71	31	7.21	34.23	-0.82	[-16.67; 15.02]	3.5
Gao 2019a	30	3.04	5.29	30	1.10	5.49	1.93	[-0.79; 4.66]	7.5
Gao 2019b	30	2.60	6.36	30	2.68	5.29	-0.08	[-3.04; 2.88]	7.5
Jiang 2020	44	3.11	9.01	44	2.82	8.92	0.29	[-3.46; 4.04]	7.3
Li 2018	28	3.22	21.38	29	2.71	21.04	0.51	[-10.50; 11.53]	4.9
Li 2019	50	45.77	17.69	50	21.51	14.78	24.26	[17.87; 30.65]	6.5
Lv 2016	100	3.66	10.89	100	3.10	11.52	0.56	[-2.55; 3.67]	7.5
Qiao 2012	30	19.77	7.19	30	14.46	6.65	5.31	[1.81; 8.81]	7.4
Qiu 2019	38	-0.35	9.59	37	-2.05	11.53	1.70	[-3.11; 6.51]	7.0
Xie 2019	50	2.69	10.90	50	3.10	11.53	-0.41	[-4.81; 3.99]	7.1
Xing 2020	34	-9.01	9.34	34	-0.35	10.93	-8.65	[-13.49; -3.82]	7.0
Xu 2020	41	49.90	14.05	41	32.30	13.90	17.60	[11.55; 23.65]	6.6
Yue 2019	30	4.30	8.78	30	4.01	7.95	0.29	[-3.94; 4.53]	7.2
Zhong Q 2019	63	1.61	4.48	63	0.54	4.52	1.07	[-0.51; 2.64]	7.7
Zhu 2015	42	1.19	15.35	40	0.80	27.88	0.39	[-9.42; 10.20]	5.3
Random effects model	**642**			**639**			**2.94**	**[-1.05; 6.93]**	**100.0**
Heterogeneity: I^2 = 86%, tau^2 = 52.9546, P < 0.01									
Test for overall effect: Z = 1.45 (P = 0.15)									

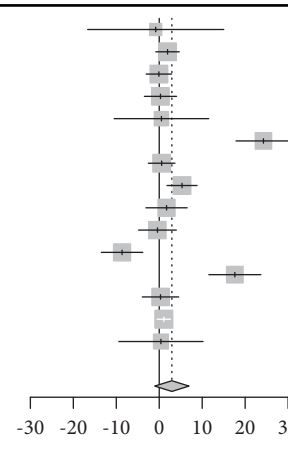

Figure 11: Forest plot of meta-analysis of changes in oestradiol (pg/mL).

in the miscarriage rate and ovarian volume was found. Additionally, patients receiving acupuncture and moxibustion also exhibited greater improvements in some sex hormones (LH level, LH/FSH ratio and total testosterone level) and indicators related to metabolic disorders (fasting insulin level and BMI). Nevertheless, acupoint stimulation

Study	Experimental group			Control group			Mean Difference	MD	95%-CI	Weight (%)
	Total	Mean	SD	Total	Mean	SD				
Body mass index										
Chen 2020	50	-3.97	3.49	50	-2.73	3.60		-1.24	[-2.63; 0.15]	9.8
Cui 2015	32	-3.01	4.29	31	-2.03	4.47		-0.98	[-3.14; 1.18]	6.6
Gao 2019a	30	-2.30	1.81	30	-1.30	2.07		-1.00	[-1.98; -0.02]	11.8
Gao 2019b	30	-4.70	2.39	30	-2.30	1.40		-2.40	[-3.39; -1.41]	11.7
Li 2018	28	-1.37	5.45	29	-0.66	6.00		-0.71	[-3.69; 2.27]	4.4
Qiu 2019	38	-0.78	2.72	37	1.91	2.90		-2.69	[-3.96; -1.42]	10.3
Wang 2021	30	-3.36	1.96	29	-1.56	2.43		-1.80	[-2.93; -0.67]	11.1
Yue 2019	30	-2.85	1.66	30	-1.57	1.43		-1.28	[-2.06; -0.50]	12.7
Zhong Q 2019	63	-4.68	3.70	63	-0.21	3.62		-4.47	[-5.75; -3.19]	10.3
Zhu 2015	42	-1.02	2.04	40	-0.49	2.87		-0.53	[-1.61; 0.55]	11.3
Random effects model	**373**			**369**				**-1.78**	**[-2.53; -1.03]**	**100.0**
Heterogeneity: I^2 = 71%, tau^2 = 0.9985, P < 0.01										
Test for overall effect: Z = -4.64 (P < 0.01)										
Fasting insulin										
Cui 2015	32	-6.08	6.30	31	-4.39	8.89		-1.69	[-5.50; 2.12]	9.8
Wang 2021	30	-4.99	2.13	29	-3.84	2.11		-1.15	[-2.23; -0.07]	32.5
Xie 2019	50	-5.88	4.68	50	-2.02	3.26		-3.86	[-5.44; -2.28]	26.5
Zhong Q 2019	63	-6.93	3.38	63	-3.98	3.42		-2.95	[-4.14; -1.76]	31.2
Random effects model	**175**			**173**				**-2.48**	**[-3.85; -1.12]**	**100.0**
Heterogeneity: I^2 = 68%, tau^2 = 1.1866, P = 0.03										
Test for overall effect: Z = -3.56 (P < 0.01)										

Figure 12: Forest plot of meta-analysis of changes in BMI (kg/m^2) and fasting insulin (mIU/L).

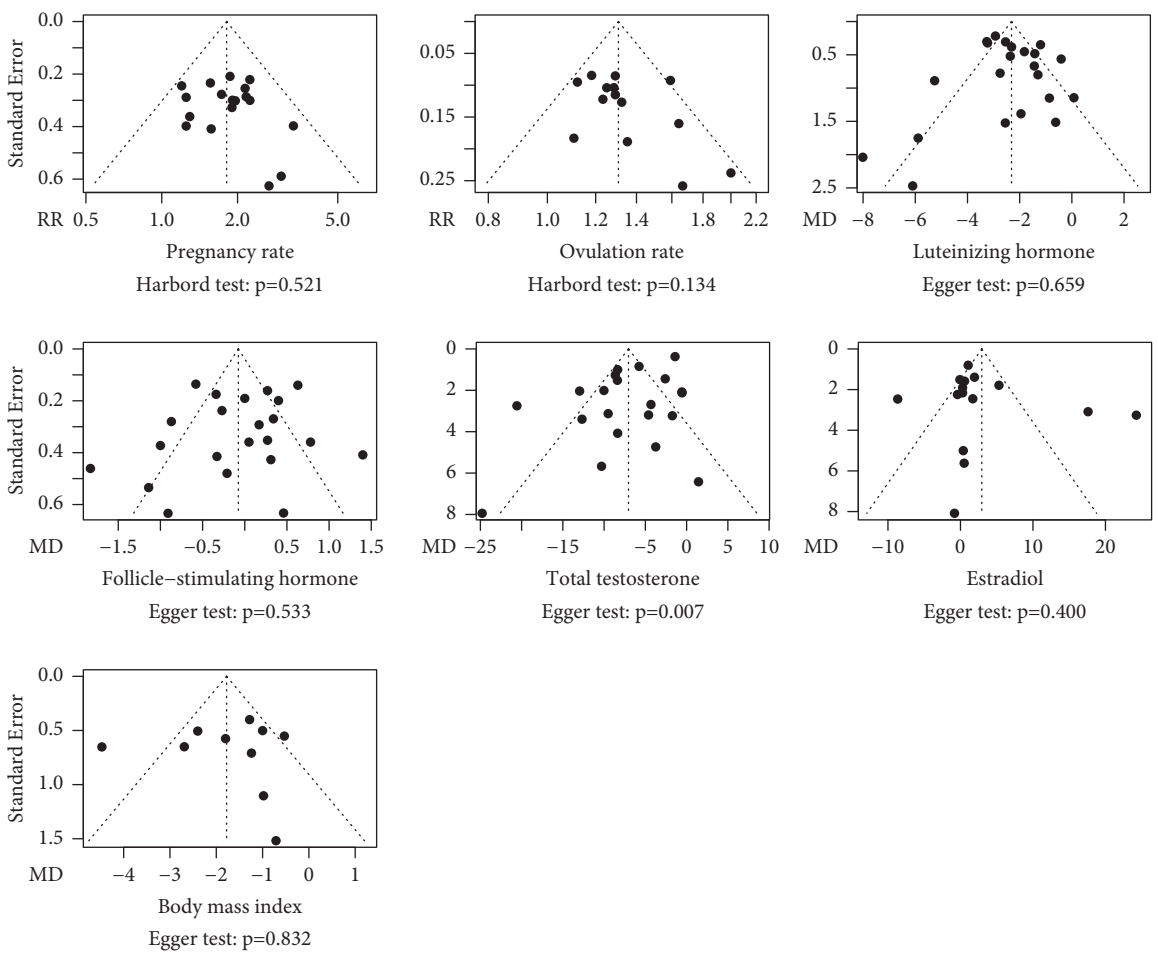

Figure 13: Funnel plot and results of Harbord's or Egger's tests. Outcomes with an insufficient number of studies to detect publication bias are not presented.

therapy had no significant effect on the levels of FSH, DHEAS, or oestradiol.

In this systematic review, the pregnancy rate among patients who received acupuncture combined with moxibustion reached 50.9%. This value represents a substantial increase compared to that of patients who did not receive acupuncture (27.5%), with an RR of 1.81 and a 4.9% additional increase compared with patients receiving acupuncture + active drugs in the PCOSAct trial (46.0%) [15]. In terms of the miscarriage rate, the additional use of moxibustion had an even greater advantage (the experimental group in this review vs. acupuncture + active drugs group in the PCOSAct trial: 13.1% vs. 35.2% [15]). Although such cross-study comparisons may not be precise, we think that moxibustion probably has a synergistic effect on acupuncture in the treatment of PCOS; notably, this synergistic effect has been observed in the treatment of other disorders [54–56].

One of the main pathophysiological states of PCOS is the disturbed hypothalamic–pituitary–ovarian/adrenal axis. This state results in increased LH release, a decreased FSH level, and a reversed increase in the LH/FSH ratio, leading to cessation of ovulation and infertility [57]. Our analysis showed that both the LH level and the LH/FSH ratio were significantly reduced after acupuncture and moxibustion treatment but that improvements were not found in the levels of FSH or oestradiol. Therefore, the role of acupuncture combined with moxibustion in lowering the LH/FSH ratio should be attributed to its inhibitory effect on LH. Mechanistically, excessive LH secretion in PCOS is related to increased pituitary sensitivity to gonadotropin-releasing hormone and changes in its secretion pattern [58]. Stimulation at the acupoint has been shown to raise the level of β-endorphin in the central endocrine system and peripheral circulation, which is involved in the direct and indirect tonic inhibition of gonadotropin-releasing hormone and subsequent LH release [59]. Moreover, altered sympathetic neurogenic control of the ovary is associated with the pathogenesis of PCOS [56], and stimulation at the acupoint can also reduce sympathetic activity by inhibiting the overexpression of nerve growth factor, which may result in a return to normal levels of the ovarian steroid response to gonadotropins [60].

Most patients with PCOS suffer from chronically elevated levels of androgens in the ovaries and circulatory system. Hyperandrogenemia can enhance the activity of mitogen-activated protein kinase in endometrial cells by activating mitogen-activated protein kinase, subsequently inducing endometrial hyperplasia [61]. It can also reduce endometrial tolerance by disrupting the expression of homeobox A10, which controls endometrial differentiation and is closely associated with infertility and recurrent abortion [62]. We found that acupuncture and moxibustion significantly reduced the total testosterone level but did not impact the level of DHEAS, suggesting that the increase in the pregnancy rate and the reduction in the miscarriage rate may also be associated with lower testosterone levels. However, the DHEAS data were derived only from three small RCTs, so the effect estimate was quite uncertain and needs further evidence.

While infertility, acne and hirsutism secondary to anovulation and hyperandrogenemia jeopardize only reproductive function and affect only the woman's appearance, the metabolic disorders that occur in 37% of patients with PCOS are more detrimental to health [63]. We selected two indicators directly related to insulin resistance and obesity, namely, the fasting insulin level and BMI, to evaluate the efficacy of acupuncture and moxibustion for treating metabolic disorders, and both methods showed a positive result. Previous meta-analyses have also supported the benefits of acupuncture and related techniques for treating metabolic disorders such as type 2 diabetes mellitus and obesity [12, 13]. This consistency increases the credibility of our findings.

In subgroup analyses, we found multiple significant subgroup differences in the ovulation rate, LH level, FSH level, LH/FSH ratio, and total testosterone level. Based on the guidance proposed by Sun et al. for assessing the credibility of subgroup effects, some of the subgroup differences are unreliable because they were in the opposite direction to that anticipated or the residual heterogeneity remained high. We are confident that the following subgroup effect claims can be made: warm-needle moxibustion and ≥3 months of treatment are better for reducing the LH/FSH ratio, and ≥3 months of treatment is better for reducing the total testosterone level. Of course, the other significant findings in the subgroup analysis could serve as clues for future studies designed to explore subgroup effects.

A small number of mild adverse events, including subcutaneous bleeding, pain at the acupuncture site, needle sickness, and pelvic pain, were reported in the included studies. Of these, pelvic pain was likely due to the administration of clomiphene, and the other adverse events were likely related to acupuncture. No moxibustion-related adverse events were reported. Based on these safety profiles, acupuncture and moxibustion therapy seems to be safe in patients with PCOS. However, in our clinical experience, the administration of acupuncture and moxibustion for PCOS requires considering contraindications in terms of timing. Specifically, the treatment should be avoided during the first five days of the menstrual period and discontinued during pregnancy to avoid excessive menstrual flow and increasing the risk of miscarriage and preterm delivery, as the effect of acupoint stimulation on these events cannot be completely ruled out [64, 65]; this was also common practice in the studies included in this systematic review (see Table S2 for details).

To our knowledge, this is the first systematic review to assess the effects of acupuncture combined with moxibustion in patients with PCOS, filling an evidence gap for complementary and alternative treatments for PCOS. The methodological strengths of our study included the systematic literature search, the reasonable statistical analysis plan, and the standardized appraisal of quality of evidence, which ensured the comprehensiveness of the body of evidence and the objectivity of the conclusions.

The study has some limitations. The first and main limitation is that most of the included studies had a moderate-to-high risk of bias, which became a reason to reduce the level of evidence for all outcomes. Second, there was substantial interstudy heterogeneity for some outcomes (e.g., FSH, oestradiol) that could not be explained by the prespecified subgroup analyses, and the prediction intervals suggested that a similar new study would yield estimates that significantly differ from the current CIs, making the direction and precision of the effect estimates for these outcomes unstable. Nevertheless, the results estimated by the random-effects model represent the average effect of acupuncture combined with moxibustion on these outcomes and may preliminarily inform an overall effective or ineffective conclusion for clinical practice. Third, tests of publication bias were not performed for some outcomes due to insufficient samples, but we did not downgrade the level of evidence for these outcomes because there was also no evidence to confirm publication bias.

5. Conclusion

Among patients with PCOS, the combined use of acupuncture and moxibustion as a complementary therapy to basic treatments has additional efficacy regarding increased pregnancy and ovulation rates and reduced miscarriage rate and can also help improve the LH level, LH/FSH ratio, fasting insulin level, and BMI. No significant effects were found for the levels of FSH, oestradiol, or DHEAS. All adverse events were mild. Based on these findings, we believe that using acupuncture combined with moxibustion rather than acupuncture alone for treating PCOS is an effective and safe approach that is also more in line with real practice in Chinese hospitals.

Abbreviations

ASRM: American society for reproductive medicine
BMI: Body mass index
CI: Confidence interval
CINeMA: Confidence in network meta-analysis
DHEAS: Dehydroepiandrosterone sulfate
ESHRE: European society of human reproduction and embryology
FSH: Follicle-stimulating hormone
GRADE: The grading of recommendations, assessment, development, and evaluations
LH: Luteinizing hormone
MD: Mean difference
PCOS: Polycystic ovary syndrome ()
PCOSAct: The PCOS acupuncture and clomiphene trial
RCT: Randomized controlled trial
RR: Risk ratio.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The funders had no role during the entire process of this study.

Conflicts of Interest

The authors claim that they have no conflicts of interest.

Authors' Contributions

PL conceived and designed the study, developed the search strategy, developed the manuscript, and drafted and revised the manuscript. PJ and DZ screened the articles, collected the data, assessed the risk of bias, and revised the manuscript. XZ revised the manuscript and provided critical methodological advice. RL conceived and designed the study, developed the manuscript, and acts as a guarantor. All authors read and approved the final manuscript.

Acknowledgments

This research was supported by Key Research and Development Program of Jiangxi Province (grant no. 20181ACG70016), Funding of Jiangxi Clinical Research Centre for Obstetrics and Gynecology Diseases of TCM (grant no. 20181BCG42003), National Natural Science Foundation of China (grant no. 81560783), and Jiangxi University of Chinese Medicine 1050 Youth Talent Project (grant no. 5142001011).

Supplementary Materials

Table S1. Search strategy. Table S2. Details of acupuncture and moxibustion treatment. Table S3. Formula of Chinese herbal medicine. Table S4. Results of subgroup analyses. Table S5. Results of sensitivity analyses. Table S6. GRADE evidence profile. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 5032681, 7 pages
https://doi.org/10.1155/2022/5032681

Research Article

Efficacy of Date Palm Pollen in the Male Sexual Dysfunction after Coronary Artery Bypass Graft: A Randomized, Double-Blind, Clinical Trial

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Received 14 October 2021; Revised 23 January 2022; Accepted 23 February 2022; Published 28 March 2022

Academic Editor: Tian-Bo Liu

Background. Bypass graft surgery of the coronary artery has a significant effect on the creation and development of sexual dysfunction among male patients. The previous studies have demonstrated that date palm pollen (DPP) increases the count and quality of sperm. Additionally, it has been shown that DPP has a protective effect against myocardial infarction and cardiac remodeling. Therefore, this is the first study investigating the impact of DPP (*Phoenix dactylifera L.*) on managing male sexual dysfunction after coronary artery bypass graft. *Methods*. This randomized, double-blind, placebo-controlled clinical trial was conducted on 60 patients (DPP group $n = 30$, control group $n = 30$) of Iranian men after coronary artery bypass graft. Two parallel groups were randomly generated from the study participants. The intervention group was prescribed 3 grams of the powder of DPP twice a day (9 AM and 9 PM) for two months, while the control group received the same prescription of the placebo powder. *Results*. The DPP consumption significantly increased the International Index of Erectile Function (IIEF) (from 23.21 to 46.57) and the Hurlbert Index of Sexual Desire (HISD) (from 59.39 to 64.45) scores over time in the intervention group. However, there were no significant changes in the control group. *Conclusion*. Daily intake of 6 g DPP for two months exhibited beneficial effects on the symptoms of male sexual dysfunction in patients who have undergone coronary artery bypass graft (CABG).

1. Introduction

Sexual dysfunction represents a highly prevalent problem among male patients who undergo coronary artery bypass graft surgery (CABG). These patients involve in issues such as erectile dysfunction, reduced libido, and premature ejaculation [1]. It is estimated that sexual dysfunction affects roughly 10–25% of middle-aged and older men [2]. Previous studies showed that around three-quarters of cardiac disease patients experience some degree of sexual dysfunction [3]. As mentioned, this condition is common among patients who have undergone CABG [4], with some patients not being able to resume regular sexual activity [5]. Such patients have an impaired perception of well-being together with an attenuated quality of life. Hence, sexual function recovery fulfills a significant role in preserving the quality of life following cardiac operations [6].

The exact cause of sexual dysfunction is not fully understood [7]. Moreover, there are relatively limited treatments in reproductive medicine being directed at these issues of male health [8]. Currently, among those facing chronic conditions, there is a trend toward using herbal medicine and other forms of complementary and alternative medicine [9–12]. Although some traditional remedies and herbal drugs have been used by patients with sexual dysfunction, a significant proportion of medicinal plants have not yet been scientifically evaluated in this regard [13–15].

Phoenix dactylifera (date palm pollen, DPP) is the male reproductive dust of palm flowers used since ancient times as an aphrodisiac and fertility enhancer in patients with sexual dysfunction or infertility [16]. Experimental studies have revealed that DPP increases both sperm count and quality [17]. Moreover, previous studies demonstrated that DPP has a protective effect against myocardial infarction (via control of hyperlipidemia) and cardiac remodeling [18]. Phytochemical research has shown that the presence of flavonoids, sterol derivatives, and amino acids in the pollen may be responsible for its pharmacological effects [19]. Despite numerous clinical and animal studies on sexual dysfunction, no studies have examined the impact of DPP on male sexual dysfunction after coronary artery bypass grafting [20]. Hence, we evaluated DPP efficacy in managing male sexual dysfunction after CABG in this randomized, placebo-controlled trial.

2. Methods

2.1. Study Design. This study took the form of a randomized, double-blind, placebo-controlled clinical trial and was carried out in Tehran Heart Center in Tehran, Iran, from September 2019 to August 2020. We evaluated the efficacy of date palm pollen in the management of the symptoms of male sexual dysfunction after CABG. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1398.915) and is registered in the Iranian Registry of Clinical Trials (IRCT20191228045911N1). Informed consent was obtained from all participants; the entire study was conducted according to the 2013 revision of the Declaration of Helsinki.

2.2. Participants. The inclusion criteria for recruiting in this trial were as follows: men aged between 40 and 70 years who had undergone CABG, complained from sexual dysfunction, were willing to participate in the study, had an International Index of Erectile Function (IIEF) score of less than 50, and had a cardiac ejection fraction above 30%. Also, the exclusion criteria of this study were as follows: having uncontrolled thyroid disease, anemia, prostate cancer, renal or hepatic dysfunction, major depressive disorder, history of myocardial infarction in the last six months, history of cardiac valve replacement, history of sexual dysfunction before cardiac surgery, and history of allergy to pollen.

2.3. Sample Size Estimation. As there was no similar study, G∗power software (version 3.1.9) [21] was used to calculate the sample size. To achieve a large effect size ($d = 0.8$) with static power of 0.8 at a significant level of 0.05, a total of 26 patients were needed in each group. However, this was increased to 30 participants to consider a 10% probable dropout rate.

2.4. Randomization, Blinding, and Allocation Concealment. Sixty patients who presented at the Tehran Heart Center (Tehran, Iran) and met the inclusion criteria were subjected to prospective sequential sampling for random allocation into the intervention and control groups. Randomization was done by a biostatistician using block randomization in the Number Cruncher Statistical System (NCSS) software (size 4 per block). The drug and placebo powder sachets were labeled by the pharmacist using the same randomization list, with the identity of each being concealed until the end of the project. Patients, researchers, and medication deliverers were not aware of the group allocation. The placebo powder sachets were similar to the pollen powder sachets in terms of color, viscosity, and weight.

2.5. Preparation of Drug and Placebo. In early spring, palm pollen was collected from male date trees in Jahrom (Fars province, Iran). An herbal pharmacologist authenticated and deposited palm pollen in the herbarium of Jahrom University of Medical Sciences (specimen voucher number: 373846). The collected pollen was kept in a glass bottle and then discharged into sachets for administration.

The placebo powder was prepared by a pharmacist using standard Avicel powder, an inert substance with the same color as date palm pollen. This product is used as a fat replacer and is composed of microcrystalline cellulose that is partially hydrolyzed with acid before being reduced to a fine powder.

2.6. Intervention. Two parallel groups were randomly generated from the study participants. The intervention group was prescribed 3 grams of the powder of DPP twice a day (9 AM and 9 PM) for two months, while the control group received the same prescription of the placebo powder. All participants were asked not to take any other herbal and alternative medicine during the study.

2.7. Outcome Measures. The outcomes of the study were collected by a staff who was not aware of group assignments. A socio-demographic questionnaire was used to take the basic characteristics of the population such as age, body mass index, smoking, blood pressure, and lipid profile.

The Hurlbert Index of Sexual Desire (HISD) and IIEF scores were considered as the outcome measures of this study. The IIEF consists of 15 questions divided into five domains: erectile function, sexual desire, orgasmic function, intercourse satisfaction, and overall satisfaction. A score of 1 to 5 is given for each item, with a "0" option sometimes being available to denote the complete absence of sexual stimulation/intercourse [7]. Higher scores represent better sexual functioning. The HISD is composed of 25 questions graded from 0 to 4 on a Likert scale, with the total score ranging from 0 to 100. The lower one's score, the lower their sexual desire [9]. All outcomes were evaluated at the beginning, the first month, and the end of the study (second month).

The signs of an allergic reaction were explained to all participants; they were asked to report any potential side effects of the prescribed drugs immediately.

2.8. Statistical Methods. Initially, for comparison of baseline characteristics between groups, an independent t-test was used for continuous variables and was expressed as mean ± SD. A chi-square test was used for categorical variables and was presented as numbers. The mean scores of HISD and IIEF were compared between groups by an independent t-test. General linear model (GLM) repeated measures analysis of variance was performed to compare intervention and placebo across the 3-time points of the baseline, after a month, and the end of the study. Statistical significance was considered when p values were below 0.05. Data analysis was done using the Statistical Package for Social Sciences (version 15; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Study Flow. During the period between September 2019 and August 2020, we evaluated a total of 81 patients for eligibility. Of these, 15 were excluded because of not having eligibility criteria and 6 for unwillingness to take part. Finally, thirty patients were allocated to each of the control and intervention groups. At the end of the follow-up, two people from the DPP group have not completed the intervention due to lack of interest and urticaria, while four of the placebo recipients were excluded (due to stomachache and lack of interest). The study flow diagram is presented in Figure 1.

3.2. Baseline Data. The age BMI matching was performed to eliminate confounders' effect in DPP and control groups. Therefore, there was no significant difference between the two groups in terms of age (I: 61.93 ± 6.36 and C: 59.38 ± 8.91 years; $p = 0.23$) and BMI (I: 28.07 ± 4.30 and C: 27.00 ± 3.84; $p = 0.398$). Furthermore, the study groups were similar in terms of all baseline characteristics, except for the IIEF scores, which were significantly higher in the control group (I: 23.21 ± 14.08 and C: 32.31 ± 16.14; $p = 0.03$) (Table 1).

3.3. Clinical Response. In terms of changes within each group, we recorded a significant improvement in IIEF (from 23.21 to 46.57) and HISD (from 59.39 to 64.45) scores over time in the intervention group. However, this was not seen in the control group (IIEF: from 32.31 to 36.08; HISD: from 62.96 to 57.33). These changes are depicted in Figure 2. Through repeated measures analysis, we found that during the study period, the intervention led to a significant boost in both the IIEF and HISD scores ($p = 0.001$; $F = 13.47$ and $p = 0.044$; $F = 4.10$, respectively) when compared with the control (Figure 2).

4. Discussion

To our knowledge, this is the first clinical trial study in Iran and the world that examined the efficacy of date palm pollen in the management of male sexual dysfunction after coronary artery bypass graft. In our study, both libido and sexual function after DPP consumption improved significantly compared to a placebo. This study demonstrated that DPP leads to a significant increase in IIEF and HISD in males with sexual dysfunction.

The popularity of varied complementary and alternative medicine modalities, particularly herbal medicine in the prevention, treatment, or rehabilitation of patients with cardiovascular diseases, have been increased over recent decades [22, 23]. Coronary artery bypass graft surgery is one of the treatments of cardiovascular diseases [24]; however, it affects different aspects of patients' quality of life and leads to problems like sexual dysfunction, so more than half of these people experience disorders in sexual life [25]. Traditional Persian medicine (TPM) has introduced DPP as a safe and effective treatment to improve sexual satisfaction in males.

A number of trial and experimental studies indicated the benefits of this herbal product on male sexual performance. For example, as thyroid hormones have an important role in gonads' development and growth, a disorder in the thyroid gland can alter spermatogenesis [26]. El-Kashlan et al. did an investigation on male rats with thyroid dysfunction and concluded that DPP extract could neutralize adverse effects of fluctuation in the level of thyroid hormones and consequently improve fertility that may be due to its antioxidant profile. In addition, the benefits of DPP extract on the hypothalamic-pituitary-testicular axis maintained steroidogenesis that regulates testicular performance [27].

In line with the current study, Marbeen et al. also reported that DPP improved fertility rate in adult men with sexual dysfunction through a significant increase in serum level of testosterone, LH, and estradiol, number and motility of sperm, and diameter of seminiferous tubules [28]. Moreover, Bahmanpour et al. evaluated the effect of different doses of date palm gemmule on seven rats groups, which results presented sperm quality, spermatogenesis, and testis morphology promoted in groups treated with date palm gemmule [29].

An additional study by Mohamed and collaborators showed that the administration of DPP suspension solely or in combination with bee pollen in diabetic rats improved the disturbance in pituitary-testicular axis and testicular histology, which

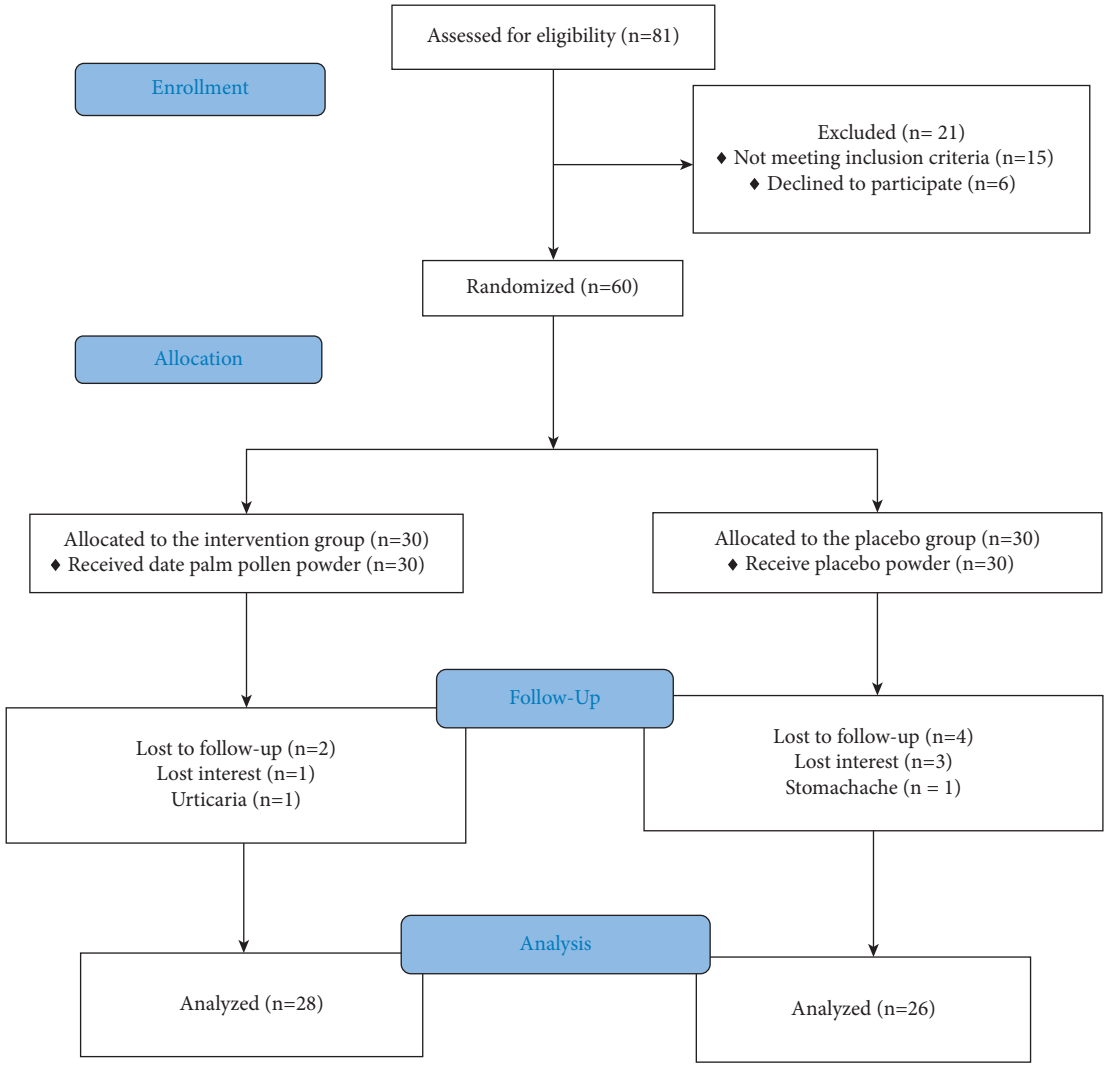

Figure 1: Flow diagram of the patients' enrolment, allocation, follow-up, and final analysis.

Table 1: Baseline characteristics of patients in the study groups.

Variables	Date palm pollen ($n = 30$)	Placebo ($n = 30$)	p value
Age (years)	61.93 ± 6.36	59.38 ± 8.91	0.23
Body mass index (kg/m^2)	28.07 ± 4.30	27.00 ± 3.84	0.39
Fasting blood sugar (mg/dL)	109.04 ± 21.53	106.50 ± 27.16	0.71
Smoker (yes/no)	15/13	13/13	0.74
Systolic blood pressure (mmHg)	113.61 ± 10.3	116.67 ± 12.4	0.05
Diastolic BP (mmHg)	75.97 ± 4.1	76.39 ± 5.8	0.33
Triglycerides (mg/dL)	174.13 ± 121.02	156.24 ± 64.12	0.54
LDL-cholesterol (mg/dL)	88.58 ± 34.10	91.10 ± 33.53	0.50
Cardiac ejection fraction (%)	44.60 ± 10.19	44.05 ± 8.30	0.84
International Index of Erectile Function score	23.21 ± 14.08	32.31 ± 16.14	0.03
Hurlbert Index of Sexual Desire score	59.39 ± 13.09	62.96 ± 12.50	0.31

Categorical variables are represented as a percentage (%) and continuous data are represented as mean ± SD.

may be because of antiglycemic and antioxidant properties of these suspensions as well as promoted sexual desire [30]. Moreover, Baharara et al. demonstrated that exposure to electromagnetic fields induced significantly reduced sperm count, viability, and motility compared with controls, which intake of DPP could fight against these adverse effects [31].

The DPP extract possesses antitoxic properties plus antioxidant and anti-inflammatory effects. For instance, cadmium (Cd) is a toxic metal that can damage various organs, particularly the reproductive system [32]. Some animal studies demonstrated that treatment with DPP lowers the level of Cd in testis and consequently attenuates reproductive damage

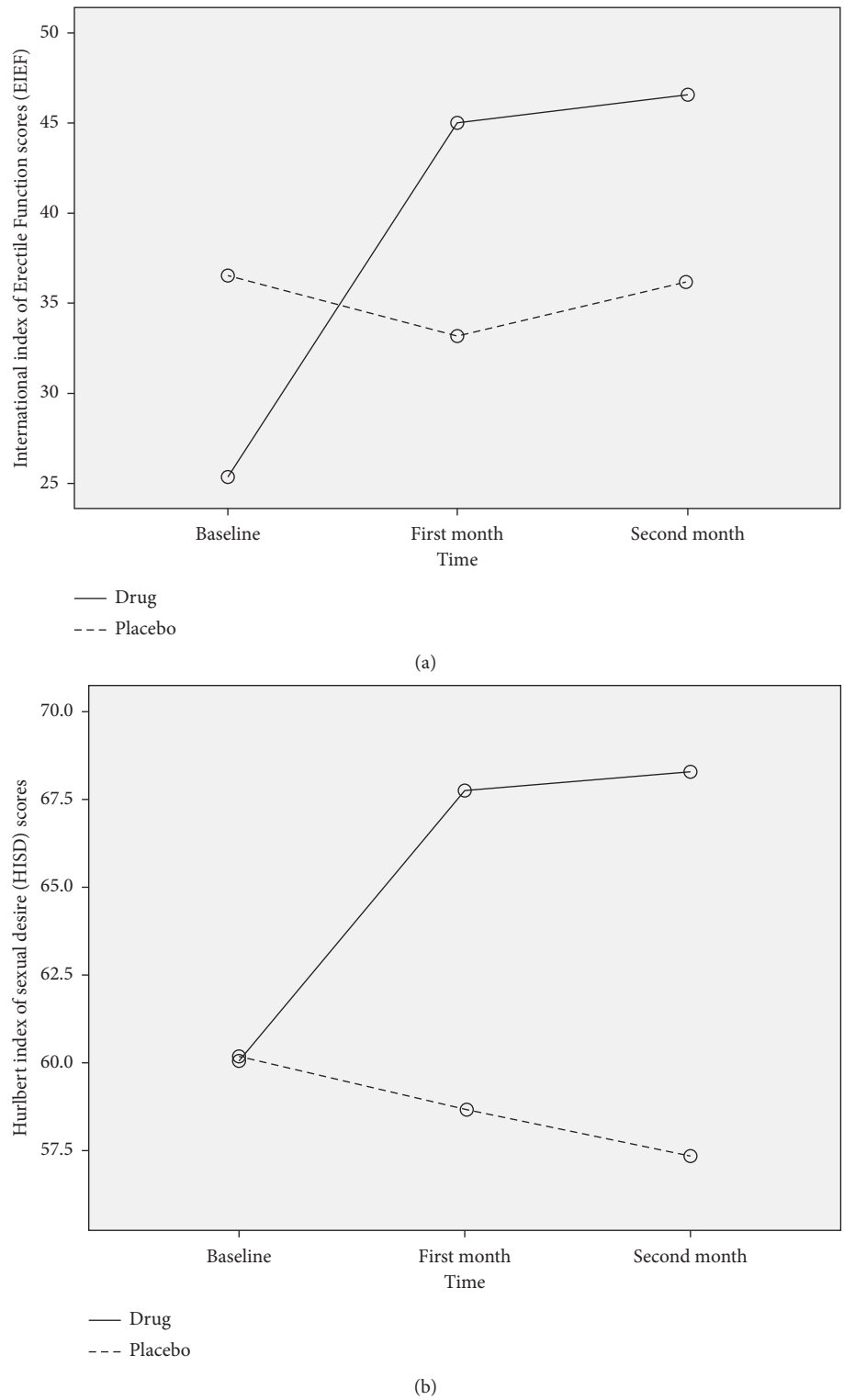

Figure 2: Changes in indices related to sexual function during the study period in each group.

[33, 34]. In another study, date palm pit powder reversed spermatotoxicity induced by nicotine considerably [35].

Besides, Forouzannia et al. showed that sexual dysfunction increased significantly after CABG surgery due to a drop in sex hormone levels [36]. Hence, an increase in the level of sex hormones may be an effective approach in treating this disorder. Research by Shariati et al. concluded that date palm pit powder rose the level of testosterone and declined dihydrotestosterone level through suppressing 5-reductase enzyme as it contains some effective ingredients including palmitic, stearic, linoleic, and oleic acids [37]. Similarly, a study revealed that daily administration of

120 mg/kg DPP suspension promoted reproductive activity and testosterone level of serum and intratesticular [38]. Additionally, a trial in infertile men demonstrated that coadministration of DPP and zinc sulphate helped to promote serum level of LH, FSH, and testosterone hormones, count and motility of sperm, and sexual desire without any toxicity [39].

In addition, Daoud et al., in their animal study, revealed that DPP had a protective effect against myocardial infarction and isoproterenol-induced cardiac remodeling in rats through the inhibition of angiotensin-converting enzyme activity [18]. Hence, another probable mechanism of action of this herb in the improvement of sexual dysfunction following CABG may be related to its cardiotonic effect. Recently, erectile dysfunction is considered as a marker of vascular health. Nitric oxide (NO), which is released in the endothelium of vascular, has a pivotal role in the penile erection [40]. Date and its drives are rich sources of cardiovascular-protective components, such as polyphenols, potassium, magnesium, folate, selenium, fiber, and vitamin C. A molecular mechanism is claimed that polyphenols promote vasodilation via the generation of NO and inhibiting free radicals [41].

There are some strengths in this study including following the principles of clinical trials and using valid and reliable tools for outcome assessment; however, some limitations should be considered.

Firstly, low sample size was recruited, while it was computed with a standard formula. But, concerning the findings of the present study, it is proposed that large-scale studies should be conducted. Secondly, the lack of drug dose adjustment and dosing assessment represent critical limitations and should be taken into account by researchers in subsequent investigations. A short duration of follow-up is another drawback of the present work. Finally, some confounders, such as smoking and diabetes, can influence the efficacy of the intervention; nevertheless, we distributed patients with these conditions approximately equally in order to lower bias.

5. Conclusion

According to the results of this study, it seems that date palm pollen can, to some extent, relieve the symptoms of male sexual dysfunction in patients who have undergone CABG. However, further studies with larger sample sizes and longer follow-ups are needed to deepen our understanding of the efficacy and safety of this herbal product.

Data Availability

The data used to support this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank the research vice chancellery of Tehran University of Medical Sciences for financial support.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3044463, 15 pages
https://doi.org/10.1155/2022/3044463

Research Article

Systematic Pharmacology-Based Strategy to Explore the Molecular Network Mechanism of Modified Taohong Siwu Decoction in the Treatment of Premature Ovarian Failure

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Received 3 October 2021; Accepted 13 December 2021; Published 21 January 2022

Academic Editor: Yong Wang

Objective. To explore the molecular network mechanism of modified Taohong Siwu Decoction (MTHSWD) to interfere with premature ovarian failure based on systematic pharmacological strategy. *Methods*. The network pharmacology strategy was used to explore the potential mechanism of MTHSWD intervention in POF, and then it was verified through animal experiments. Mouse zona pellucida 3 was used as an antigen to subcutaneously immunize BALB/c female mice to establish an immune POF model. Mice were divided into MTHSWD low-, medium-, and high-dose groups, positive control group, model group, and normal group. After 30 days of drug intervention, ovarian tissue was taken for pathological hematoxylin-eosin (HE) staining, and immunohistochemical methods were used to detect the expression of TGF-β1 and TGF-βRII and Smad2/3 protein expression in follicular wall granular cells and ovarian tissue, respectively. *Results*. Network pharmacology studies have shown that MTHSWD may interfere with the TGF-β signaling pathway. Animal experimental research shows that, compared with the model group, the number of ovarian mature follicles in the MTHSWD groups and the positive group was significantly increased, and the number of atresia follicles decreased. Immunohistochemistry showed that, compared with the control group, the expression of TGF-β1, TGF-βRII, and Smad2/3 in the follicular wall granulosa cells and ovarian tissues of MTHSWD groups was significantly higher than that of the model group ($P < 0.05$). *Conclusion*. MTHSWD may improve the ovarian function of POF mice by upregulating the protein expression of granulosa cells TGF-β1, TGF-βRII, and Smad2/3.

1. Introduction

Premature ovarian failure (POF) refers to a disease that causes amenorrhea, infertility, menopause, and genitourinary symptoms before the age of 40 due to ovarian failure [1]. Epidemiological studies have shown that the incidence rate in women is about 1% [1, 2]. Hormone detection indicators showed that it has hypogonadotropic hypogonadism [3]. At present, hormone replacement therapy (HRT) is the most popular choice for women with POF to get rid of menopausal syndrome [4]. However, HRT has its own indications and contraindications [5]. For example, unexplained vaginal bleeding, acute liver injury, liver insufficiency, vascular embolism, and breast cancer are contraindications to HRT [6, 7]. In alternative medicine, ancient Chinese medicine has accumulated a lot of clinical experience. With the increase of clinical evidence, TCM has shown a good effect in the treatment of POF [8–10]. The systematic review and meta-analysis of Bushen Huoxue (nourishing the kidney and promoting blood circulation) Chinese herbal medicine for POF showed that compared with the western medicine group, Chinese medicine may improve the total effective rate, menstrual improvement rate, symptom score improvement, and so on, and the incidence of adverse

reactions is low [11, 12]. Nourishing the kidney and promoting blood circulation, Chinese medicine may also reduce the level of follicle-stimulating hormone, increase the level of estrogen, reduce clinical symptoms, promote the growth of antral follicles, increase the volume of the ovary, increase the blood flow speed and blood flow pulsation of the ovary, and reduce the blood flow resistance of the ovary. It may also reduce the level of osteocalcin and serum alkaline phosphatase in the body, increase the level of calcitonin, delay the occurrence of osteoporosis, reduce triglycerides and total cholesterol, and reduce the risk of cardiovascular and cerebrovascular diseases. It may also increase the level of CD3+ and CD4+ lymphocytes, improve the body's immunity, and reduce the recurrence rate after drug withdrawal [13–16].

Siwu Decoction [17] was first published in the "Secret Recipe of Xianshou Li Shang" by Lin Taoist in the Tang Dynasty. It was used to treat traumatic diseases, iron beating injuries, blood loss, and blood stasis. In the Song Dynasty, "Tai Ping Hui Min He Ji Ju Fang" began to develop Siwu Decoction into a special prescription for the treatment of gynecological diseases, enriching blood, promoting blood circulation, and regulating menstruation [18, 19]. Since then, physicians of the past generations have elaborated and exerted the use of Siwu Decoction in the treatment of gynecological diseases. They believed that the effect of Siwu Decoction was to enrich blood, promote blood circulation, regulate menstruation, and treat many diseases caused by blood deficiency and blood addiction [19]. On this basis, the addition and subtraction changes have formed many Siwu Decoctions as the core to treat women's abdominal pain during menstruation, that is, Siwu Decoction prescriptions for gynecological blood stasis dysmenorrhea [20]. Among them, Taohong Siwu Decoction is the main representative prescription of nourishing blood and promoting blood circulation in Siwu Decoctions. Recent studies have shown that Modified Taohong Siwu Decoction (MTHSWD) combined with HRT may significantly improve ovarian function and improve the clinical efficacy of the treatment of POF [21]. MTHSWD can improve the symptoms of late menstruation, decreased menstrual flow, irritability, and vaginal dryness in patients with decreased ovarian reserve, reduce FSH levels, improve ovarian blood supply, and increase the number of antral follicles [22]. In addition, the treatment of infertility patients with Shou Tai Wan combined with Taohong Siwu Decoction can effectively improve the quality of pregnancy and improve the immune function and ovarian function of the body [23]. Further studies have shown that Siwu Decoction can improve ovarian reserve and improve follicular dysplasia [24, 25].

However, the molecular biological network mechanism of MTHSWD in the treatment of POF is still not clear. Network pharmacology combines the ideas of systems biology and multidirectional pharmacology. It analyzes the mechanism of action of the effective ingredients of drugs by constructing a complex network within "component targets-pathways-disease," which turns pharmacological research from the traditional research concept of finding a single target to the network comprehensive analysis thinking [26, 27]. In network pharmacology research, the same disease can be regulated by different genes at different stages of development, and some genes can also play a central regulatory role in multiple diseases. This coincides with the traditional Chinese medicine theory of "different treatment of the same disease" and "treatment of the same disease at the same time" [28, 29]. Therefore, this study hopes to explore the molecular biological network effect and the basis of pharmacodynamic active ingredients of MTHSWD in the treatment of POF by combining systemic pharmacology and experimental pharmacology strategies, so as to provide a scientific basis for the clinical application of MTHSWD.

2. Materials and Methods

2.1. Potential Compounds and Targets of MTHSWD and POF Gene Collection. The potential components and targets of MTHSWD were searched from TCMSP (https://tcmsp-e.com/) [30] according to the pharmacokinetic parameters of chemical components (absorption, distribution, metabolism, and excretion (ADME)). The standard was oral bioavailability (OB) $\geq 30\%$, Caco-2 parameter > -0.4, and drug-like activity (DL) ≥ 0.18 [30]. The POF genes were collected to search the Online Mendelian Inheritance in Man (OMIM, http://omim.org/) [31] and GeneCards (http://www.genecards.org/) [32]. The official gene symbol of MTHSWD potential targets and POF genes were collected from UniProt KB (https://www.uniprot.org/uniprot/), with the species restricted to human (Table S1 and Table S2).

2.2. Network Construction and Analysis Methods. The String database (https://string-db.org/) was utilized to collect the PPI data of MTHSWD targets and POF genes [33]. The Cytoscape 3.7.2 was utilized to construct and analyze the MTHSWD-POF PPI network [34]. The DAVID ver 6.8 (https://david.ncifcrf.gov/) was utilized to perform gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis [35].

2.3. Experimental Materials

2.3.1. Experimental Animal. Sixty-seven healthy female BALB/c mice (SYXK (Xiang) 2013–0005), aged 7–8 weeks and weighing 20–22 g, were selected. The vaginal exfoliated cell smear showed that the estrus cycle was normal. Mice were kept in a clean environment, room temperature 18~22°C, relative humidity 40%~60%, and light for 12 h.

2.3.2. Experimental Drugs. MTHSWD is composed of *Rehmanniae Radix Praeparata* (Di Huang) 15 g, *Polygonatum sibiricum* Red. (Huang Jing) 12 g, *Cornus officinalis* Sieb. et Zucc. (Shan Zhu Yu) 12 g, *Lycium barbarum* L. (Gou Qi Zi) 12 g, *Angelica sinensis* (Oliv.) Diels (Dang Gui) 15 g, *Paeonia lactiflora* Pall. (Bai Shao) 12 g, *Ligusticum chuanxiong* Hort. (Chuan Xiong) 9 g, *Salvia miltiorrhiza* Bge. (Dan Shen) 12 g, and *Prunus persica* (L.). Batsch (Tao Ren) 9 g. Those medical materials were purchased from Pharmacy Department of the First Affiliated Hospital of Hunan University of Chinese Medicine. The Department of Pharmacy, the First Affiliated

Hospital of Hunan University of Chinese Medicine, identified, screened, washed, processed, sliced, dried, and crushed the source and variety of the same batch of Chinese medicinal materials. The batch number was 20150701. Estradiol valerate tablets were obtained from Bayer Healthcare Co., Ltd., National Medicine Standard J20130009.

2.3.3. Instruments and Reagents. Mouse zona pellucida polypeptide solution and the 330–342 amino acid sequence of mouse zona pellucida 3 (ZP3) (NSSSSQFQIHGPR), with an analytical purity of 91.5%, were obtained from Hangzhou Zhongpi Biochemical Co., Ltd. (P00105). Freund's complete adjuvant (F5881) and Freund's incomplete adjuvant (F5506) were obtained from American Sigma company. Rabbit anti-mouse TGF-β1, TGF-βRII, and Smad2/3 polyclonal antibodies were obtained from Wuhan Boster Bioengineering Co., Ltd. (BA0290, BA0526, BA1395). TRIzol reagent was obtained from Thermo Fisher Technology (China) Co., Ltd. [lot number 267309]. MonScri RTIII All-in-One Mix with sdDNase was obtained from Mona Biotechnology Co., Ltd. (lot number 130449). Universal SYBR qPCR Master Mix Universal Real-Time PCR Kit was obtained from China Biosharp Company (lot number 70090100). Horseradish peroxidase- (HRP-) labeled secondary antibody was obtained from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (ZDR-5118). Whole protein extraction kit was obtained from Nanjing KGI Biotechnology Development Co., Ltd. (KGP2100). BCA protein quantitative detection kit was obtained from Shanghai Shenggong Biological Engineering Co., Ltd. (C503031). LEICADMLB2 binocular microscope and LEICARM2255 automatic rotary microtome were obtained from German LEICA company.

2.4. Quality Control of MTHSWD

2.4.1. Preparation of Sample. Control solution preparation: morroniside 4.07 mg, loganin 1.16 mg, and paeoniflorin 2.34 mg were accurately weighed into a 10 mL volumetric flask. Then methanol : water (1 : 1) was added, dissolved, and diluted to the mark and filtered through a 0.45 μm filter membrane to make a mixed reference solution.

MTHSWD solution preparation: the medicinal materials of MTHSWD were placed in a round-bottomed flask, 1000 mL of pure water was added, and the mixture was refluxed, extracted for 1.5 h, and filtered. Then, 1000 mL of pure water was added, refluxed for extraction for 1 h, and filtered. The two filtrates were combined and concentrated to 500 mL by rotary evaporation. 2 mL of the concentrated filtrate was added into a 5 mL volumetric flask, and anhydrous methanol was added to the volume. Then, the filtrate was ultrasonically processed (power 300 W, frequency 40 Hz) for 30 min and filtered by suction. The subsequent filtrate was filtered with a 0.45 μm organic microporous filter membrane.

2.4.2. HPLC Condition. Chromatographic column was Hypersil ODS C18 chromatographic column (200 mm $\times$ 4.6 mm, 5 μm). Flow rate was 1.0 mL/min. Detection wavelength was 237 nm. Mobile phase was acetonitrile (A)-0.1% phosphoric acid solution (B) gradient elution. Injection volum was 10 μL. Column temperature was 30°C. The contents of those components are morroniside 4.612 ± 0.013 mg/g, loganin 1.291 ± 0.003 mg/g, and paeoniflorin 3.084 ± 0.009 mg/g (Figure S2).

2.5. Experimental Methods

2.5.1. Experimental Grouping, Modeling, and Intervention. According to the random number table method, 67 female BALB/c mice were divided into (N) 10 mice as the blank group, and the remaining 57 were prepared for modeling. Add 6 mg of ZP3 transparent polypeptide powder, add 6 mL of double-distilled water to make a solution, and make a 1 : 1 immune reagent with Freund's complete adjuvant; it is formulated with Freund's incomplete adjuvant at a ratio of 1 : 1 to prepare immune enhancement reagents (both in the form of porous white viscous oil). Mice were given 0.15 mL of immune reagent injected into the soles of both hind feet and subcutaneously in the abdominal cavity. 14 days later, 0.15 mL of immunoenhancing reagent was injected again into the soles of both hind feet and subcutaneously in the abdominal cavity to establish an immune POF model. Mice in the blank group was injected with 0.15 mL of normal saline into the same area. Beginning on the 8th day after modeling, all mice were subjected to cervical mucus smears and HE staining of vaginal exfoliated cells at 9 : 00 every morning. The observation lasted for 12 days and the estrus cycle of the mice was checked. The results showed that 5 mice still did not have any estrous cycle disorder and were eliminated.

In the remaining 52 mice, 2 were randomly selected to observe follicular morphology to confirm the POF model. The other 50 mice were randomly divided into model group, positive control group, MTHSWD low-dose group, middle-dose group, and high-dose group, with 10 mice in each group. The dosages of MTHSWD low-, medium-, and high-dose groups were 0.54 g, 1.08 g, and 2.16 g of crude drug/mL, respectively. The low, medium, and high doses of MTHSWD were converted by 1, 2, and 4 times the adult clinical dose according to the "Equivalent Dose Table for Conversion of Human and Animal Body Surface Areas," respectively [36, 37]. The positive control group was given 0.03 mg of Estradiol. The intragastric administration was started 1 week after the model was established. Both the blank group and the model group were given 0.3 mL of normal saline. The intervention lasted 30 days and was given by gavage. The mice were weighed every 7 days.

2.5.2. Pathological Observation. The ovaries were fixed in 4% paraformaldehyde solution, dehydrated with gradient alcohol, embedded in paraffin, sectioned, deparaffinized, and stained with the conventional hematoxylin-eosin (HE) method.

2.5.3. Expression of TGF-β1, TGF-BβRII, and Smad2/3 Protein in Ovarian Tissue Detected by Immunohistochemistry. The ovaries were fixed in 4%

paraformaldehyde solution, dehydrated with gradient alcohol, embedded in paraffin, sectioned, and deparaffinized. Then the expression of TGF-β1, TGF-βRII, and Smad2/3 protein in ovarian tissue was detected by immunohistochemistry SP two-step method. Then the areas under the microscope were randomly selected and analyzed with Image-Pro Plus 6.0. The cumulative optical density (IOD) and area and mean density were measured according to the standard operation method.

2.5.4. Determination of Smad2, Smad3, and Smad7 mRNA Expression in Ovarian Tissue. The total RNA of mouse ovarian tissue was extracted according to the TRIzol method, and the first-strand cDNA was synthesized by reverse transcription, and Smad2, Smad3, and Smad7 mRNA were detected according to the Real-Time PCR method, and β-actin was used as the internal control. The reaction was prepared according to the operating instructions of the kit, and the primer sequence was synthesized by Shenggong Bioengineering (Shanghai) Co., Ltd. The reaction conditions were 95°C predenaturation for 2 min, 95°C denaturation for 15 s, 60°C annealing for 20 s, 72°C extension for 30 s, and 40 cycles of amplification. The 2-△△Ct method was used to analyze mRNA expression levels (Table 1).

2.6. Statistical Analysis. The SPSS 21.0 statistical software was used for analysis, and the data were expressed as mean ± standard deviation. Multigroup analysis was performed by single-factor analysis of variance. $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. MTHSWD Potential Targets and POF Targets. A total of 247 MTHSWD potential targets were obtained and 754 POF genes were collected from OMIM and GeneCards. The relationship among compounds and targets of MTHSWD is shown in Figure 1. This network consists of 100 compound nodes, 247 potential target nodes, and 1527 edges. In this network, the targets near the center can be regulated by more components than targets near the periphery.

3.2. MTHSWD-POF PPI Network Analysis. The MTHSWD potential targets, POF genes, and the PPI data were input into Cytoscape 3.7.2 to construct MTHSWD-POF PPI network. This network consists of 823 nodes (578 POF gene nodes, 183 MTHSWD potential target nodes, and 62 MTHSWD-POF target nodes) and 19442 edges. The targets are arranged according to degree from large to small, and the top 20 targets can be divided into 3 categories: (1) MTHSWD potential targets: JUN, EGFR, IL1B, EGF, HIF1A, and FOS; (2) POF genes: ACTB, ALB, INS, and IGF1; (3) MTHSWD-POF targets: TP53, AKT1, IL6, MYC, TNF, ESR1, VEGFA, STAT3, CASP3, and PTEN (Figure 2).

3.3. Enrichment Analysis of MTHSWD-POF PPI Network

3.3.1. Biological Processes of MTHSWD-POF PPI Network. The biological processes include positive regulation of pathway-restricted SMAD protein phosphorylation, positive regulation of transcription from RNA polymerase II promoter, response to drug, SMAD protein signal transduction, positive regulation of DNA-templated transcription, aging, response to ethanol, positive regulation of gene expression, response to hypoxia, positive regulation of cell proliferation, cholinergic synaptic transmission BMP signaling pathway, negative regulation of apoptotic process, ovarian follicle development, regulation of apoptotic process, transforming growth factor beta receptor signaling pathway, and signal transduction (Figure 3) (Table S3).

3.3.2. Cell Components of MTHSWD-POF PPI Network. The cell components include extracellular space, cytosol, extracellular region, acetylcholine-gated channel complex, receptor complex, nucleoplasm, integral component of plasma membrane, postsynaptic membrane, membrane raft, external side of plasma membrane, transcription factor complex, cell surface, plasma membrane, neuron projection, and cytoplasm (Figure 4) (Table S3).

3.3.3. Molecular Function of MTHSWD-POF PPI Network. The molecular function includes transforming growth factor beta receptor binding, growth factor activity, enzyme binding, cytokine activity, protein binding, protein homodimerization activity, drug binding, acetylcholine binding, acetylcholine-activated cation-selective channel activity, hormone activity, identical protein binding, acetylcholine receptor activity, ligand-gated ion channel activity, and protein heterodimerization activity (Figure 5) (Table S3).

3.3.4. Signaling Pathway of MTHSWD-POF PPI Network. The signaling pathway includes neuroactive ligand-receptor interaction, TGF-beta signaling pathway, FoxO signaling pathway, ovarian steroidogenesis, TNF signaling pathway, prolactin signaling pathway, apoptosis, PI3K-Akt signaling pathway, T cell receptor signaling pathway, steroid hormone biosynthesis, HIF-1 signaling pathway, cytokine-cytokine receptor interaction, neurotrophin signaling pathway, p53 signaling pathway, NF-kappa B signaling pathway, and NOD-like receptor signaling pathway (Figures 6 and 7). The TGF-beta signaling pathway was shown in Figure 8 (Table S3).

3.4. Morphological Changes of Ovarian Tissue. In model group, the ovarian volume was reduced; a few primary follicles and growing follicles were seen in the ovarian cortex. The number of mature follicles was significantly reduced, the atretic follicles increased, and the secondary follicles were loosely arranged. In MTHSWD medium- and low-dose groups, compared with the model group,

Table 1: Primer sequence of RCR.

Sequence	Upstream 5′–3′	Downstream 5′–3′
TGF-β1	CCAAGGAGACGGAATACAGG	GTGTTGGTTGTAGAGGGCAAG
Smad 2	AGCCGCCCGAAGGGTA	AGACCCACCGGCTGATTTTT
Smad 3	CGAGCTGCCTCTGTGCG	CCATCCAGTGACCTGGGGAT
β-Asctin	CGCGAGTACAACCTTCTTGC	CGTCATCCATGGCGAACTGG

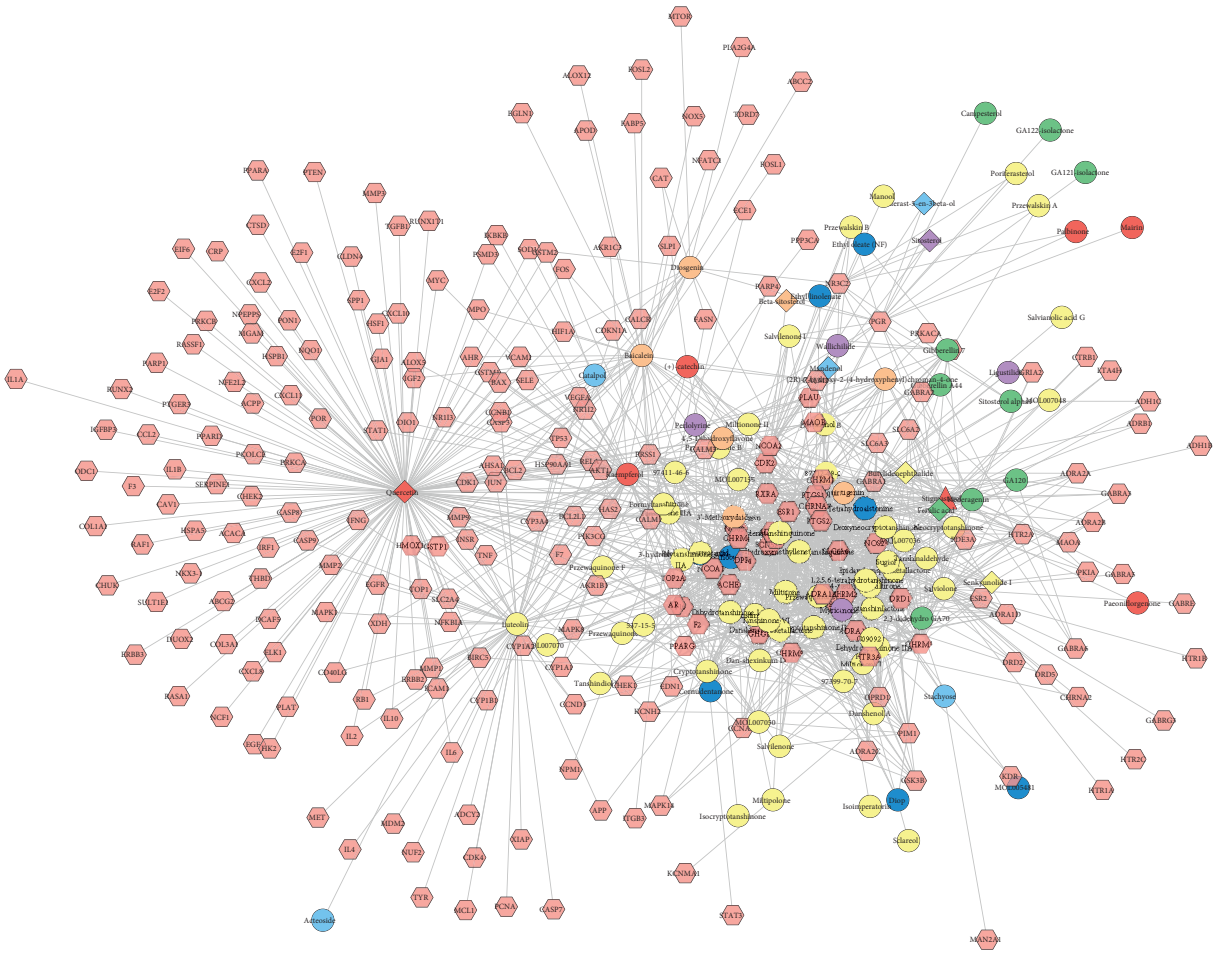

Figure 1: Compound-compound target of MTHSWD (red, orange, yellow, green, blue, indigo, and purple circles stand for components of *Paeonia lactiflora* Pall., *Polygonatum sibiricum* Red., *Salvia miltiorrhiza* Bge., *Prunus persica* (L.) Batsch, *Rehmanniae Radix Praeparata*, *Cornus officinalis* Sieb. et Zucc., and *Ligusticum chuanxiong* Hort., respectively. Red diamond stands for component of *Lycium barbarum* L. Orange diamond stands for common component of *Polygonatum sibiricum* Red., *Prunus persica* (L.) Batsch, *Rehmanniae Radix Praeparata, Cornus officinalis* Sieb. et Zucc., and *Angelica sinensis* (Oliv.) Diels. Yellow diamond stands for the common components of *Angelica sinensis* (Oliv.) Diels and *Ligusticum chuanxiong* Hort. Green diamond stands for the common components of *Angelica sinensis* (Oliv.) Diels and *Lycium barbarum* L. Blue diamond stands for the common components of *Ligusticum chuanxiong* Hort. and *Cornus officinalis* Sieb. et Zucc. Indigo diamond stands for the common components of *Cornus officinalis* Sieb. et Zucc. and *Salvia miltiorrhiza* Bge. Purple diamond stands for the common components of *Paeonia lactiflora* Pall., *Polygonatum sibiricum* Red., *Cornus officinalis* Sieb. et Zucc., and *Ligusticum chuanxiong* Hort. Red triangle stands for the common components of *Rehmanniae Radix Praeparata, Cornus officinalis* Sieb. et Zucc., and *Angelica sinensis* (Oliv.) Diels.).

the number of primary and mature follicles increased and the number of atretic follicles decreased. In MTHSWD high-dose group and the positive control group, a large number of primary follicles and antral follicles are seen in the ovarian cortex, and there are many near-mature follicles, and the corpus luteum increases and grows well (Figure 9).

3.5. Expression of TGF-β1, Smad2, and Smad3 mRNA in Ovarian Tissue. Compared with the normal group, the expression levels of TGF-β1, Smad2, and Smad3 mRNA in the ovarian tissue of the model group were significantly decreased ($P < 0.05$). Compared with the model group, the expression levels of TGF-β1, Smad2, and Smad 3 mRNA in the ovarian tissue of the MTSWD group increased ($P < 0.05$) (Figure 10).

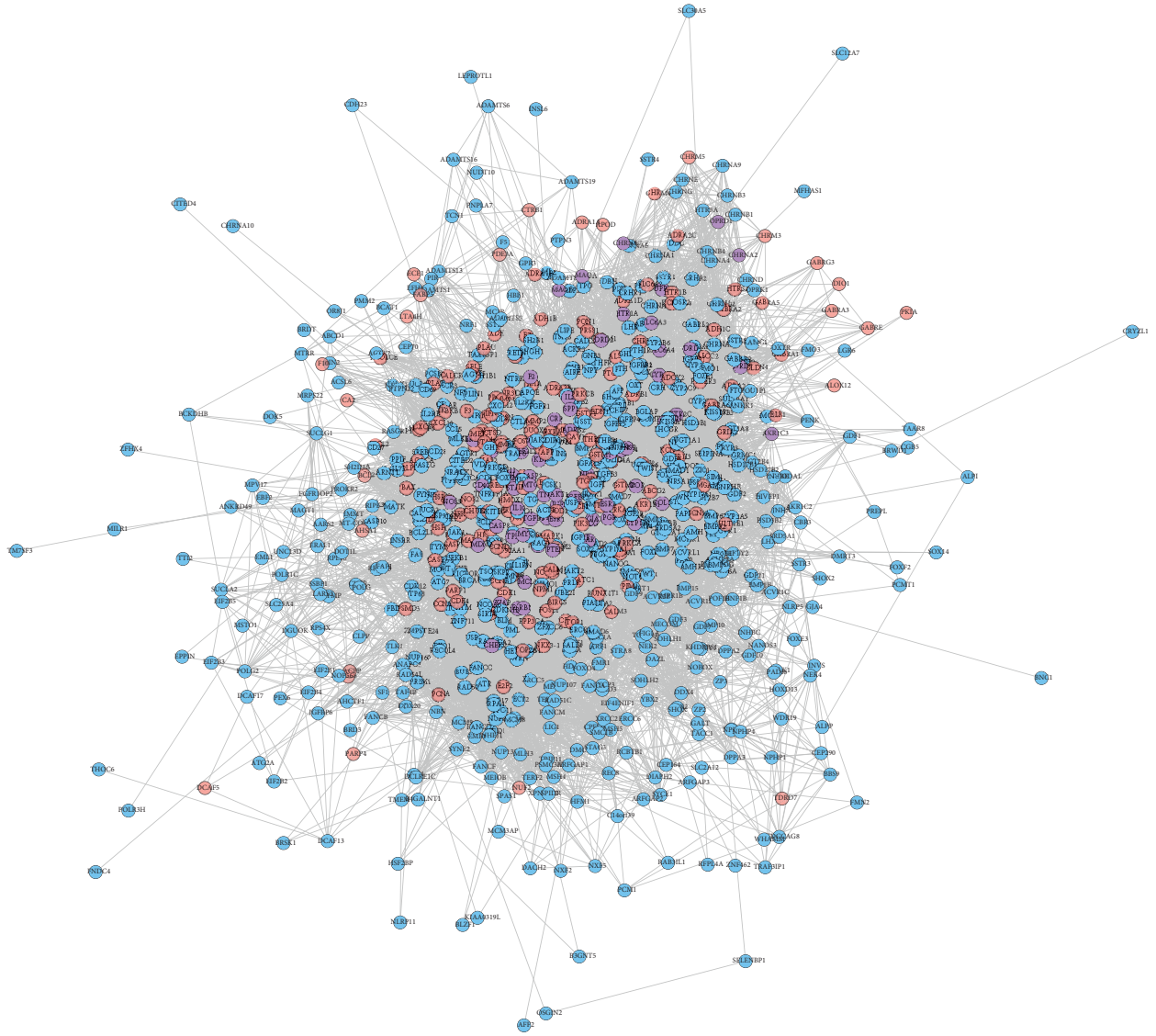

Figure 2: MTHSWD-POF PPI network (blue, pink, and purple circles stand for POF gene, MTHSWD target, MTHSWD-POF target, resp.).

3.6. Expression of TGF-β1, TGF-BβRII, and Smad2/3 in Granulosa Cells of Ovarian Follicle Wall Detected by Immunohistochemistry. In the model group, TGF-β1, Smad2/3, and TGF-βRII were weakly expressed in the cytoplasm of granulosa cells of antral follicles. In the MTHSWD low-dose and middle-dose groups, TGF-β1 and Smad2/3 were strongly expressed in some areas of antral follicles. In the MTHSWD high-dose group, positive control group, and blank group, TGF-β1 and Smad2/3 were strongly expressed in most areas of antral follicles. TGF-βRII was strongly expressed in some areas of the antral follicles of MTHSWD low-, medium-, and high-dose groups, positive control group, and blank group (Figures 11–13).

Compared with the model group, the expression of TGF-β1, TGF-βRII, and Smad2/3 in the MTHSWD medium-dose, high-dose group, positive control group, and blank group increased ($P < 0.05$). There was no difference in the expression of TGF-β1, TGF-βRII, and Smad2/3 in the MTHSWD medium-dose and high-dose groups compared with the positive control group ($P < 0.05$) (Figure 14).

4. Discussion

At present, it is more recognized that the factors that cause POF involve genetics, immunity, infection, iatrogenic, psychological, and other factors. In the treatment of POF, hormone replacement or combined use of ovulation induction and assisted reproductive technology is also used for those who have fertility requirements [38]. Previous studies have shown that TCM, which focuses on invigorating the kidney, can reduce the damage of cisplatin to the ovary, promote follicular development, and improve and enhance ovarian function by regulating the content of various hormones in the serum of POF rat models and changing the expression of apoptotic cell-related proteins [39]. Further studies have shown that the Chinese

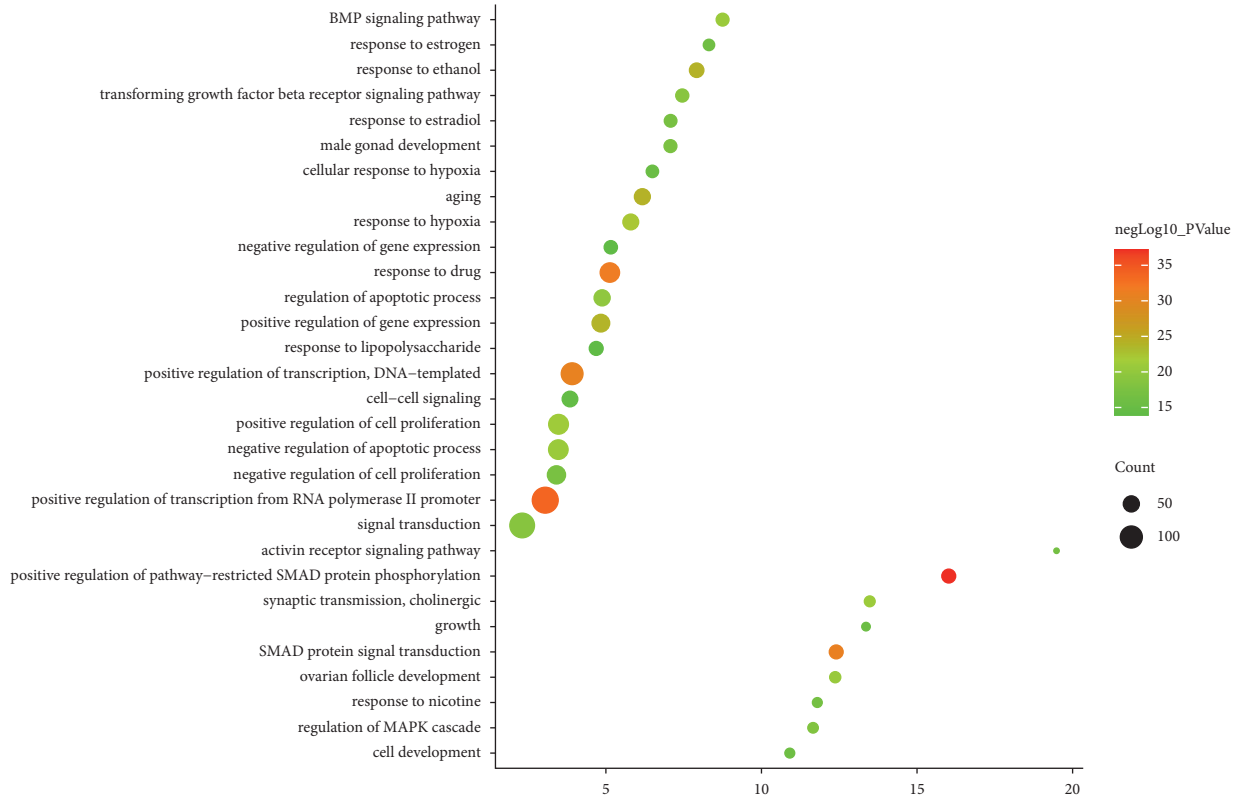

Figure 3: Bubble chart of biological processes (*X*-axis stands for fold enrichment).

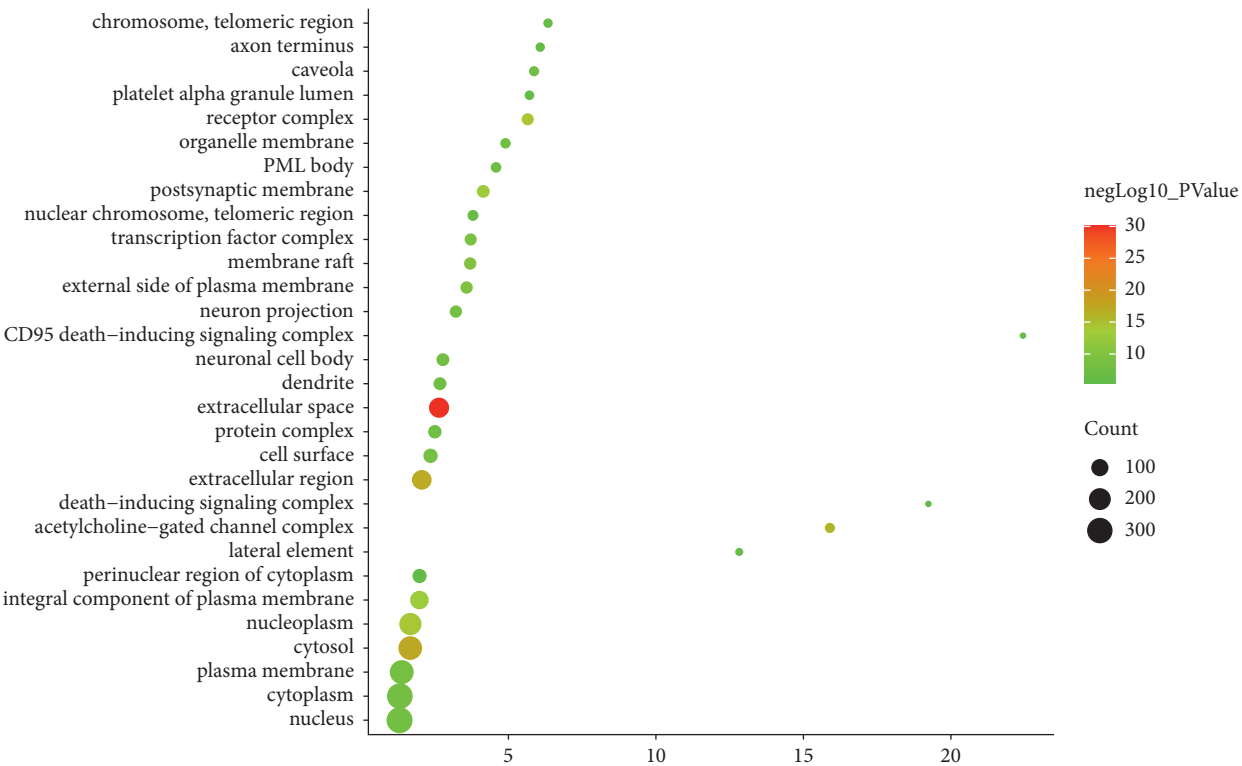

Figure 4: Bubble chart of cell components (*X*-axis stands for fold enrichment).

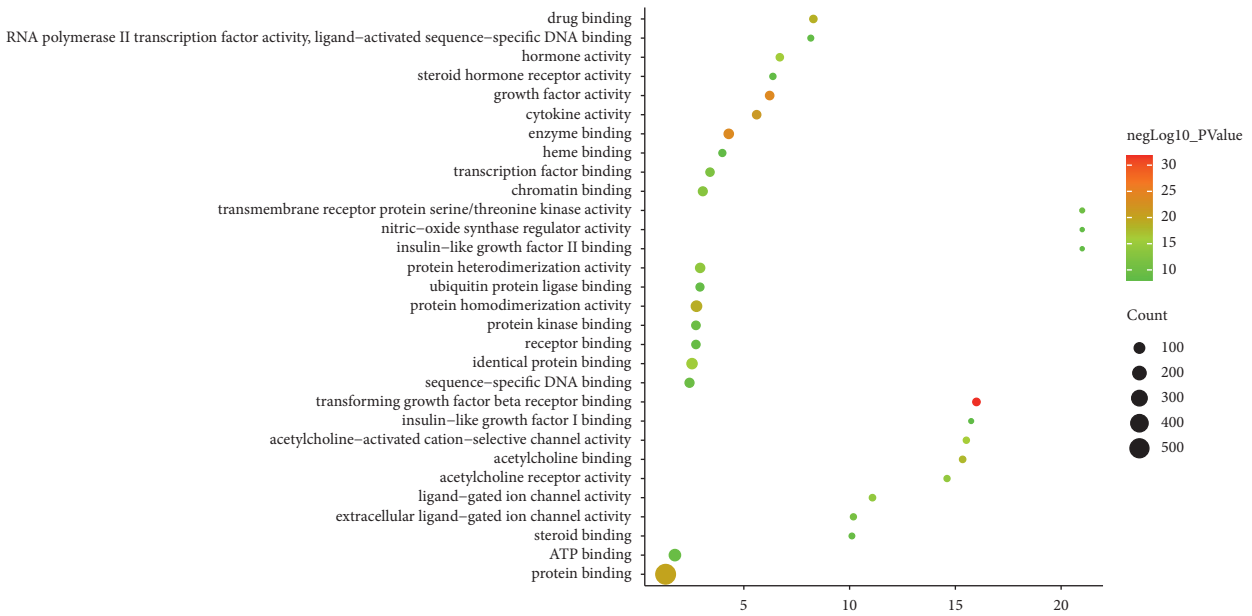

Figure 5: Bubble chart of molecular function (*X*-axis stands for fold enrichment).

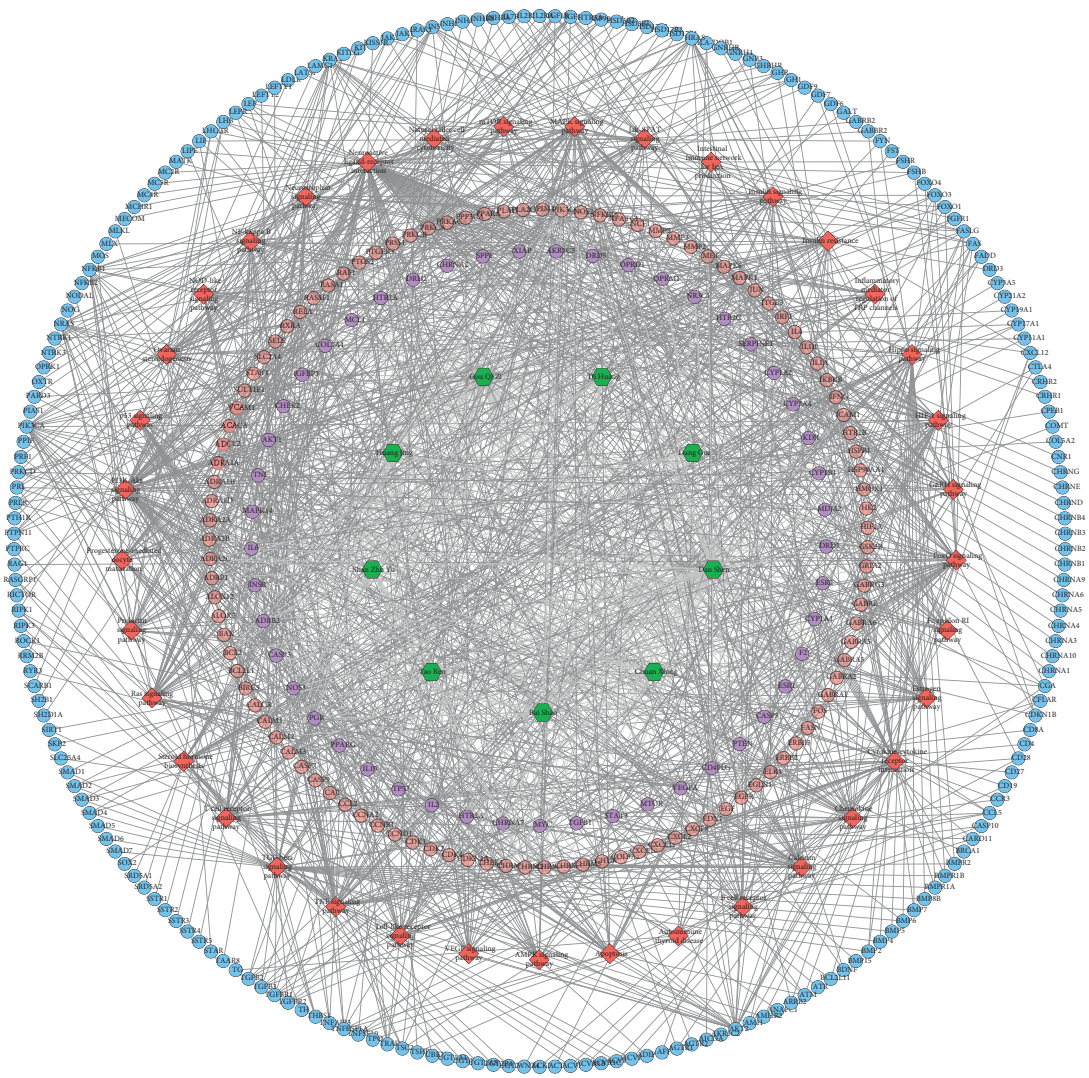

Figure 6: Herb-Target-Signaling pathway network (red diamond stands for signaling pathway. Blue, pink, and purple circles stand for POF gene, MTHSWD target, and MTHSWD-POF target, respectively. Green hexagon stands for herbs).

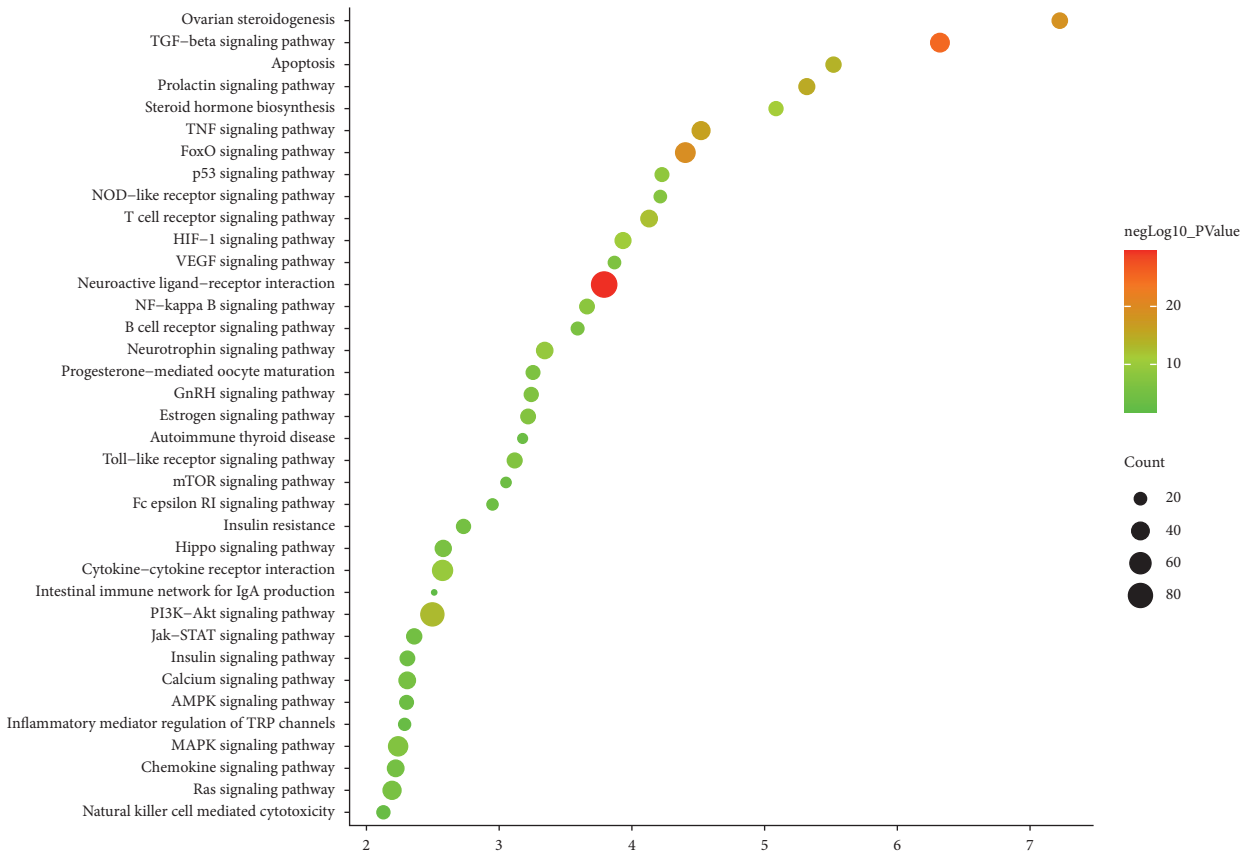

Figure 7: Bubble diagram of signaling pathways (*X*-axis stands for fold enrichment).

medicine compound for replenishing qi and blood can regulate the rate of estrous cycle disorder in mice with primary ovarian insufficiency, reduce serum LH and FSH levels, and reduce the rate of ovarian granulosa cell apoptosis. It may also increase serum E2 and AMH levels, increase the quality of uterus and ovaries, and increase the expression of TGF-β, GDF-9, and BMP15 proteins in ovarian tissue, thereby improving the ovarian function of POF mice [40].

Current studies have shown that mutations in the bone morphogenetic proteins (BMP) gene, which is a member of the transforming growth factor (TGF-β) superfamily, will cause serious abnormalities in follicular development and ovulation. The TGF-β/Smad signaling pathway involves the growth and development of follicles, the proliferation and apoptosis of granulosa cells and membrane cells, the synthesis of steroid hormones, the maturation of oocytes, ovulation, and luteinization [41, 42]. Smad2 and Smad3 proteins are members of the Smads protein family receptor regulation, and their main function is to participate in signal transduction in the TGF-β and activin signaling pathways. At present, siRNA transfection studies have shown that Smad2 and Smad3 are involved in the upregulation of TGF-β1 and the production of PGE2, respectively, and then participate in the occurrence and development of ovarian regulation of ovulation [41]. Further studies have shown that Smad2/3 plays an important role in the transition from primordial follicles to primary follicles and the formation of antral follicles [43–45]. The fertility of mice with knocked out Smad2 and Smad3 genes was greatly reduced, and they cannot form normal cumulus expansion and mediate the signal transduction between granulosa cells and oocytes [46]. Researches have showed that Bushen Tiaochong method can effectively increase the expression of TGF-β1, Smad3, and P-Smad3 in rat ovarian cells [46]. However, the specific role and mechanism of TGF-β/Smad signaling pathway in the granulosa cells of immune POF mice have not been reported yet. The factors that affect follicle development and cell proliferation and differentiation involve multiple signaling pathways, including wnt/β-catenin signaling pathway [47], Nodal signaling pathway [48], FGF signaling pathway [49], and BMP/Smads signaling pathway [50]. Smad2 and Smad3 are important factors for maintaining ovarian development and function [51, 52]. A number of studies have shown that the fertility of Smad3 knockout mice is reduced, and the proliferation of granulosa cells is inhibited [53, 54]. Smad2/3 plays an important factor in the granular cells of newly formed primordial follicles [55]. Smad2 and Smad3 knockout mice have greatly reduced fertility [45]. Yang et al. further found that TGF-β inhibits the degradation of CyclinD2 through Smad2 and Smad3, activates CDK4, and promotes the synthesis of granular cell DNA [56]. This study found that, compared with the blank group, the protein expression of granulosa cells TGF-β1, TGF-βRII, and Smad2/3 in the mouse follicles of the model

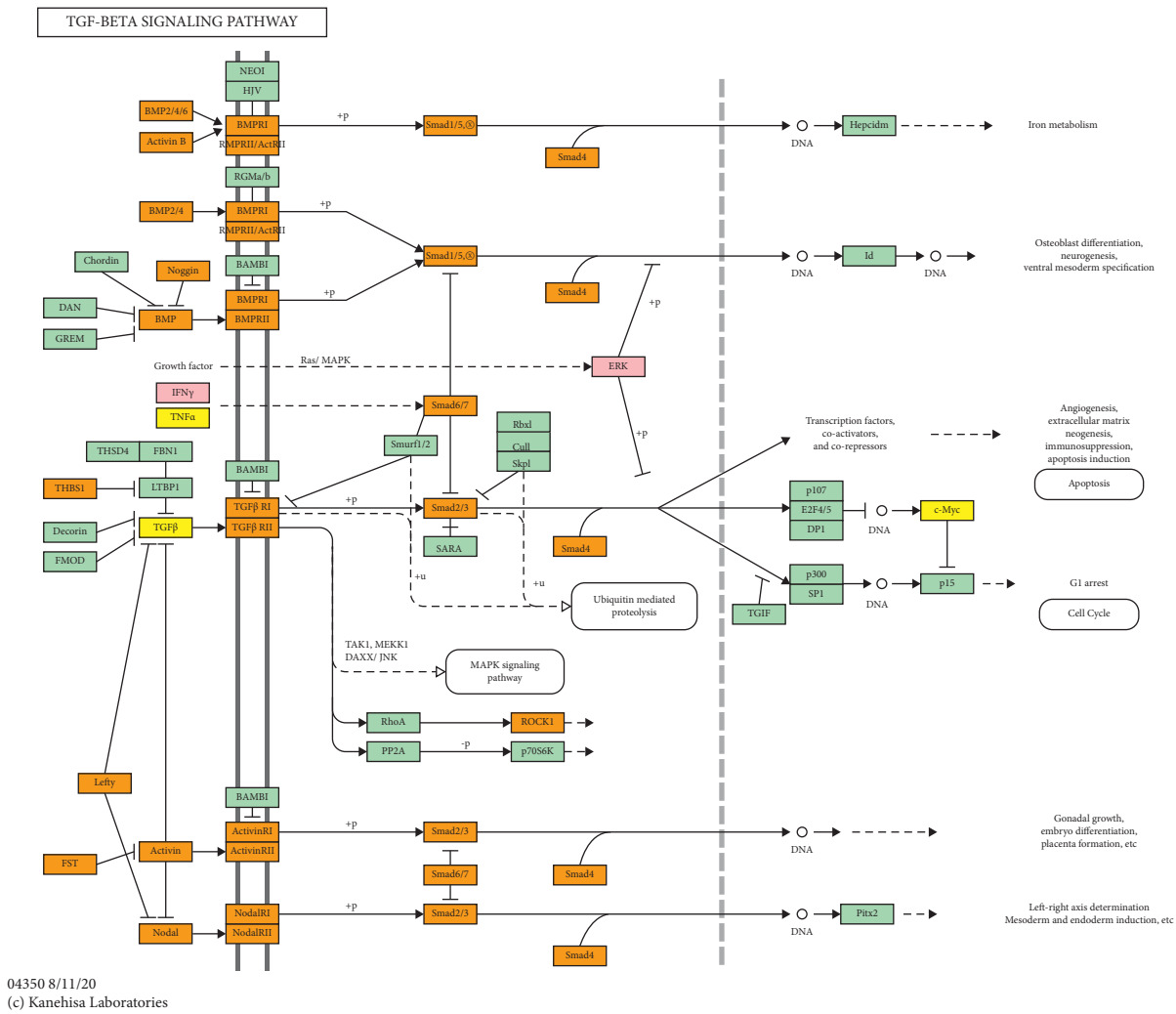

Figure 8: The KEGG Mapper modified from hsa04350 (pink is MTHSWD target, yellow is POF genes, and orange is MTHSWD-POF target).

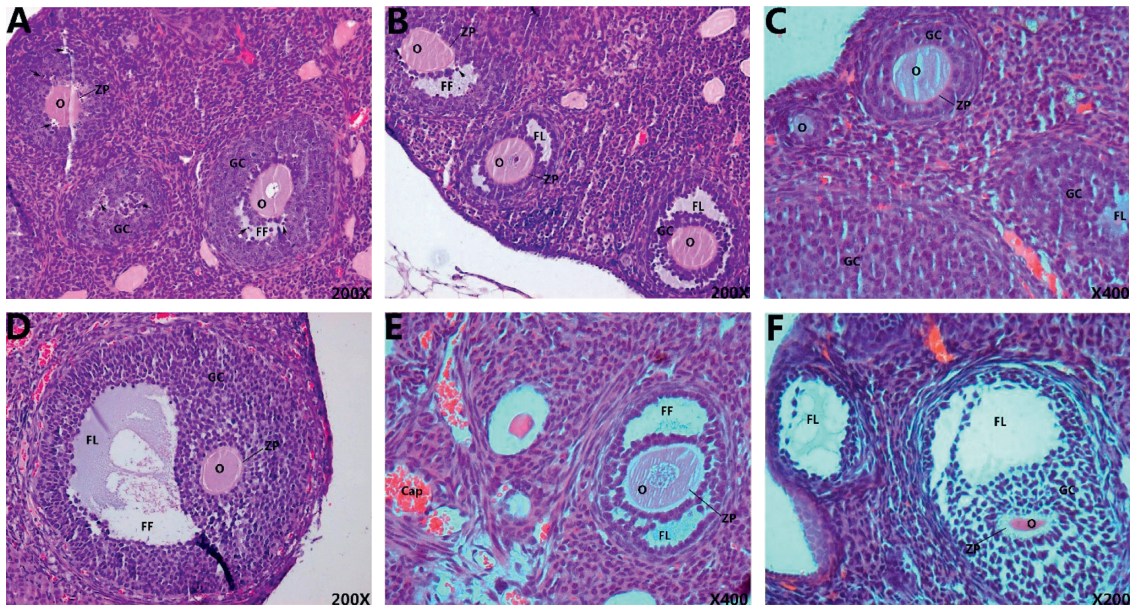

Figure 9: Morphological changes of ovarian tissue (HE staining: 200X). (a) Model group, (b) MTHSWD low-dose group, (c) MTHSWD medium-dose group, (d) MTHSWD high-dose group, (e) positive control group, and (f) blank group. O: oocyte, FL: follicular fluid, FF: follicular cavity, GC: granular cell, ZP: zona pellucida, Cap: blood vessel, and black arrow points to white blood cell.

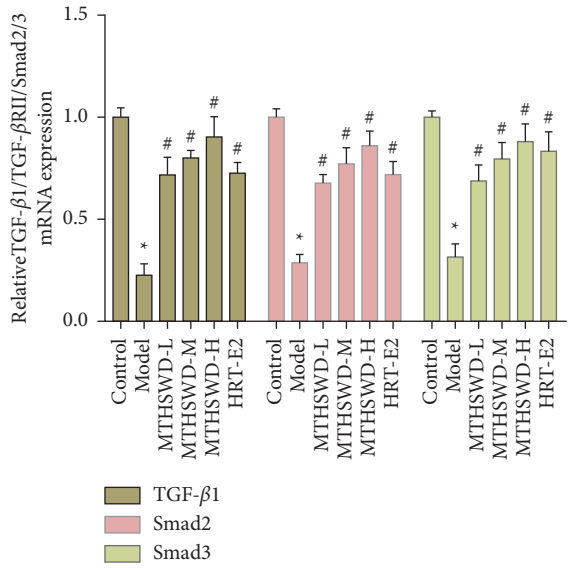

Figure 10: Expression of Smad2, Smad3, and Smad7 mRNA in ovarian tissue (compared with normal group, $^{*}p < 0.05$. Compared with model group, $^{\#}P < 0.05$).

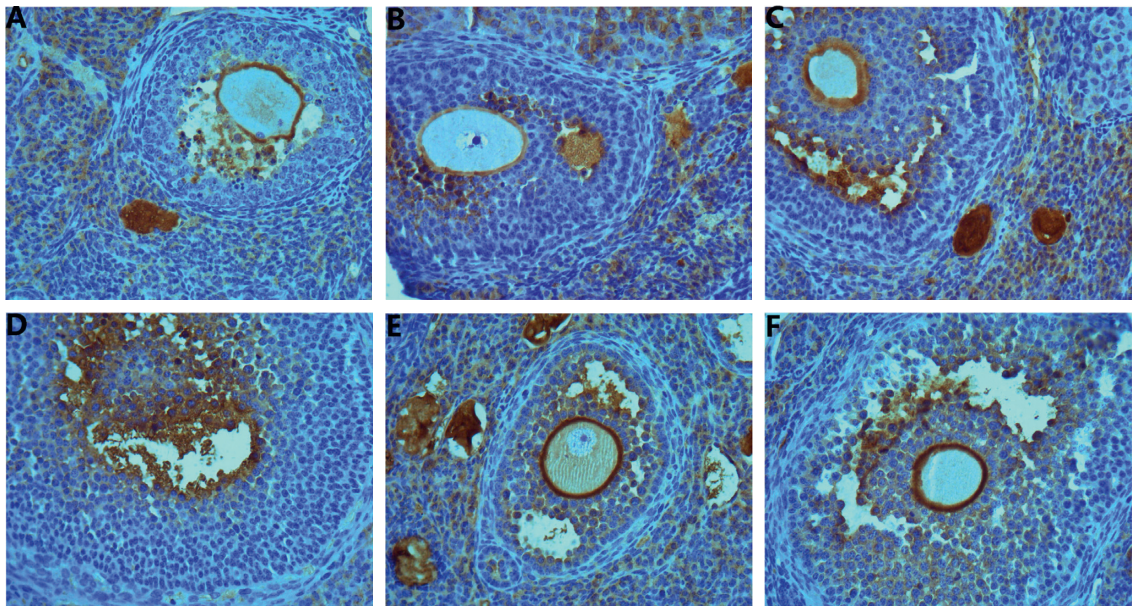

Figure 11: Expression of TGF-β1 in granulosa cells (immunohistochemistry: 400X). (a) Model group, (b) MTHSWD low-dose group, (c) MTHSWD medium-dose group, (d) MTHSWD high-dose group, (e) positive control group, and (f) blank group.

group was significantly reduced, and the follicular atresia was significantly increased. After MTHSWD intervention, the protein expression of TGF-β1, TGF-βRII, and Smad2/3 was significantly increased ($P < 0.05$), and the expression of MTHSWD high-dose group was significantly higher than that of MTHSWD low-dose group ($P < 0.05$).

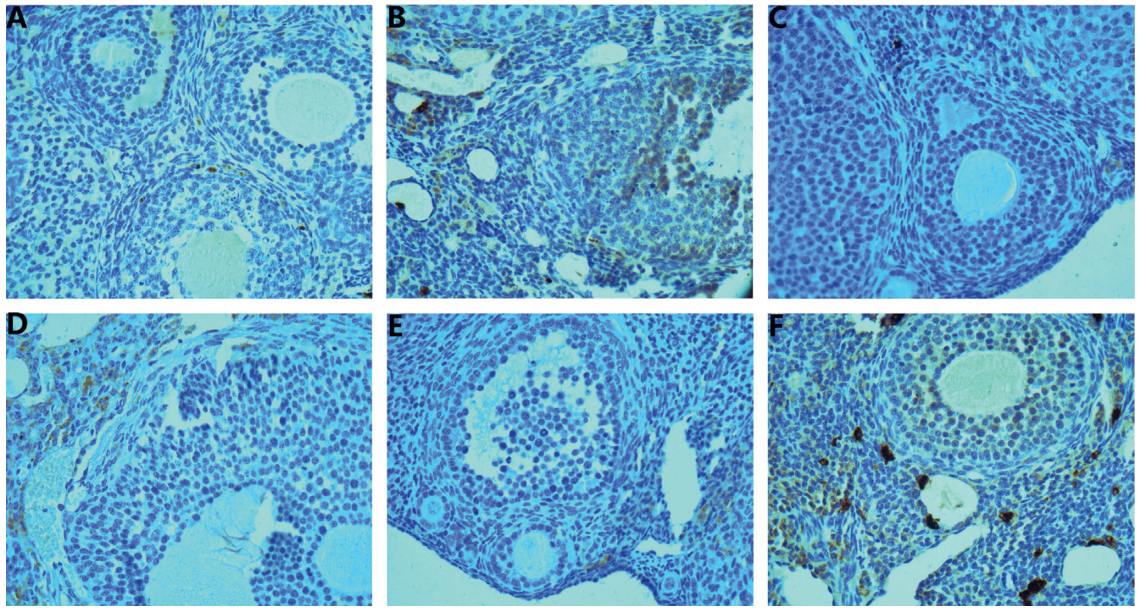

Figure 12: Expression of TGF-βRII in granulosa cells (immunohistochemistry: 400X). (a) Model group, (b) MTHSWD low-dose group, (c) MTHSWD medium-dose group, (d) MTHSWD high-dose group, (e) positive control group, and (f) blank group.

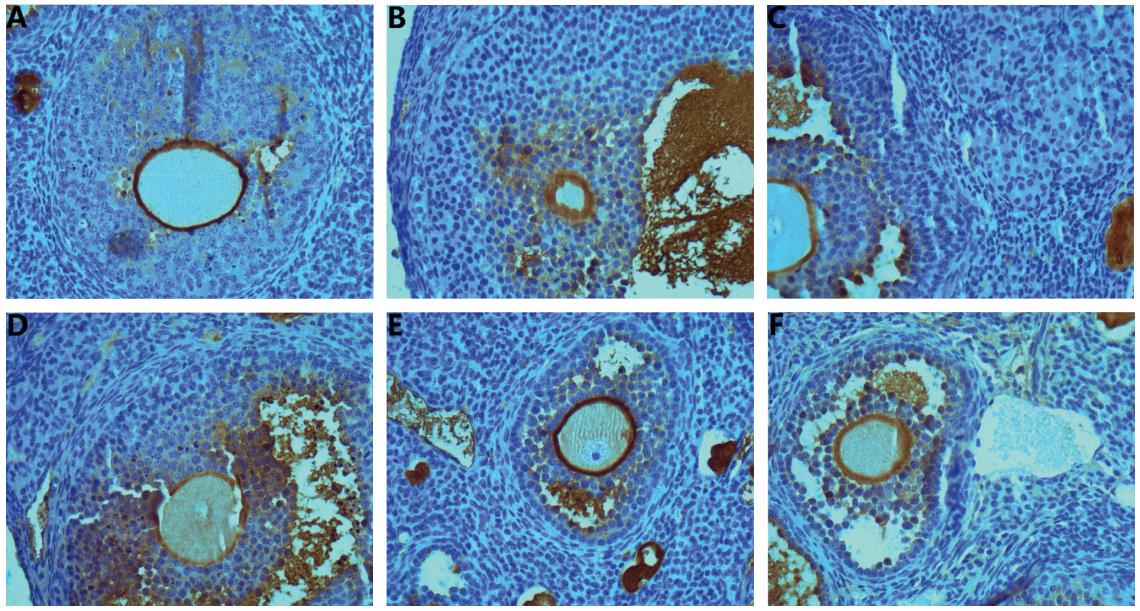

Figure 13: Expression of Smad2/3 in granulosa cells (immunohistochemistry: 400X). (a) Model group, (b) MTHSWD low-dose group, (c) MTHSWD medium-dose group, (d) MTHSWD high-dose group, (e) positive control group, and (f) blank group.

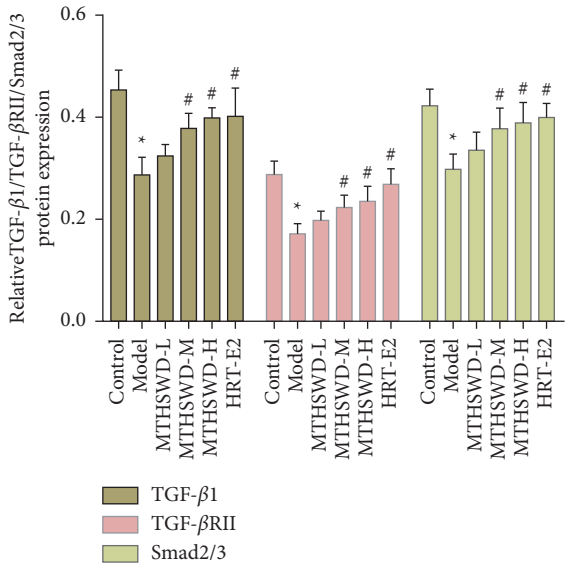

Figure 14: Expression of TGF-β1, TGF-βRII, and Smad2/3 proteins in granulosa cells (compared with normal group, $^{*}p < 0.05$. Compared with model group, $^{\#}P < 0.05$).

5. Conclusion

In summary, this study showed that MTHSWD can significantly promote the transmission of the TGF-β1/Smads signaling pathway in POF mice, thereby promoting the proliferation and differentiation of granulosa cells. However, since we only studied the TGF-β1 ligand in the TGF-β pathway, the changes in the local microenvironment formed by the interaction of various factors within the ovary during the development of POF still need to be clearly elucidated. Meanwhile, whether MTHSWD can enhance the signal transcription of other cytokines by increasing the expression of Smads in the treatment of POF mice remains to be further studied.

Data Availability

All datasets for this study are included in the manuscript and the supplementary files.

Disclosure

Hunan University of Chinese Medicine is the first and corresponding address.

Conflicts of Interest

All authors have no financial or scientific conflicts of interest with regard to the research described in this manuscript.

Authors' Contributions

K. Yang, X. Yuan, W. Xiang, H. Liu, and G. Zhang participated in the concept and design. K. Yang, X. Yuan, Q. He, W. Xiang, and J. Fan are responsible for data analysis and interpretation in the network pharmacological section. H. Liu and G. Zhang are responsible for data analysis and interpretation in experiments. K. Yang and Y. Xiao drafted the paper. G. Zhang and H. Liu supervised the study. All authors participated in the analysis and interpretation of data and approved the final paper.

Acknowledgments

This research was supported by Key Project of Scientific Research Fund of Hunan Provincial Education Department (nos. 19A370 and 21A0233), Hunan Province Domestic First-Class Cultivation Discipline Integrated Traditional Chinese and Western Medicine Discipline Project (no. 2021ZXYJH10), Key Projects of Hunan Province Traditional Chinese Medicine Scientific Research Program (no. 2021025), and Key Projects of the Scientific Research Fund of Hunan University of Chinese Medicine (no. 2020XJJJ003).

Supplementary Materials

Figure S1: the results of HPLC. Table S1: components and targets of MTHSWD. Table S2: POF genes. Table S3: enrichment analysis of MTHSWD-POF PPI network. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 1364297, 20 pages
https://doi.org/10.1155/2022/1364297

Review Article

Evidence for the Use of Complementary and Alternative Medicine for Pelvic Inflammatory Disease: A Literature Review

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Received 21 October 2021; Accepted 13 December 2021; Published 19 January 2022

Academic Editor: Yong Wang

Pelvic inflammatory disease (PID), a common infectious disease of the female reproductive tract, is mainly characterized by abdominal/pelvic pain and tenderness of the uterus, cervix, or adnexa on physical exam. In recent years, its incidence has gradually increased yearly due to numerous factors, including sexually transmitted diseases and intrauterine operations. Based on self-report of PID in the National Health and Nutrition Examination Survey (NHANES) 2013–2014 survey, PID impacts approximately 2.5 million women in the US during their reproductive age. Although empiric treatments such as antibiotics or surgery could alleviate the related symptoms of PID, its unsatisfactory obstetric outcome and high relapse bring heavy physical and psychological burden to women. Complementary and alternative medicine (CAM), a complementary therapy other than Western medicine with a complete theoretical and practical system, has been attached to importance in the world due to its remarkable efficacy. More people are accepting and trying to use CAM to treat gynecological diseases, including infertility, polycystic ovary syndrome, and PID, but its efficacy and mechanism are still controversial. This article reviews the previous literature systematically focusing on the effectiveness, safety, and mechanism of CAM in the treatment of PID to provide an evidence-based basis for the clinical application of CAM in patients with PID.

1. Introduction

Pelvic inflammatory disease (PID) refers to the inflammation of the female organs located at the upper genital tract and their surrounding tissues (uterus, fallopian tubes, ovaries, parauterine tissues, and peritoneum) caused by pathogen infection, often involving adjacent tissues. Inflammation can involve one site or spread to several sites simultaneously, mainly including endometritis, salpingitis, tubo-ovarian abscess, and pelvioperitonitis, with the highest incidence rate of salpingitis [1, 2]. The investigation in the recent ten years has shown that the prevalence of PID shows a significant upward trend. Approximately, 4%–12% of women during childbearing age worldwide suffer from PID [3]. In most developing countries, PID is more difficult to be controlled and effectively treated [4]. In China, the incidence rate of CPID in women who have more than 5 years of intercourse exceeds 20% [5], which seriously affects women's physical and mental health. From July 2013 to March 2014, a survey of 1010 women in a certain area of China showed that the prevalence rate of CPID was 5.8% and pointed out that abortion was one of the crucial factors of PID infection [6]. A survey of 1100 patients in a hospital found that the proportion of patients with reproductive tract infection was 4.36%, of which PID constituted 14.6% [7]. A census for diseases of married women in rural areas in China showed that the prevalence of PID accounted for 13.5% among all diseases on average, and in some areas, it could be highly 23.53% [8].

Neisseria gonorrhoeae and *Chlamydia trachomatis* are the main pathogenic microorganisms. In addition, some aerobic bacteria, anaerobic bacteria, viruses, and mycoplasma also participate in its occurrence. Pathogenic microorganisms are mostly mixed infections ascending from the vagina, leading to local tissue congestion, edema, inflammatory exudation, connective tissue hyperplasia, followed by irregular menstruation, abnormal leucorrhea, etc. [9, 10]. On most occasions, the symptoms of PID vary from none to severe. PID can be divided into two categories, namely acute PID and chronic PID. Chronic pelvic inflammatory disease (CPID), caused by PID not receiving prompt and effective treatment, can be accompanied by inflammatory lesions such as pelvic adhesions and tubal obstruction, leading to infertility, ectopic pregnancy, and chronic pelvic pain [11]. Due to the great variation of the clinical manifestations, the diagnosis of PID could not be completely accurate [12]. The diagnostic criteria recommended by the Centers for Disease Control and Prevention (CDC) are lower abdominal or pelvic pain and at least one of the following: adnexal tenderness or cervical motion tenderness or uterine tenderness [13].

PID has the characteristics of the long course, low cure rate, and high recurrence rate. Empirical therapies involves broad-spectrum combination regimens of antimicrobial agents to cover likely pathogens, and surgery can be performed if necessary [14]. Although the incidence and severity of PID in North America and Western Europe had reduced by using antibiotics in the past two decades, the ultimate efficacy was still not satisfactory. Complementary and alternative medicine (CAM), a supplement to conventional medicine, mainly includes the following methods: alternative medical systems, physical and mental intervention, biological therapy, manipulation and body-based methods, energy therapy, etc. [15]. The relevant report suggested that the rate of CAM application has reached 9.8%–76.0% globally [16]. At present, CAM has been widely utilized in female genital infections. Several randomized controlled trials (RCTs) have found that CAM has antibacterial and anti-inflammatory therapeutic effects, which could be effective in treating PID [17], but its efficacy and mechanism still exist in dispute. This article summarizes the efficacy of Chinese herbal medicine (CHM), acupuncture and moxibustion, pelvic exercises, hyperbaric oxygen therapy, and microwave physiotherapy in the treatment of PID and further explores their possible mechanism and safety in treating PID.

2. Researching the Overview of CHM in Treating PID

CHM, an integral part of traditional Chinese medicine (TCM), has a long history of treating diseases. In ancient times, Chinese people used certain animals, plants, and minerals to relieve the symptoms of diseases. Through long-term practice, the theories and methods of TCM have gradually formed and are recorded in written works, *such as* The Yellow Emperor's Inner Classic, Treatise on Cold Damage, and A Hundred Records on "Shen Nong's Classic of the Materia Medica". Its basic characteristics are the concept of holism and syndrome differentiation and treatment. TCM has been widely used in different stages of disease, such as prevention, diagnosis, treatment, and rehabilitation. With the development of science and technology, TCM staff have provided a more theoretical basis for TCM to treat diseases by combining modern technology with original theories [18]. CHM has various dosage forms (e.g., pills, powders, granules, wines, tinctures, and ointments). It can also be processed into Chinese patent medicines. PID always threatens women, which needs to attract people's attention. For patients suffering from PID, adjuvant treatment of CHM seems to be in high demand. Studies have shown that CHM mainly administered by oral and retention enema has a definite curative effect and obvious advantages in treating gynecological diseases, especially for PID [19].

2.1. Clinical Effects of Oral Chinese Medicine Compound Formula (CMCF) in Treating PID. Clinically, oral CMCF combined with conventional therapy is used to treat PID. More and more RCTs showed that the combination therapy could greatly improve the clinical effective rate and reduce adverse reaction and recurrence rate [20]. Zhang and Zhang [21] found that CMCF combined with antibiotics in treating acute PID can shrink pelvic mass; reduce pelvic fluid, inflammatory factors, and adverse reactions; and improve immune function. Xiangli took the same method to treat PID. After treatment, the patients' symptoms such as fever, lower abdominal pain, and abnormal leucorrhea were significantly improved [22]. Feng's research showed that CMCF combined with antibiotics could effectively decrease the time of symptom relief and reduce the TCM syndrome score and the deepest diameter of pelvic effusion [23]. Wang and Gan added another CMCF (treatment arm) to treat PID based on CMCF combined with antibiotics (control arm) and found that the cure rate of treatment arm was higher than that of the other arm ($P < 0.05$). After treatment, TCM syndrome score, inflammatory mass diameter, pelvic effusion depth, serum ICAM-1, D-dimer, plasma viscosity, erythrocyte sedimentation rate, and platelet aggregation rate of treatment arm decreased more significantly than those of control arm. IL-2, IL-10, MMP-2, complement C3, and C4 were significantly higher than those of control arm [24]. Xiong et al. [25] also found that CHM combined with antibiotics has a more obvious effect on CPID. After treatment, the number of leukocytes, the proportion of neutrophils, and CD8+ and Th2 in treatment arm were significantly lower than those in the control group ($P < 0.01$); the levels of CD3+, CD4+, CD4+/CD8+, Th1, and Th1/Th2 were significantly higher than those in the control group, and the total effective rate was significantly higher than that in the control group ($P < 0.05$). Xiong [26] found that the serum estradiol (E2), cancer antigen 125 (CA125), and hemorheological indicators of PID patients treated with CMCF combined with antibiotics were lower than those treated with antibiotics alone. Besides, some studies have reached the same conclusion [27–30]. In a double-blind, multicenter, placebo-controlled clinical trial, 155 patients

with PID were randomly assigned into treatment arm ($n = 77$) and control arm ($n = 78$). The treatment arm was given CMCF combined with antibiotics placebo in the first two weeks. Subsequently, the patient received oral CMCF merely for the remaining weeks. While the control arm was administered two kinds of medicines together with CMCF placebo in the first two weeks and CMCF placebo for the remaining weeks. The follow-up results after treatment showed that the cure rate together with the effective rate of TCM syndrome in treatment arm was 81.82%, significantly higher than 67.95% in control arm ($P < 0.05$), which demonstrated that the oral CMCF could reduce the antibiotic dosage required for PID treatment and improved symptoms in PID patients [31]. In addition, some studies are comparing the efficacy of oral CMCF with conventional therapy. Zhou and Chen [32] compared the efficacy of CMCF with antibiotics in treating endometritis by observing the morphological changes of the endometrium through hysteroscopy and performing an endometrial biopsy at a fixed position. The results showed that the markedly effective rate of clinical symptoms in the CMCF group was 91.3%, significantly higher than that of the control group 60.0% ($P < 0.05$), and plasma cell CD38 infiltration of the endometrial stroma was significantly decreased, the positive expression of MUC-1 increased, and the expression of HIF-1α was reduced, indicating that CMCF has a better therapeutic effect on chronic endometritis compared with antibiotics. The clinical effects were also not completely consistent among different oral CHMs. Wu et al. compared the clinical efficacy of two CMCFs (Tongluo Qingre Decoction and Gexia Zhuyu Decoction) on PID through an RCT and found that the effective rate of Tongluo Qingre Decoction was higher than that of Gexia Zhuyu Decoction ($P < 0.05$). After treatment, compared with the patients administered with Gexia Zhuyu Decoction, those taken Tongluo Qingre Decoction had lower TCM syndrome scores, and the patients' peak systolic flow velocity of uterine blood flow levels was higher ($P < 0.05$), and the flow resistance index and pulsatility index were lower ($P < 0.05$) than those administered with Gexia Zhuyu Decoction. The study concluded that Tongluo Qingre Decoction had better efficacy in treating PID and was more conducive to improving uterine blood microcirculation [33]. Huang randomized 180 CPID patients to CMCF arm, physiotherapy arm, and combination therapy arm. Patients in CMCF arm were given oral CMCF, physical therapy arm was given physical therapy, and combined treatment arm was given oral CMCF combined with physical therapy. After treatment, the effective rate of combined therapy arm was 98.3%, which was significantly higher than that of CMCF arm (86.7%) and physical therapy arm (76.7%), thus demonstrating that oral CMCF combined with physiotherapy could significantly improve the effect of CPID patients, strengthen the immunity of patients, and reduce inflammation. In addition, it can improve blood rheology indicators as well [34]. Furthermore, studies have found that the combination of CMCF and antibiotics for PID is not as good as expected. Lan et al. combined CMCF with antibiotics to treat CPID. The total effective rate of treatment arm was 95.00%. Although the levels of C-reactive protein and IL-10 were significantly lower than those in control arm after treatment, the incidence of adverse reactions and recurrence rate at 3 and 6 months were similar, without any significant difference [35]. Table 1 lists some of the above study protocols and some unmentioned protocols [24–38].

2.2. The Mechanism of Oral CMCF in Treating PID. Extensive research has proved that the mechanism of CHM in treating PID is associated with inflammatory cells, inflammatory factors, and related pathways. Zhang et al. found that the administration of CHM to CPID-like rats could decrease the levels of IL-2, IL-6, IL-10, TNF-α, and TGF-β1 in serum, increased the mRNA of P53, Fas/FasL, and MMP-2 mRNA in the uterus, while decreased TGF-β 1 mRNA, and inhibited NF-κB p65 in the uterus and ovary tissue. These results indicated that the possible mechanism of CHM in treating CPID was achieved by inhibiting the inflammatory response, inducing the inflammatory cell apoptosis, and downregulating serum inflammatory cytokines [36]. A study conducted by Zou et al. [37] has suggested that CHM could inhibit the infiltration of lymphocytes and neutrophils in the fallopian tubes of PID-like rats, reduce the release of IL-1β, IL-6, IL-8, and MCP-1, promote the production of LXA4, and found that CHM could regulate LPS-stimulated NF-κB signaling activity and promote FPR2 expression in THP-1 cell line, therefore contributing its anti-inflammatory effect. The experimental results of Zheng et al. showed that compared with PID-like rats not administered with CHM, the levels of serum IL-6, IL-8, and TNF-α in PID-like rats treated with CHM significantly decreased. It was concluded that CHM had strong anti-inflammatory and anti-infective effects, and could be used to treat PID and relieve pain [38]. Sun [39] explored the efficacy of CHM combined with levofloxacin in treating CPID. 120 patients were randomized into control arm and observation arm. The control arm was given levofloxacin lactate injection, and the observation arm was combined with CHM on the basis of control arm. After treatment, the levels of IL-2 and IL-10 in the observation arm were higher than those in the control arm, and the levels of CRP and TNF-α were lower than those in the control arm ($P < 0.05$). Xia et al. [40] analyzed endogenous small-molecule metabolites in the serum of rats after CHM treatment based on gas chromatography-mass spectrometry (GC-MS)-based metabolic profiling method combined with multivariate statistical analysis, such as PCA, PLS-DA, and OPLS-DA. The results showed that CHM treatment could significantly improve the inflammatory pathological characteristics and tissue damages of model rats. Based on the principle of VIP > 1 and $P < 0.05$, six different metabolic biomarkers, i.e., L-valine, L-isoleucine, L-threonine, butanedioic acid, serine, and D-glucose, were identified, and their contents were significantly reversed after administration. Further analysis of the metabolic pathways in the KEGG database showed that CHM could achieve this effect through the metabolism of glycine, serine, and threonine, biosynthesis of aminoacyl tRNA, and biosynthesis of valine, leucine, and isoleucine. Li et al. [41] used the network

Table 1: The RCTs of effective CHM formula for PID with oral administration.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitations
[21]	RCT	144	Treatment arm: Dachaihu Decoction + cefoxitin + doxycycline hyclate Control arm: cefoxitin + doxycycline hyclate	Treatment arm: TFR, 97.22% (70 of 72) Control arm: TFR, 84.72%* (61 of 72)	Dachaihu Decoction: White peony root (Bai shao), Corydalis tuber (Yan hu suo), Herba Patriniae (Bai jiang cao), *Bupleurum* (Chai hu), Coix seed (Yi yi ren), rhubarb root and rhizome (Da huang), dandelion (Pu gong ying), dried ginger rhizome (Gan jiang), fruit of citron or trifoliate orange (Zhi shi), *Pinellia ternata* (Ban xia), Trogopterus dung (Wu ling zhi), Radix scutellariae (Huang qin), Jujube (Da zao) , Delavay honeysuckle (Jin yin hua), Monkshood (Fu zi)	Not mentioned blindness and drop-out rate
[22]	RCT	360	Treatment arm: Gongying Tuling Decoction + moxifloxacin Control arm: moxifloxacin	Treatment arm: TFR, 91.67% (165 of 180) Control arm: TFR, 84.44%* (152 of 180)	Gongying Tuling Decoction: dandelion (Pu gong ying), bearded scutellaria (Ban zhi lian), Coix seed (Yi yi ren), glabrous greenbrier rhizome (Tu fu ling), white Atractylodes rhizome (Bai zhu), Pinellia rhizome (Ban xia), Amur cork-tree bark (Huang bai), Corydalis tuber (Yan hu suo), Atractylodes rhizome (Cang zhu)	Not mentioned blindness and drop-out rate
[23]	RCT	78	Treatment arm: Hongteng Baijiang Decoction + levofloxacin + metronidazole Control arm: levofloxacin + metronidazole	Treatment arm: TFR, 94.87% (37 of 39) Control arm: TFR, 79.49%* (31 of 39)	Hongteng Baijiang Decoction: Herba Patriniae (Bai jiang cao), weeping forsythia capsule (Lian qiao), Delavay honeysuckle (Jin yin hua), Sargentodoxa cuneata (Hong teng), dandelion (Pu gong ying), Tokyo violet (Zi hua di ding), Poria (Fu ling), wild chrysanthemum flower (Ye ju hua), Salvia (Dan shen), combined spicebush root (Wu yao) Modifications: Inflammation: safflower (Hong hua), Chinese wax gourd seed (Dong gua ren), Chinese honeylocust spine (Zao jiao ci) Chest oppression: *Bupleurum* (Chai hu), long stamen onion bulb (Xie bai), snake gourd fruit (Gua lou) Severe phlegm-heat: Moutan (Dan pi), weeping forsythia capsule (Lian qiao)	Not mentioned blindness and drop-out rate

Table 1: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitations
[24]	RCT	96	Treatment arm: Yiqi Huayu Penyan Decoction + levofloxacin + Guizhi Fuling capsules Control arm: levofloxacin + Guizhi Fuling capsules	Treatment arm: TFR, 95.83% (46 of 48) Control arm: TFR, 83.33%* (40 of 48)	Yiqi Huayu Penyan Decoction: Radix codonopsis (Dang shen), Astragalus (Huang qi), *Angelica sinensis* (Dang gui), Salvia (Dan shen), *Ligusticum wallichii* (Chuan xiong), Cyperus (Xiang fu), *Liquidambar formosana* Hance (Lu lu tong), white peony root (Bai shao), leech (Shuizhi), licorice (Gancao) Modifications: Abnormal vaginal discharge: add heartleaf houttuynia (Yu xing cao), bearded scutellaria (Ban zhi lian), Amur cork-tree bark (Huang bai) Pruritus vulvae: add Coix seed (Yi yi ren), Hypoglaucous Collett Yam Rhizome (Bi xie) Diarrhea: add Psoralea (Bu gu zhi), common yam rhizome (Shan yao)	Not mentioned blindness and drop-out rate
[25]	RCT	108	Treatment arm: Dahuangmudan Decoction + cefoxitin + doxycycline Control arm: cefoxitin + doxycycline	Treatment arm: TFR, 94.55% (52 of 55) Control arm: TFR, 79.25%* (42 of 53)	Dahuangmudan Decoction: rhubarb root and rhizome (Da huang), Moutan (Mu dan pi), Semen Persicae (Tao ren), Chinese wax gourd seed (Dong gua zi), sodium sulfate (Mang xiao), Herba Patriniae (Bai jiang cao), Delavay honeysuckle (Jin yin hua), red peony (Chi shao), Coix seed (Yi yi ren), licorice (Gan cao)	Not mentioned blindness and drop-out rate
[26]	RCT	100	Treatment arm: self-made Qingre Huayu Decoction + levofloxacin Control arm: levofloxacin	Treatment arm: TFR, 94% (47 of 50) Control arm: TFR, 80% * (40 of 50)	Self-made Qingre Huayu Decoction: licorice (Gan cao), Amur cork-tree bark (Huang bai), Semen Persicae (Tao ren), Chinese wax gourd seed (Dong gua zi), Herba Leonuri (Yi mu cao), Corydalis tuber (Yan hu suo), Indian bread (Fu ling), Danshen root (Dan shen), red peony root (Chi shao), Sargentodoxa cuneata (Hong teng), *Patrinia* (Bai jiang cao), Tokyo violet (Zi hua di ding), dandelion (Pu gong ying), Delavay honeysuckle (Jin ying hua)	Not mentioned blindness and drop-out rate
[27]	RCT	118	Treatment arm: Fuyanshu capsule + levofloxacin hydrochloride + metronidazole Control arm: Fuyanshu capsule simulator + levofloxacin hydrochloride + metronidazole	Treatment arm: RR, 18.64% (11 of 59) Control arm: RR, 86.44%* (51 of 59)	Fuyanshu capsule: honeysuckle stem (Ren dong teng), Sargent gloryvine stem (Da xue teng), licorice (Gan cao), woad root (Da qing ye), dandelion (Pu gong ying), red peony root (Chi shao), rhubarb root and rhizome (Da huang), Danshen root (Dan shen), giant knotweed rhizome (Hu zhang), Toosendan fruit (Chuan lian zi), Corydalis tuber (Yan hu suo)	Not mentioned blindness and drop-out rate

Table 1: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitations
[28]	RCT	110	Treatment arm: Danbai granules + azithromycin Control arm: azithromycin	Treatment arm: TFR, 98.2% (54 of 55) Control arm: TFR, 90.9%* (50 of 55)	Danbai granules: white peony root (Bai shao), Moutan (Mu dan pi), Tokyo violet (Zi hua di ding), common burr reed tuber (San leng), Ailanthus bark or root bark (Chuan pi), *Patrinia* root (Mu tou hui), *Patrinia* (Bai jiang cao), *Sparganium* (San leng), Curcumae rhizome (E zhu), Nightshade (Bai ying), Sichuan lovage root (Chuan xun), Sargent gloryvine stem (Da xue teng), glabrous greenbrier rhizome (Tu fu ling), Chinese Angelica (Dang gui), *Oldenlandia* (Bai hua she she cao)	Not mentioned blindness and drop-out rate
[29]	RCT	96	Treatment arm: Fuyanning Decoction + levofloxacin Control arm: levofloxacin	Treatment arm: TFR, 97.92% (47 of 48) Control arm: TFR, 87.50%* (42 of 48)	Fuyanning Decoction: Danshen root (Dan shen), Astragalus root (Huang qi), Myrrh (Mo yao), Sargentodoxa cuneata (Hong teng), Dodder seed (Tu si zi), Morinda root (Ba ji tian), heartleaf houttuynia (Yu xing cao), flying squirrel faeces (Wu ling zhi), Herba Patriniae (Bai jiang cao), white Atractylodes rhizome (Bai zhu), degelatinated deer antler powder (Lu jiao shaung), Coix seed (Yi yi ren), Indian bread (Fu ling), Lychee seed (Li zhi he), Cassia twig (Gui zhi), Licorice (Gan cao)	Not mentioned blindness and drop-out rate
[30]	RCT	120	Treatment arm: Guizhi Fuling pills + ornidazole Control arm: ornidazole	Treatment arm: TFR, 91.67% (55 of 60) Control arm: TFR, 78.33%* (47 of 60)	Guizhi Fuling pills: *Cassia* twig (Gui zhi), Indian bread (Fu ling), Moutan (Mu dan pi), red peony root (Chi shao), Semen Persicae (Tao ren)	Not mentioned blindness and drop-out rate
[31]	RCT	155	Treatment arm: the first 14 days: placebo (levofloxacin + metronidazole) + Jinying capsules; remaining 14 days: Jinying capsules Control arm: the first 14 days: Jinying capsules placebo + levofloxacin + metronidazole; remaining 14 days: Jinying capsule placebo	Treatment arm: TFR, 76.32% (60 of 78) Control arm: TFR, 59.46%*(46 of 77)	Jinying capsules: Delavay honeysuckle (Jin yin hua), Amur cork-tree bark (Huang Bai), Tokyo violet (Zi hua di ding), wild chrysanthemum flower (Ye ju hua), dandelion (Pu gong ying), Atractylodes rhizome (Cang zhu), Chinese honeylocust spine (Zao jiao ci), Salvia (Dan shen), red peony (Chi shao), Corydalis tuber (Yan hu suo)	Not mentioned blindness and drop-out rate

Table 1: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitations
[32]	RCT	38	Treatment arm: Penning granule Control arm: levofloxacin + metronidazole	Treatment arm: TFR, 91.3% (21 of 23) Control arm: TFR, 60%* (9 of 15)	Penning granule: Sargent gloryvine stem (Da xue teng), Patrinia (Bai jiang cao), *Oldenlandia* (Bai hua she she cao), red peony (Chi shao), *Angelica sinensis* (Dang gui), Frankincense (Ru Xiang), Myrrh (Mo yao), common burr reed tuber (San leng), Curcumae rhizome (E zhu), Chinese honeylocust spine (Zao jiao ci), Salvia (Dan shen), *Bupleurum* (Chai hu), Manchurian wild ginger (Xi xin), Astragalus (Huang qi), Pangolin scales (Chuan shan jia)	Not mentioned blindness, drop-out rate, and small sample size
[33]	RCT	90	Tongluo Qingre Decoction arm GeXia Zhuyu decoction arm	Tongluo Qingre Decoction arm: TFR, 91.11% (41 of 45) GeXia Zhuyu Decoction arm: TFR, 82.22% * (37 of 45)	Tongluo Qingre Decoction: giant knotweed rhizome (Hu zhang), glabrous greenbrier rhizome (Tu fu ling), Amur cork-tree bark (Huang bai), ground beetle (Tu bie chong), Red peony (Chi shao), Moutan (Mu dan pi), Semen Persicae (Tao ren), Sichuan lovage root (Chuan xiong), Toosendan fruit (Chuan lian zi), leech (Shui zhi), Chinese Angelica (Dang gui), safflower (Hong hua), turmeric root tuber (Yu jin) GeXia Zhuyu Decoction: flying squirrel faeces (Wu ling zhi), Chinese Angelica (Dang gui), Sichuan lovage root (Chuan xiong), Semen Persicae (Tao ren), Moutan (Mu dan pi), red peony (Chi shao), combined spicebush root (Wu yao), Corydalis tuber (Yan hu suo), licorice (Gan cao), Cyperus (San leng cao), nutgrass galingale rhizome (Xiang fu), safflower (Hong hua), bitter orange (Zhi qiao)	Not mentioned blindness and drop-out rate
[34]	RCT	180	Treatment arm: pelvic inflammatory decoction Decoction arm: physical therapy Physiotherapy arm: inflammatory decoction + physical therapy	Treatment arm: TFR, 96.3% (59 of 60) * decoction arm: TFR, 86.7% (52 of 60) Physiotherapy arm: TFR, 76.7% (46 of 60)	Pelvic inflammatory decoction: Atractylodes rhizome (Cang zhu), Astragalus (Huang qi), licorice (Gan cao), Radix codonopsis (Dang shen), *Liquidambar formosana* Hance (Lu lu tong), weeping forsythia capsule (Lian qiao), Platycodon root (Jie geng), combined spicebush root (Wu yao), common self-heal fruit-spike (Xia ku cao), Delavay honeysuckle (Jin yin hua)	Not mentioned blindness and drop-out rate

Table 1: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitations
[35]	RCT	80	Treatment arm: Shaofu Zhuyu Decoction + tinidazole + levofloxacin Control arm: tinidazole + levofloxacin	Treatment arm: TFR, 95% (38 of 40) Control arm: TFR, 80%*(32 of 40)	Shaofu Zhuyu Decoction: Fennel (Xiao hui xiang), dried ginger rhizome (Gan jiang), Corydalis tuber (Yuan hu), Trogopterus dung (Wu ling zhi), *Ligusticum wallichii* (Chuan xiong), Cattail Pollen (Pu huang), *Angelica sinensis* (Dang gui), red peony (Chi shao), cinnamon bark (Rou gui) Modifications: Pain: Toosendan fruit (Chuan lian zi) Chest oppression: Cyperus (Xiang fu), turmeric root tuber (Yu jin) Waist pain: *Eucommia* (Du zhong), Psoralea (Bu gu zhi) Lack of strength: Radix codonopsis (Dang shen), *Astragalus* (Huang qi)	Not mentioned blindness, drop-out rate, and small sample size

Note. RCT: randomized clinical trial; TFR: total effective rate; RR: recurrence rate. *$p < 0.05$ versus treatment arm.

pharmacology method to screen targets and found that the PTGS2 target in the arachidonic acid (AA) pathway was significantly correlated with CPID, which further confirmed that CHM could reduce the development of CPID by regulating PTGS2 target.

2.3. Clinical Effects and Mechanism of CHM Retention Enema (CHMRE) in Treating PID. CHMRE evolved from the enema method in "Treatise on Cold Damage" is one of the most commonly used external treatment methods and also known as anorectal administration. Pouring CHM into the rectum and keeping it for four to five hours to be absorbed fully by intestinal mucosa to treat the disease. In line with physiological and anatomical characteristics of the female pelvic cavity, the transrectal administration of CHM can make the drug absorbed through the mucosal venous plexus [42–44], directly arrive at the lesion, accurately and quickly exert the drug effect, improve local microcirculation, and promote the absorption of effusion and mass [45, 46]. The possible mechanism of CHMRE in treating PID is related to its ability to improve blood rheology, reduce oxidative stress response, reduce inflammation, etc. [47]. Due to its simplicity and convenience, CHMRE has a wide range of clinical applications. In addition, compared with conventional therapies, the medicine enters the internal iliac vein through the inferior rectal vein and/or anal vein, then finally into the systemic circulation, which can reduce the irritation of the digestive system, avoid the first pass elimination to improve the bioavailability of drugs, and reduce the damage to the liver and other organs, therefore greatly reducing the adverse effects and side effects [48–50]. To assess the efficacy of CHMRE in treating PID, Liang and Ling carried out a comparative study on 184 patients who suffered from sequelae of PID and found that the total effective rate of CHM staining therapy combined with CHMRM arm was higher than that of abdominal ultrasound drug delivery therapy arm, with statistically significant difference ($P < 0.05$) [51]. A clinical study [52] on a larger number of people has reported that compared with 55 patients with routine anti-inflammatory treatment, patients with CHMRE combined with anti-inflammatory treatment had a significantly higher effective rate, and serum TNF, IL-2, and IL-10 levels were statistically significant. Research conducted by Shao demonstrated that the *CHMRE* has a significant effect on PID, especially in terms of pain relief, recurrence reduction, and prevention of long-term complications [53]. More recently, numerous clinical studies have drawn the same conclusion [54–56]. Some researchers utilized *CHMRE* after hysteroscopy, which effectively reduced the levels of TNF-α, IL-6, and IL-8, improved the unobstructed fallopian tube, and relieved abdominal pain and other symptoms, thereby promoting the recovery of fertility [57–60]. *CHMRE* could also reduce the recurrence rate of PID for half a year or more [61, 62]. Based on the above-mentioned research works, CHMRE has significant efficacy in treating PID with a low incidence of adverse reactions, which is worthy of clinical application. However, it should be noted that the temperature of CHM should be between 39 and 41°C to prevent intestinal spasms or scald of intestinal mucosa caused by inappropriate temperature. Of course, CHMRE also has its shortcomings. For very few patients, the intolerance of it manifested as severe diarrhea and abdominal distension affects their quality of life seriously. In addition, for patients after intestinal tumor surgery, since the normal barrier function of the intestine has been broken, special attention should be paid to the medication. The above studies are listed in Table 2.

2.4. The Application of Chinese Medicine Monomer in Treating PID. Apart from CMCF, Chinese medicine monomers are also generally used in treating PID. Sargent gloryvine stem (SC) and *Patrinia scabiosifolia* (PS) have the effects of clearing heat and resolving toxins, invigorating blood, and dissolving stasis, which are the key to treating PID. Modern pharmacological studies have suggested that SC and PS contained various active ingredients such as phenols, flavonoids, phenylpropenes, and triterpenes, which have antioxidant, antibacterial, anti-inflammatory, and antiviral effects [63]. Their active ingredients act on key targets such as VEGFA, VWF, IL6, TNF, and NFKB1, therefore regulating AGE-RAGE, FA, Toll-like receptors, PI3K/Akt, NF-κB, apoptosis, and cancer signaling pathways. Zhang Y used Chinese medicine *Smilax china* L. to treat PID. Its active ingredient *Smilax china* polysaccharide is considered to have an anti-inflammatory effect. Through extraction, purification, and structural identification, it was discovered for the first time that *Smilax china* L. polysaccharide can be purified to produce polysaccharides SCLP1 (*Smilax china* L. polysaccharide 1,42.1 kDa) and SCLP3-2 (*Smilax china* L. polysaccharide 3,2, 16.8 kDa), which structure had been identified by chemical and spectral analyses. The results showed that SCLP1 and SCLP3-2 could inhibit the production of NO and IL-6 in RAW264.7 cells stimulated by LPS through NF-κB and MAPKs (ERK1/2, JNK) pathways [64]. Kong D studied the therapeutic efficacy and potential mechanism of Asian acid (AA) on PID-like rats. The results showed that AA treatment significantly reduced the overproduction of cytokines and chemokines and inhibited MPO activity, NLRP3 inflammasome, activation of NF-κB and caspase-3, and oxidative stress, indicating that AA had stronger anti-inflammatory and antioxidant effects on PID-like rats. Its anti-inflammatory mechanism may be related to the inhibition of NLRP3 inflammasome activity and the NF-κB pathway [65].

3. Researching the Overview of Acupuncture and Moxibustion in Treating PID

Acupuncture and moxibustion, a widely practiced traditional medical system that existed for more than 3,000 years, is considered to be rooted in naturalistic theories compatible with Confucianism and Taoism [66–68]. Since the reform and opening up, acupuncture and moxibustion have been gradually accepted by Western countries, which have been playing an important role in the internationalization of TCM. With its unique advantages (e.g., simple operation,

Table 2: The RCTs of effective CHMRE for PID.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
[44]	RCT	184	Control arm: abdominal ultrasound drug delivery therapy Treatment arm: CHM staining therapy combined with CHMRM	Control arm: TFR, 91.95%* (88 of 92) Treatment arm: TFR, 97.82% (90 of 92)	CHMRE prescription: common burr reed tuber (San leng), Curcumae rhizome (E zhu), Corydalis tuber (Yan hu suo), Toosendan fruit (Chuan lian zi), Cassia twig (Gui zhi), Sargentodoxa cuneata (Hong teng), Herba Patriniae (Bai jiang cao), Delavay honeysuckle (Jin yin hua), weeping forsythia capsule (Lian qiao), Poria (Fu ling)	Not mentioned drop-out rate
[45]	RCT	110	Control arm: cefotaxime sodium + metronidazole + fluconazole (mycotic infection)/doxycycline (mycoplasma infection) Treatment arm: combined with enema Chinese prescription on the basis of the control arm	Control arm: TFR, 61.82%* (34 of 55) Treatment arm: TFR, 90.91% (34 of 55)	Enema Chinese prescription: Herba Patriniae (Bai jiang cao), Salvia (Dan shen), Tokyo violet (Zi hua di ding), dandelion (Pu gong ying), Toosendan fruit (Chuan lian zi), heartleaf houttuynia (Yu xing cao), red peony (Chi shao) Modifications: Abdominal distention: Semen Vaccariae (Wang bu liu xing), *Liquidambar formosana* Hance (Lu lu tong) Cold-damp constraining: combined spicebush root (Wu yao), Poria (Fu ling), Cassia twig (Gui zhi)	Not mentioned blindness and drop-out rate
[47]	RCT	90	Control arm: levofloxacin + ornidazole Treatment arm: self-made Gynecological Anti-Inflammatory No. 1 Decoction on the basis of the control arm	Control arm: TFR, 75.56%* (34 of 45) Treatment arm: TFR, 93.33% (42 of 45)	Self-made Gynecological Anti-Inflammatory No.1 Decoction: Delavay honeysuckle (Jin yin hua), dandelion (Pu gong ying), weeping forsythia capsule (Lian qiao), Oldenlandia (Bai hua she she cao), Herba Patriniae (Bai jiang cao), heartleaf houttuynia (Yu xing cao), Sargent gloryvine stem (Da xue teng), red peony (Chi shao), Moutan (Mu dan pi), cinnamon bark (Rou gui), green tangerine peer (Qing pi), Morinda root (Ba ji tian), white Atractylodes rhizome (Bai zhu), citrus (Chen pi), Coix seed (Yi yi ren), Poria (Fu ling)	Not mentioned drop-out rate and small sample size
[48]	RCT	78	Control arm: levofloxacin + ornidazole Treatment arm: self-made pelvic inflammation decoction (oral and retention enema)	Control arm: TFR, 79.49%* (31 of 39) Treatment arm: TFR, 92.31% (36 of 39)	Self-made pelvic inflammation decoction: Sargentodoxa cuneata (Hong teng), white Atractylodes rhizome (Bai zhu), Euryale seed (Qian shi), common yam rhizome (Huai shan), Dragon bones (Long gu), Concha Ostreae (Mu li), Radix Pulsatillae (Bai tou weng), Haematitum (Dai zhe shi), Dodder seed (Tu si zi), heartleaf houttuynia (Yu xing cao), dandelion (Pu gong ying), Angelica root (Bai zhi), cuttlebone (Hai piao xiao), Radix et Rhizoma Rubiae (Qian cao), Semen Plantaginis (Che qian zi), Semen Ginkgo (Bai guo), Amur cork-tree bark (Huang bai)	Not mentioned drop-out rate and small sample size

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
[49]	Case-control study	70	Control arm: Oral Xiaoyan Decoction Treatment arm: Xiaoyan Decoction (oral and retention enema)	Control arm: TFR, 80.00%* (28 of 35) Treatment arm: TFR, 97.14% (34 of 35)	Xiaoyan Decoction: Cyperus (Xiang fu), Radix Bupleuri (Cai hu), *Ligusticum wallichii* (Chuan xiong), red peony (Chi shao), *Angelica sinensis* (Dang gui), Corydalis tuber (Yan hu suo), Rhizoma Cibotii (Gou ji), Psoralea (Bu gu zhi), Sargentodoxa cuneata (Hong teng), Herba Patriniae (Bai jiang cao), Dandelion (Pu gong ying), white Atractylodes rhizome (Bai zhu), Semen Euryales (Qian shi), fresh ginger (Sheng jiang)	Not mentioned blindness, drop-out rate, and small sample size
[51]	RCT	92	Control arm: tubal hydrotubation Treatment arm: pelvic inflammation recipe (oral and retention enema)	Control arm: TFR, 80.43* (37 of 46) Treatment arm: TFR, 95.65% (44 of 46)	Pelvic inflammation recipe: *Liquidambar formosana* Hance (Lu lu tong), red peony (Chi shao), Sargentodoxa cuneata (Hong teng), Herba Patriniae (Bai jiang cao), Caulis Lonicerae Japonicae (Ren dong teng), Tokyo violet (Zi hua di ding), lychee seed (Li zhi he)	Not mentioned drop-out rate and small sample size
[53]	RCT	50	Control arm: laparoscopic surgery Treatment arm: combined with Pen Yan Qing on the basis of the control arm	Control arm: TFR, 52% * (13 of 25) Treatment arm: TFR, 84% (21 of 25)	Pen Yan Qing: Danshen root (Dan shen), common burr reed tuber (San leng), Curcumae rhizome (E zhu), Sargentodoxa cuneata (Hong teng) (because the drug is applied for a new drug, the full prescription is not disclosed)	Not mentioned drop-out rate and small sample size
[54]	RCT	86	Control arm: antibiotic Treatment arm: combined with CHMRE on the basis of the control arm	Control arm: PR, 41.8% * treatment arm: PR, 62.7%	CHMRE: Delavay honeysuckle (Jin yin hua), Sargentodoxa cuneata (Hong teng), weeping forsythia capsule (Lian qiao), Chinese honeylocust spine (Zao jiao ci), Patrinia (Bai jiang cao), Amur cork-tree bark (Huang bai), Moutan (Mu dan pi), Frankincense (Ru xiang), Myrrh (Mo yao), Curcumae rhizome (E zhu), Corydalis tuber (Yan hu suo)	Not mentioned drop-out rate and small sample size
[55]	RCT	74	Control arm: ceftizoxime sodium/levofloxacin lactate + ornidazole Treatment arm: combined with CHMRE on the basis of the control arm	Control arm: TFR, 75.68%* (28 of 37) Treatment arm: TFR, 94.59% (35 of 37)	CHMRE: Radix et Rhizoma Cynanchi Paniculata (Xu chang qing), Chinese honeylocust spine (Zao jiao ci), Sargentodoxa cuneata (Hong teng), Dandelion (Pu gong ying), Delavay honeysuckle (Jin yin hua), Herba Patriniae (Bai jiang cao), Danshen root (Dan shen), Radix Scutellariae (Huang qin)	Not mentioned drop-out rate and small sample size

Note. RCT: randomized clinical trial; TFR: total effective rate; RR: recurrence rate, PR: pregnancy rate. $^{*}P < 0.05$ versus treatment arm.

durable, and strong quantity of stimulus), acupuncture and moxibustion have been recognized and applied in 183 countries around the world. There are about 200,000 practitioners [69–73]. In clinic, acupuncture and moxibustion are usually divided separately. Guided by the theory of meridians and acupoints, practitioners use needles and artemisia as tools and materials to stimulate specific parts of the body through needles or burning artemisia to adjust the balance of Yin-Yang, therefore achieving the purpose of treating and preventing diseases. PID usually has longer treatment courses and a relatively low cure rate due to its complicated etiology. As a branch of CAM, acupuncture can enhance immunity and relieve the lower abdominal pain of PID patients. Moreover, it is simple in operation, available, and accepted by patients because of its fewer side effects. Due to such superiorities, acupuncture and moxibustion have aroused the attention of many gynecologists, so a series of clinical and animal studies have been carried out to evaluate the effect and therapeutic mechanism in PID.

3.1. Clinical Effect of Acupuncture in Treating PID. Acupuncture can effectively treat more than 500 types of diseases. In 1971, the acupuncture anesthesia test was successful, which set off an upsurge of acupuncture anesthesia. Later, when US President Nixon visited China in 1972, the accompanying journalists experienced acupuncture for analgesia, which made medical workers worldwide arouse great interest in acupuncture for treating diseases [74, 75]. Acupuncture has a long history of treating gynecological diseases, and abundant studies have shown that acupuncture is beneficial, particularly in treating CPID [76, 77]. There are many categories of acupuncture treating CPID, including filiform acupuncture, warming-acupuncture, etc. Selecting appropriate acupuncture based on patients' clinical manifestations is conducive to their recovery [78]. Before the 1980s, there were fewer reports on the related studies about acupuncture in PID, let alone the large-sample clinical RCTs. In 1989, Wang [79] randomly assigned 95 patients with CPID into treatment arm (electroacupuncture (EA) combined with moxibustion) and control arm (antibiotic). The results showed that the therapeutic effect of EA combined with moxibustion was better than antibiotics ($P < 0.05$). This was the first clinical trial to show that acupuncture was effective in treating PID. Subsequently, in 2008, Zhen and Wang [80] randomized 85 CPID patients into the warming-acupuncture arm and the CHM arm and concluded that the effective rate was 95.6% versus 77.5%, respectively, with a significant statistical difference ($P < 0.05$). During the follow-up, the recurrence rate of the treatment arm was 8.7%, while the other arm was 22.2%. Zhang [81] compared the efficacy of Western medicine with acupuncture combined with CHMRE in treating patients with acute PID. The results showed that the effective rate of acupuncture combined with CHMRE was higher than that of Western medicine ($P < 0.05$). Therefore, it is concluded that acupuncture combined with CHMRE is effective in treating acute PID, and the two therapies have a synergistic effect, which can better relieve pain and symptoms. Wu et al. [82] investigated the effect of acupuncture on the inflammation and symptoms of patients suffering from acute PID. The efficacy of observation arm was better than that of control arm (95.24% vs 81.08%, $P < 0.05$). After treatment, the levels of TNF-α and CRP in the serum were significantly decreased ($P < 0.05$). Shi [83] also got a similar conclusion. Liu et al. [84] conducted a multicenter RCT. The results showed that acupuncture combined with ibuprofen sustained-release capsule can effectively improve the symptoms and signs of patients with chronic pelvic pain caused by PID and improve their quality of life, which was more effective than ibuprofen sustained-release capsule alone. Several systematic reviews and meta-analyses of acupuncture treatment of PID have been published successively. Although these studies had varying degrees of bias risk, the conclusions still provided strong evidence for acupuncture as supplements to substitute Western medicine to treat PID. Zheng et al. [85] used data mining technology to analyze the rule of acupoint selection in treating CPID. The results showed that acupoints of 14 meridians were mainly selected for treating CPID, of which CV4 (168 times) and SP6 (155 times) were used most frequently, with the proportion as high as 76.02% and 70.14%, respectively. According to the analysis of the regularity of the use of extra meridian acupoints, EX-CA1 has the highest frequency followed by EX-B7 and Changyi. He et al. [86] conducted a meta-analysis on the efficacy and safety of acupuncture in treating CPID. The results showed that, in terms of efficacy, the total effective rates of acupuncture and its combination with medicine were higher than that of medicine alone; there was no significant difference in the incidence of adverse reactions in acupuncture arm compared with the control arm. We have listed the 10 most commonly used acupoints for the treatment of PID in Table 3.

3.2. The Mechanism of Acupuncture in Treating PID. Studies have shown that acupuncture induces reactions such as activation of nerve, endocrine, and immune signaling pathways by stimulating skin tissue [87]. There are many pathogenic factors of PID [88], including a variety of microbial infections, decreased autoimmunity, and pelvic floor muscle dysfunction. The main mechanisms of acupuncture in treating PID are as follows: first of all, acupuncture can promote blood circulation, increase the permeability of the cell membrane, and accelerate the absorption of inflammation, thereby treating PID; second, acupuncture can enhance immunity by stimulating health-care acupoints and specific acupoints, thereby preventing the occurrence of PID and promoting the recovery of the disease; and finally, acupuncture treats PID by improving persistent pain in the lower abdomen. Now, we will discuss these three aspects in detail in the following sections.

3.2.1. Acupuncture Could Promote Blood Circulation and Accelerate Inflammation Absorbed. Acupuncture can promote blood circulation, dilate blood vessels and lymphatic vessels, increase the permeability of cell membranes, exhibit an anti-inflammatory effect to a certain extent, and promote

Table 3: The location, regional anatomy, and innervation of common acupoints for treating PID.

Acupoint	Location	Muscle	Innervation
CV4 (Guanyuan)	3 cun below the center of the umbilicus on the lower abdomen and on the anterior midline	Fibrous tissue, linea alba	L1
SP6 (Sanyinjiao)	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4-5, S1-2
EX-CA1 (Zigong)	4 cun below the umbilicus and 3 cun lateral to the anterior midline	Obliquus internus abdominis, musculus transversus abdominis	T10-L2
CV3 (Zhongji)	4 cun caudal to the umbilicus	Fibrous tissue, linea alba	L1
ST36 (Zusanli)	3 cun below ST35, one finger breadth from the anterior crest of the tibia, (front ridge of tibia), between fibula and tibia	Anterior tibial muscle, extensor digitorum longus	L4-5, S1-2
CV6 (Qihai)	1.5 cun inferior to the center of the umbilicus, on the anterior midline	Fibrous tissue, linea alba	Th11
ST29 (Guilai)	4 cun inferior to the center of the umbilicus, 2 cun lateral to the anterior midline	M. rectus abdominis	Th6-12
BL32 (Ciliao)	At the 2nd posterior sacral foramen on the sacrum and the posterior ramus of the S2 nerve	Erector spinae	L2-S4
BL23 (Shenshu)	Under the 2nd spinous process of lumbar vertebra, next to 1.5 cun	Erector spinae	L1
SP9 (Yinlingquan)	Below medial tibia condyle	M. gastrocnemius	S1-2

the dissipation and absorption of pathological products and inflammatory exudates [89–91]. Shi [83] randomly divided 70 CPID patients into control arm (conventional therapy) and observation arm (conventional therapy combined with acupuncture). After treatment, the imageology showed that the time of inflammation absorbed and abdominal pain relief in the observation arm were shorter than those of control arm, with a statistically significant difference ($P < 0.05$). The levels of TNF-α, hs-CRP, and IL-6 in the two arms were decreased, and the observation arm was lower than the control arm ($P < 0.05$). Therefore, acupuncture can effectively increase the absorption of inflammation, which could be recommended for the treatment of PID [71].

3.2.2. Acupuncture Can Improve Immunity. The body mainly relies on the immune system to fight infection, with innate immunity in the early stage and acquired immunity in the later stage. Under the stimulation of pathogenic bacteria, the body activates its autoimmune system and releases plenty of cytokines and chemokines through a series of signal transduction, thereby killing the pathogenic bacteria. In the reproductive system, the innate immune system, namely Toll-like receptors (TLRs), is the first to be activated [91]. It plays an important role in the host's antimicrobial infection by regulating innate and acquired immunity and is the key to linking infection, inflammation, and injury [92]. Studies have shown that the variation of Toll-like receptors is closely related to the occurrence and development of PID [93]. Huang conducted an RCT on 80 patients with PID, and the conclusion showed that the application of acupuncture combined with acupoint injection in treating patients with CPID can improve patients' immunity, enhance efficacy, and reduce relapse [94]. Research by Jiang Yu et al. pointed out that acupuncture at Dong Qi points (a special acupuncture therapy popular in Taiwan, Europe, and the United States) can enhance the immunity of patients with CPID [95].

3.2.3. Acupuncture Can Relieve Lower Abdominal Pain. Pain located at the lower abdomen and lumbosacrum is the most common symptom of PID, which affects the life quality of most patients [96]. Numerous studies have confirmed that acupuncture can effectively relieve pain [88]. The experiment by Wang explored the efficacy of warming-needle moxibustion in treating CPID with the type of qi stagnation and blood stasis and concluded that warming-needle moxibustion could significantly eliminate pain and other clinical manifestations of CPID patients and relieve their anxiety and depression [97]. Gr [98] explored the efficacy of acupuncture combined with moxibustion in treating CPID. The results showed that, after treatment, the NRS pain score of the treatment arm was lower than that of the control arm ($P < 0.05$), and the total effective rate was higher than that of the control arm ($P < 0.05$).

3.3. The Safety of Acupuncture in Treating PID. Acupuncture is relatively safe on most occasions, only improper operation performed by unqualified acupuncturists can cause adverse reactions such as faint during treatment, stuck needle, bending of the needle, needle breakage, and hematoma. Compared with conventional therapies, treating PID with acupuncture has fewer adverse reactions, most of which are skin erythema, bruising, and pain [99]. These problems usually can be reduced or avoided by careful operation. According to an RCT, the incidence of adverse reactions in the acupuncture arm was 0.75%, which was significantly lower than 25.00% in the control arm ($P < 0.05$) [84]. In another clinical trial of 62 patients with pain treated by acupuncture, only 5 patients had adverse events [100]. Compared with significant efficacy, most minor adverse reactions can be ignored [101].

At present, a large number of clinical trials have shown that acupuncture has a clear effect on PID, and the mechanism is also relatively clear. However, there are still some limitations such as insufficient sample size and lack of high-

quality evidence. Therefore, large-scale RCTs are needed to further verify the efficacy and mechanism of acupuncture in treating PID.

3.4. The Efficacy and Mechanism of Moxibustion in Treating PID. As a natural therapy, moxibustion is to fumigate acupoints by igniting moxa-sticks made from artemisia to treat diseases. Its mechanism is similar to acupuncture, and they have a synergistic effect in treating diseases. Moxibustion is commonly used to treat gynecological diseases such as PID, dysmenorrhea, premature ovarian failure, blocked fallopian tubes, and infertility [102–106]. Among different types of moxibustion, heat-sensitive moxibustion and medicine-separated moxibustion are most frequently utilized to treat PID. Heat-sensitive moxibustion, also known as "heat-sensitive suspension moxibustion," refers to using the heat generated by burning moxa-sticks to stimulate heat-sensitive moxibustion sensation (e.g., heat permeability, heat expansion, heat transfer, local heatless but remote heat, nonheat sensation) to promote pelvic blood circulation, accelerate the absorption, and dissipation of inflammation, thereby treating PID [107, 108]. It has the characteristics of without touching the body, injury, side effects, etc. Yin's RCT compared the efficacy of acupuncture and heat-sensitive moxibustion in treating CPID. The results showed that the efficacy of heat-sensitive moxibustion was better than that of acupuncture, and the effective rates were 93.33% vs 77.78% ($P < 0.05$) [109]. Wang et al. randomly assigned 208 cases with TCM syndrome type of qi stagnation and blood stasis CPID into Xuefu Zhuyu capsule arm and heat-sensitive moxibustion Ren Du meridian combined with Xuefu Zhuyu capsule arm. The results showed that the effect of heat-sensitive moxibustion on Ren Du meridian combined with Xuefu Zhuyu capsule was better. Meanwhile, it can significantly reduce the levels of CA125 and IL-8 and increase the level of TGF-β1 in serum. Therefore, heat-sensitive moxibustion plays an important role in treating CPID [110]. Medicine-separated moxibustion is a combination of moxibustion, acupoints, and medicine. Medicine-separated moxibustion is an effective treatment for CPID with TCM syndrome type of cold-damp stagnation, which fully combines the efficacy of TCM with the heat produced by moxibustion. Because the skin around the umbilical cord is thin, it is easier for the drug to reach the lesion directly, thereby improving the arterial blood supply of the uterus, promoting the absorption of pelvic inflammation, and achieving the purpose of treating disease [111]. Some studies have shown that moxibustion can promote metabolism, increase hematopoiesis of erythrocytes, leukocytes, hemoglobin, and enhance immunity [112, 113]. In addition, studies have confirmed that moxibustion could significantly reduce the levels of CRP, IL-2, IL-6, and TNF-α in the serum of CPID patients and could effectively control the patient's infection status [114, 115]. At present, there are few adverse reactions related to moxibustion in treating PID. The adverse reactions could be that the burning moxa-sticks fall off then scald the skin and the symptoms such as fever, thirst, and skin pruritus after moxibustion. In short, moxibustion has its unique advantages in treating PID. However, there still lacks research on moxibustion's heat-sensitive effect and thermal radiation effect, which should be the focus of future research.

4. The Overview of Other CAMs in Treating PID

In addition to acupuncture and CHM, pelvic exercises, hyperbaric oxygen therapy, and other therapies can also treat PID.

4.1. The Application of Pelvic Exercises in Treating PID. Pelvic exercise belongs to aerobic exercise, which can increase the tension of pelvic ligaments and blood vessels, improve pelvic blood circulation, and promote inflammatory absorption [116]. In addition, pelvic exercise can enhance the pelvic floor ligament and muscle strength, promote local oxygen uptake, and relieve symptoms such as lumbosacral pain and lower abdominal distension [117, 118]. Pelvic exercise, the behavioral therapy in psychotherapy, can improve symptoms, at the same time, it can also help patients adjust their mentality in time, establish the confidence to cure the disease, ensure patient compliance with treatment, and improve clinical efficacy [119]. A large number of studies have found that pelvic exercise can effectively reduce the level of immune factors, such as TNF-α, CRP, IL-2, IL-6, IL-10, and MDA, and help to relieve abdominal pain. It is safe and worthy of clinical application [120–123].

4.2. The Application of Hyperbaric Oxygen Therapy in Treating PID. Hyperbaric oxygen therapy has the function of increasing blood oxygen content and oxygen partial pressure and improving the state of systemic organs, which can be used to treat gynecological diseases such as infertility, premature ovarian failure, and PID [124–126]. Studies have shown that hyperbaric oxygen has a bactericidal effect, especially on anaerobic bacteria, and can inhibit the growth of aerobic bacteria [127, 128]. Other studies have shown that hyperbaric oxygen plays a role in the treatment of PID by downregulating CRP and inflammatory cytokines [129]. Hyperbaric oxygen can also improve the body's immunity and reduce the recurrence and complications of PID [130]. Many pieces of literature reported that hyperbaric oxygen combined with the drug could increase its effect, which was worthy of further clinical application [131, 132].

4.3. The Application of Microwave Physiotherapy in Treating PID. Microwave physiotherapy, complementary therapy for PID, can dilate blood vessels, accelerate blood circulation and metabolism, and improve tissue nutrition. A large number of experiments have confirmed that microwave physiotherapy combined with TCMRE can accelerate the absorption of pathological and inflammatory products, promote local blood circulation, and significantly improve inflammatory factors and hemorheological indicators [133–136]. Microwave physiotherapy can also improve the

patients' ability to prevent disease and effectively prevent the recurrence of PID [137, 138]. Some RCTs have shown that mild moxibustion combined with microwave physiotherapy can promote blood circulation, accelerate metabolism, and effectively alleviate the symptoms of CPID patients with qi stagnation and blood stasis [139–141].

4.4. The Application of Cupping in Treating PID. Cupping is an external therapy of TCM to prevent and treat diseases. A negative pressure environment is formed in the cupping through methods such as combustion and suction. After cupping is adsorbed on acupoints or corresponding parts of the body, the local tissue would be congested, thereby achieving the purpose of warming the channels and unblocking the collaterals, relieving swelling and pain, drawing out the toxin, and expelling pus [142]. Modern studies have shown that cupping can regulate the function of the nervous system, improve the function of phagocytes, and promote blood circulation. Therefore, it has been proven to be one of the unique ways to treat PID [143]. The negative pressure stimulation produced by cupping can not only promote pelvic blood circulation, increase metabolism, be beneficial to the absorption of inflammation, and repair damaged tissues but also enhance immunity, shorten the course of treatment, and reduce the recurrence rate [144–146].

4.5. The Application of Ozone Therapy in Treating PID. Ozone is a highly active molecule with antioxidant activity, which can be used as a complementary alternative therapy for PID [147–149]. Through animal comparative studies, Wei et al. found that O3 can treat PID by inhibiting the necrosis of endometrial epithelial cells and reducing the inflammatory response, which provides a new target for the treatment of PID [150]. Escandón et al. [151] researched that ozone can reduce endometritis and improve the fertility of dairy cows. Ozone therapy is becoming a new adjuvant therapy for female reproductive health.

5. Summary

In recent years, the incidence of PID has reached as high as 12%, and the risk of depression secondary to PID has also increased yearly [152]. Clinical treatment is mainly focusing on antibiotics. However, antibiotic abuse is severe, and the potential risks such as flora imbalance, bacterial resistance, super bacteria production, and increased adverse reactions have become increasingly prominent. The most commonly used CAMs for treating PID are acupuncture and CHM. In addition, this review also mentioned the application of pelvic exercises, hyperbaric oxygen therapy, microwave physiotherapy, cupping, and ozone therapy in PID. In summary, the merits of CAM in the treatment of PID mainly include the following: (1) CAM effectively alleviates the symptoms caused by PID and accelerates the disappearance time of symptoms; (2) CAM can greatly improve clinical effective rate and reduce adverse reactions and recurrence rates; and (3) the efficacy produced by CAM can replace antibiotics and reduce the dose of antibiotics required for PID treatment. As an adjuvant treatment of PID, CAM's controversial efficacy and mechanism have aroused the horizon of many Western medical scholars. At present, due to the limitations such as small sample size, low quality, and lack of uniform standards, the effectiveness of CAM in the treatment of PID has been controversial. Therefore, we are looking forward to more high-quality research on CAM in the treatment of PID to provide a more convincing basis for CAM treatment of PID.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Dongmei Wang, Yue Jiang, and Jiaxing Feng contributed equally to this work.

Acknowledgments

This work was supported by the Natural Science Foundation of Heilongjiang (grant no. H2017051), the Scientific Research Project of Heilongjiang Administration of Chinese Medicine (grant no. SHY18-068), and the General Project of Heilongjiang Provincial Department of Education (grant no. SJGY20170613).

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