

Research Article

Network Pharmacology Analysis and Molecular Docking to Identify the Mechanism of Kuntai Capsules: Brief Research on Its Action in Premature Ovarian Insufficiency

Ruihong Cai,¹ Hailiang Wang,² Qiuping Lin,³ Jintuo Zhou,¹ and Jinhua Zhang¹ 

¹College of Clinical Medicine for Obstetric & Gynecology and Pediatrics, Fujian Medical University, Department of Pharmacy, Fujian Maternity and Child Health Hospital, Fuzhou, Fujian 350001, China

²Department of VIP Dental Service, Fujian Key Laboratory of Oral Diseases, School and Hospital of Stomatology, Fujian Medical University, Fuzhou, Fujian 350001, China

³Traditional Chinese Medicine Department, Fujian Maternity and Child Health Hospital, College of Clinical Medicine for Obstetric & Gynecology and Pediatrics, Fujian Medical University, Fuzhou, Fujian 350001, China

⁴Department of Pharmacy, Fujian Maternity and Child Health Hospital, Fuzhou, Fujian 350001, China

Correspondence should be addressed to Jinhua Zhang; pollyzhang2006@126.com

Received 12 September 2023; Revised 21 February 2024; Accepted 23 February 2024; Published 5 March 2024

Academic Editor: Muhammad Iqhrammullah

Copyright © 2024 Ruihong Cai et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. This study aimed to explore the therapeutic targets and related pathways of Kuntai capsules (KTCs) for premature ovarian insufficiency (POI) using network pharmacology and molecular docking. **Methods.** The active components and their targets of KTCs were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) website, and disease therapeutic targets of POI were downloaded from DisGeNET, GeneCards, and OMIM databases and combined with the disease differential genes of POI microarray dataset from the Gene Expression Omnibus (GEO) database. The intersecting genes of drug potential therapeutic targets and disease therapeutic targets were uploaded to the STRING database to form a protein-protein interaction network. Also, the possible pathway of KTCs in the treatment of POI was explored by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis; through the core therapeutic targets, the corresponding active ingredients of KTCs were found. Finally molecular docking was conducted to verify the accuracy of the drug action. **Results.** 120 potential therapeutic targets of KTCs for POI were found. The bioinformatics analysis revealed that KTCs may regulate the recruitment, growth, and development of follicles by controlling various pathways such as fluid shear stress, atherosclerosis, PI3K/AKT, and p53 signaling. They can inhibit granulosa cell atrophy and apoptosis, promote follicle maturation, regulate follicle sensitivity to follicle-stimulating hormone, and ultimately impact ovarian function. The core therapeutic targets were TP53, AKT1, and TNF, and the corresponding active ingredients were quercetin, kaempferol, and stigmaterol. The molecular docking results showed that all the root mean square deviations were less than 2. **Conclusions.** KTCs improve ovarian function probably by acting on regulating the recruitment of follicles, reducing the apoptosis of granulosa cells, promoting their growth and development, reducing oxidative stress damage, and improving the sensitivity of follicles to FSH.

1. Introduction

Premature ovarian insufficiency (POI) is ovarian failure in women prior to 40 years old. This generally manifests as menstrual abnormalities (amenorrhea, sparse, or frequent menstruation), high follicle-stimulating hormone levels

(FSH >25 U/L), and decreased or volatile estrogen levels [1]. POI may be accompanied by a range of symptoms, including hot flushes, sweating, and irritability. POI is the depletion and quality decrease of ovarian follicles, leading to infertility and other complications, such as perimenopausal symptoms that can seriously affect a woman's quality of life. Patients

with reproductive needs often turn to assisted reproductive technology, but the results are often unsatisfactory. Kuntai capsule (KTC) is a patented Traditional Chinese Medicine (TCM) composed of *Rehmanniae Radix Praeparata*, *Paeoniae Radix Alba*, *Coptidis Rhizoma*, *Asini Corii Colla*, *Scutellariae Radix*, and *Poria*. In TCM terms, they have the effect of nourishing yin, clearing heat, transpiring nerves, and getting rid of annoyance. KTCs are recommended for various syndromes including both pre- and postmenopause. Previous clinical trials reported that these capsules can improve ovarian function and associated reproductive outcomes [2–4]. However, owing to the multiple components, targets, and pathways, it has not been able to fully determine the mechanism of KTCs in POI treatment through experimental research. Recently, the rise of network pharmacology approaches and molecular docking techniques has brought light to explore the mechanism of action of TCM [5, 6]. Based on the previously proposed TCM pathogenesis of POI, the potential therapeutic targets of KTCs in the treatment of POI were identified and validated using network pharmacology. In addition, GO and KEGG analyses were performed to predict the probable pathway, and the main components of KTCs were also molecularly docked with the core targets of POI, hoping to enrich the clinical application and translation of KTCs in POI. The workflow of the study is shown in Figure 1.

2. Material

The following databases and tools were utilized for the analysis: Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP) (<https://old.tcm-sp-e.com/tcm-sp.php>), UniProt Database (<https://www.uniprot.org>), STRING Database (<https://cn.string-db.org>), GeneCards Database (<https://www.genecards.org>), OMIM Database (<https://www.omim.org>), DisGeNET Database (<https://www.disgenet.org>), GEO Database (<https://www.ncbi.nlm.nih.gov/geo>), Protein Data Bank (PDB, <https://www.rcsb.org/>), Venny 2.0 (<https://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html>), PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>), as well as software tools like Cytoscape 3.7.2, PyMOL 2.5.2, AutoDockTool-1.5.6, Open Babel 2.4.1, Discovery Studio 2019 Client, Adobe Illustrator CS6, and R3.6.2.

3. Methods

3.1. Screening the Active Ingredients and Targets of KTCs. The chemical constituents of KTCs were retrieved in TCMSP and screened according to absorption, distribution, metabolism, and excretion (ADME) parameters based on the conditions of drug-like properties ≥ 0.18 and bioavailability $\geq 40\%$. Then, we searched for the targets of the active ingredients through the same database and converted the target names to the gene symbols through the UniProt database. Finally, the Cytoscape 3.7.2 software was used to construct the active ingredients of KTCs and their corresponding target genes' interaction network. The period of time searched was May 2023.

3.2. Collecting the Therapeutic Targets of POI. The therapeutic targets were attained by searching DisGeNET, GeneCards, and OMIM with “POI,” “premature ovarian insufficiency,” “POF,” and “premature ovarian failure” as keywords. The period of time searched was May 2023. In GeneCards database, the higher the relevance score, the closer relationship between the targets and POI. As a rule of thumb, those with score value greater than the median were set as potential therapeutic targets if there are too many genes. Duplicate ones from the three disease databases were deleted after merging. Then, we converted the target names to the gene names by the UniProt database.

3.3. Screening Differentially Expressed Genes Related to POI. GEO is a public genomic data repository of high throughput gene expression data and microarrays. POI expression data were retrieved from GEO using the search terms “premature ovarian insufficiency,” “primary ovarian insufficiency,” “POI,” and “premature ovarian failure.” The period of time searched was from the establishment of the GEO database to May 2023. Then the R 3.6.2 “limma” package was further used for differentially expressed genes (DEGs) screening ($|\log_2(\text{fold change})| = 1$ and adjust $p = 0.05$), and volcanoes and heatmaps of DEGs were mapped.

3.4. Potential Therapeutic Targets of KTCs in the Treatment of POI. Therapeutic targets related to POI obtained from DisGeNET, GeneCards, and OMIM were combined with DEGs from POI dataset retrieved from the GEO database. These targets were then intersected with KTC therapeutic targets by Venny 2.0 to identify prospective KTC therapeutic targets for POI.

3.5. The Analysis of Protein-Protein Interaction (PPI) Network, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG). Potential therapeutic targets of KTCs for POI were uploaded to the STRING database to obtain their interactions by setting species as “*Homo sapiens*,” the minimum interaction threshold value to 0.95, and hiding disconnected nodes in the network. The PPI network was illustrated by Cytoscape 3.7.2, and the core therapeutic targets were further screened according to the degree value. To investigate the probable molecular mechanisms of KTCs in the treatment of POI, R3.6.2 “clusterProfiler” and “org.Hs.eg.db” packages were used to perform GO and KEGG enrichment analysis (p value cutoff = 0.05).

3.6. Identifying the Core Ingredients of KTCs. The venny 2.0 website was used to intersect the targets of each ingredient in KTCs with the potential therapeutic targets of KTCs for POI. The components were then ranked based on the number of intersecting targets, from highest to lowest. The first 3 ones with the most overlapping targets were identified as the core ingredients. Targets corresponding to the main core ingredient were uploaded to the STRING database to obtain

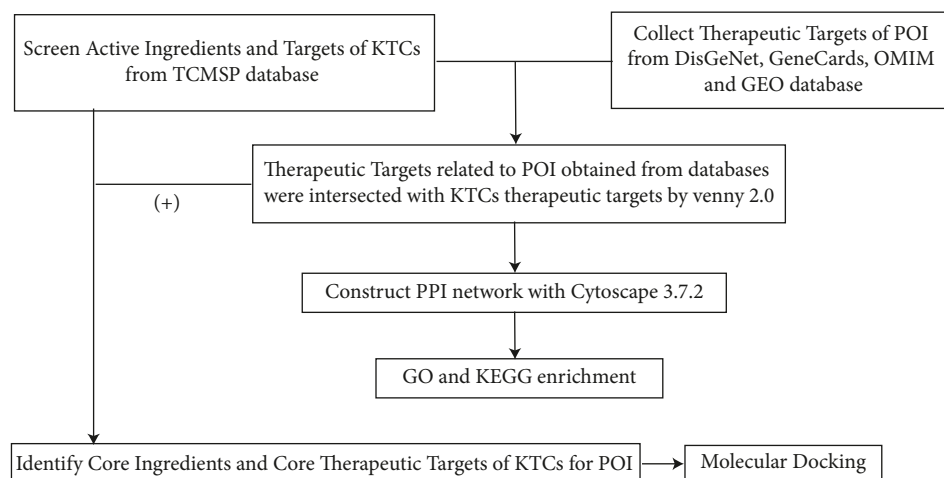


FIGURE 1: Flowchart of the network pharmacology analysis and molecular docking of KTC treatment for POI. KTCs = Kuntai capsules; POI = premature ovarian insufficiency.

the PPI interaction network by setting species as “*Homo sapiens*,” the minimum interaction threshold value to 0.95, and hiding disconnected nodes in the network. The core therapeutic targets were further screened according to the degree value.

3.7. Molecular Docking. Molecular docking was performed using CB Dock server adopting blind approach [7, 8]. In our study, the three-dimensional (3D) structure of the main core active ingredients was obtained from the PubChem website. Their chemical structures were downloaded in * mol2 format, processed using Mgltools1.5.6, hydrogenated, had their charges calculated, combined with non-polar hydrogen, and saved in * pdbqt format. The core targets of KTCs in treating POI were obtained from the Protein Data Bank (PDB), processed with Mgltools1.5.6 by hydrogenation, charge calculation, and addition of non-polar hydrogen. Subsequently, the active sites were defined (Table 1). AutoDock Vina 1.1.2 was used to dock small molecules with proteins, and the conformation with the highest score was selected for analysis and mapping by Discovery Studio 2019. The binding energy between each active ingredient and the receptor protein was obtained, a lower binding energy between the ligand and receptor in docking indicates a stronger molecular binding and a higher likelihood of interaction [9]. Finally, the visualization of molecular docking was optimized with the “Adobe Illustrator CS6” software.

4. Results

4.1. The Active Ingredients and Targets of KTCs. According to the screening conditions, 41 active ingredients and 207 therapeutic targets of KTCs were obtained through the TCMSP database (Supplementary Tables 1 and 2). The regulatory network of the active ingredients and their corresponding targets were drawn by Cytoscape 3.7.2 (Figure 2). The middle diamonds represent genes, the circles around represent drugs, and different colours represent different drugs. The larger the pattern area and the darker

the colour, the more genes the drug acts on. The degree value represented the number of edges connected to the node in the graph. In terms of degree value, MOL000098, MOL000422, and A2 were the top three pharmaceutical ingredients, corresponding to quercetin (CID: 5280343), kaempferol (CID: 5280863), and stigmasterol (CID: 5280794).

4.2. Therapeutic Targets for POI. We found 299, 1264, and 1172 therapeutic targets in the DisGeNET, GeneCards (relevance score = 7.86), and OMIM databases, respectively, by using keywords mentioned above (Supplementary Table 3).

4.3. DEGs Related to POI in the GEO Dataset. The qualified chip expression dataset GSE135697 was obtained from the GEO database. This study included 10 patients with biologically abnormal POI who were undergoing IVF/ICSI-ET at the Reproductive Medicine Center of Shandong University and 10 normal controls [10]. Biologically abnormal POI is defined by the basal serum FSH of ≥ 10 IU/L, age of < 40 years, regular menstruation, and unilateral ovarian antral follicle counts of < 5 . Differential analysis was performed on mRNAs with $|\log_2(\text{fold change})| \geq 1$ and adjust p value < 0.05 . The fold change refers to the comparison of the mean expression level of serum mRNAs in POI patients with normal patients. We detected 60 DEGs from this dataset (Supplementary Table 4). As shown in Figure 3(a), volcano plot of DEGs in GSE135697 from POI patients was plotted using ggplot2 package in R 3.6.2; fold change ≥ 2 and adjust p value < 0.05 mean that the gene expression is upregulated, indicated by red dots; fold change ≤ -2 and adjust $p < 0.05$ mean that the gene expression is downregulated, indicated by blue dots; $-2 < \text{fold change} < 2$ and adjust $p \geq 0.05$ mean that the gene expression is stable expression, indicated by grey dots. Heatmap for cluster analysis of DEGs between the two groups is shown in Figure 3(b); it was plotted using pheatmap function in R3.6.2. Each column represents

TABLE 1: Docking data between the main ingredients and the core therapeutic targets of KTCs in POI.

Receptor	Ligand (core ingredient of KTCs)	Grid box size (grid points)	Grid point (nm)	Center grid box (x, y, z)	Binding energy (kcal/mol)	Interacting amino acids	Amino acids with H-bond
TNF	Quercetin	102 × 126 × 92	0.519	16.171, 5.398, -1.374	-7.81	Pro 351, Lys79, Leu75, Leu86, His80, Val83	Leu75, Leu86, His80, Val83
TNF	Kaempferol	100 × 126 × 126	0.508	16.087, 5.255, -0.282	-7.39	Pro29, Thr175, Arg173, Val30, Ala172, Ser32, Gly31, Tyr35, Asn14, His174	Ser32
AKT1	Stigmasterol	126 × 94 × 112	0.475	18.877, -17.816, -16.252	-8.64	Arg179, Val118, Phe182, Pro180, Tyr156, His181, Lys143	Arg 179
TP53	Stigmasterol	108 × 106 × 126	0.558	8.103, -1.146, -5.988	-7.88	Tyr344, Val349, Leu317, Pro314, Tyr311, Lys309, Leu351	Tyr311

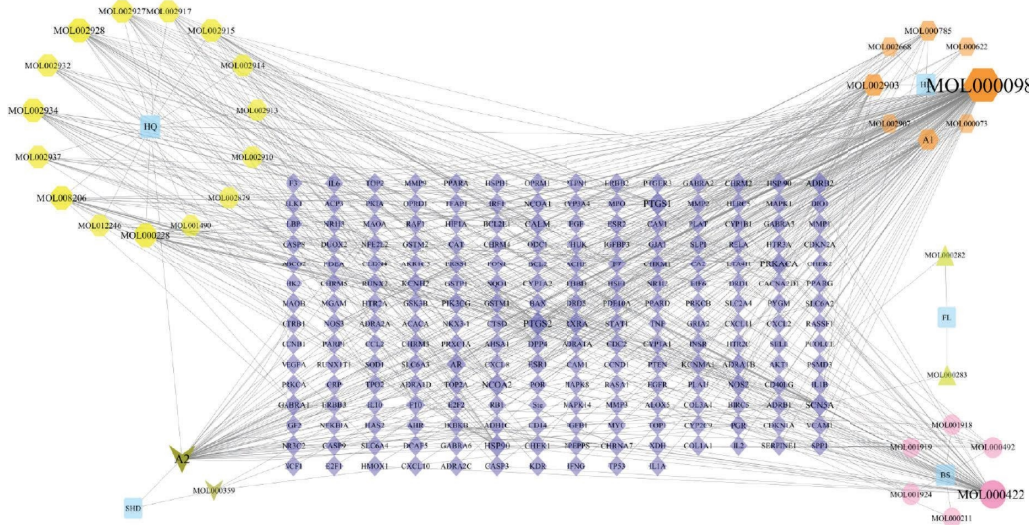


FIGURE 2: “Traditional Chinese medicine-active ingredient-target of the active ingredient” network of KTCs in the treatment of POI. KTCs = Kuntai capsules; POI = premature ovarian insufficiency. HQ: Huangqin, Scutellariae Radix. HL: Huanglian, Coptidis Rhizoma. FL: Fuling, Poria. BS: Baishao, Paeoniae Radix Alba. SDH: Shudihuang, Rehmanniae Radix Praeparata.

a sample, and each row represents a differentially expressed gene (DEG). Red indicates that the DEG was upregulated compared with another group ($p < 0.05$). Green indicates that the DEG was downregulated compared with another group ($p < 0.05$). Black indicates that the DEG was intermediate expression.

4.4. Potential Therapeutic Targets of KTCs. The genes obtained from GSE135697 were combined with POI-related targets from the DisGeNET, GeneCards, and OMIM databases. Subsequently, all these genes were intersected with therapeutic targets of KTCs, resulting in 120 potential therapeutic targets for POI (Figures 4(a) and 4(b); Supplementary Table 5).

4.5. PPI Network and GO and KEGG Analyses of Potential Therapeutic Targets for POI. These 120 potential therapeutic targets were submitted to the STRING database. The PPI network is shown in Figure 5(a). Then, Cytoscape 3.7.2 was used to optimize the interaction (Figure 5(b)). The degree value represents the number of edges connected to the node in the graph. The core targets have the largest area and the darkest color. According to the degree value, AKT1, TP53, HSP90AA1, TNF, and RELA were found to be the core therapeutic targets, with UniProt id and RCSB PDB protein structure database protein numbers of AKT1 (P31749, 3OS5), TP53 (P04637, 3TG5), HSP90AA1 (P07900, 3BM9), TNF (P01375, 5WJJ), and RELA (Q04206, 6HL6). The GO enrichment of the potential targets is shown in Figure 6(a), the vertical axis represents the GO term name, the horizontal axis represents the gene ratio, the size of the dot indicates the number of genes expressed in the GO term, and the color of the dot corresponds to the adjusted p value range. The GO enrichment revealed that the targets were mostly enriched in

growth factor, ubiquitin protein ligase and protease bindings as well as nuclear and steroid hormone receptor activities, etc. Further, KEGG enrichment analysis reported that the targets were mostly enriched in PI3K-Akt and p53 signaling pathways as well as endocrine resistance (Figure 6(b)). The vertical axis represents the KEGG term name, the horizontal axis represents the number of genes expressed in the KEGG term, and the color of the histogram corresponds to the adjusted p value range. The PPI network and the result of GO and KEGG analyses are shown in Supplementary Table 6.

4.6. The Core Ingredients of KTCs. The 148 quercetin corresponding therapeutic targets from the TCMSP database have 102 intersection genes with the 120 potential targets of KTCs for POI treatment (Figure 7(a)), which shows that quercetin is the core ingredient of KTCs. Quercetin corresponding targets were uploaded to the STRING database, and the optimized PPI interaction network by Cytoscape 3.7.2 suggested that the core therapeutic targets of quercetin were AKT1, TP53, TNF, VEGFA, and IL 6, which are highly similar to KTCs (Figure 7(b)). Then, according to the same steps, we found that kaempferol and stigmaterol are the next two main ingredients.

4.7. The Results of Molecular Docking. We performed molecular docking according to the steps described in Methods section and obtained the corresponding data (Supplementary Table 7). We found that the root mean square deviations (RMSDs) between the conformation of the docked ligand and the conformation of the original crystal structure of all docking results were less than 2. The docking data between the main ingredients and the core therapeutic targets of KTCs in POI treatment are shown in Table 1. Also, images of docking results for quercetin-TNF, kaempferol-TNF, stigmaterol-AKT1, and stigmaterol-TP53 are shown in Figure 8.

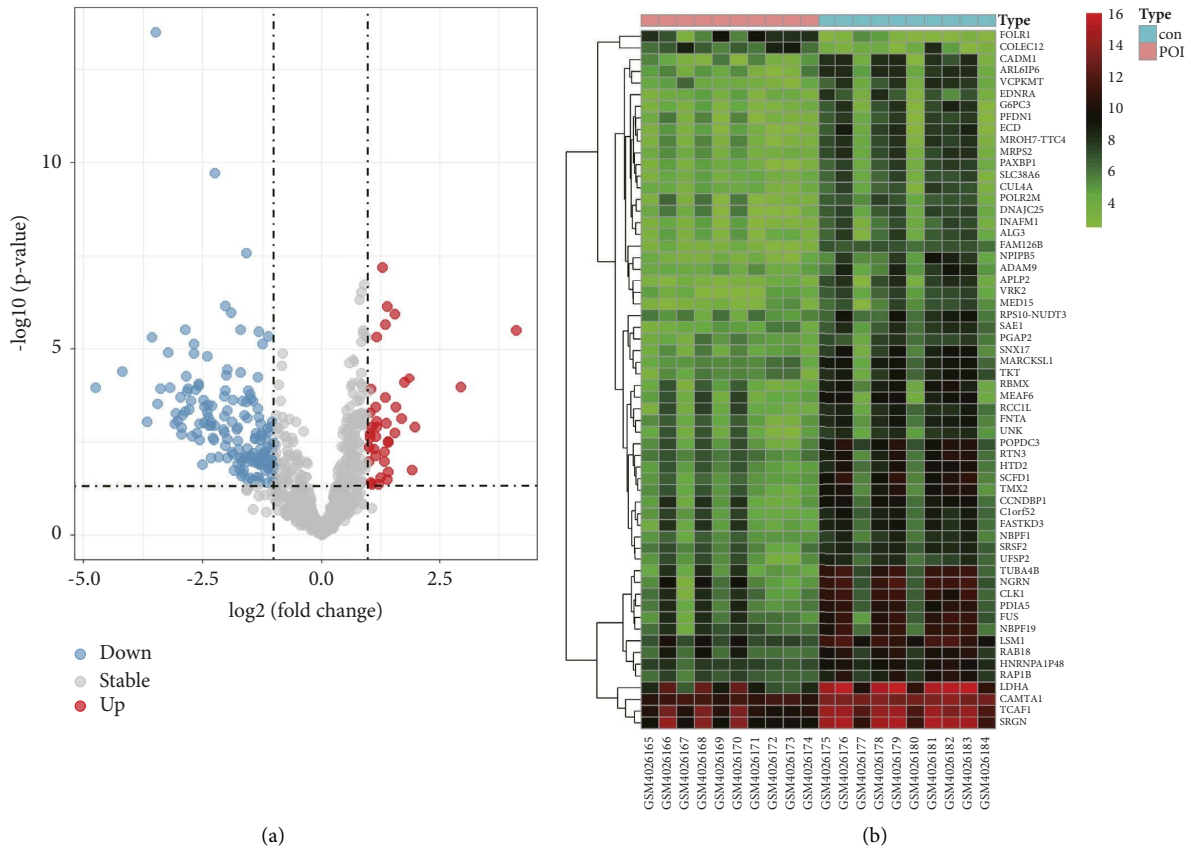


FIGURE 3: Volcano plot and heatmap of the DEGs in POI patients. DEGs: differentially expressed genes; POI = premature ovarian insufficiency. (a) Volcano plot of DEGs in GSE135697 from POI patients. (b) Heatmap for cluster analysis of DEGs. X-axis: serum samples of the POI patients in GSE135697 and the control group; Y-axis: DEGs of GSE135697.

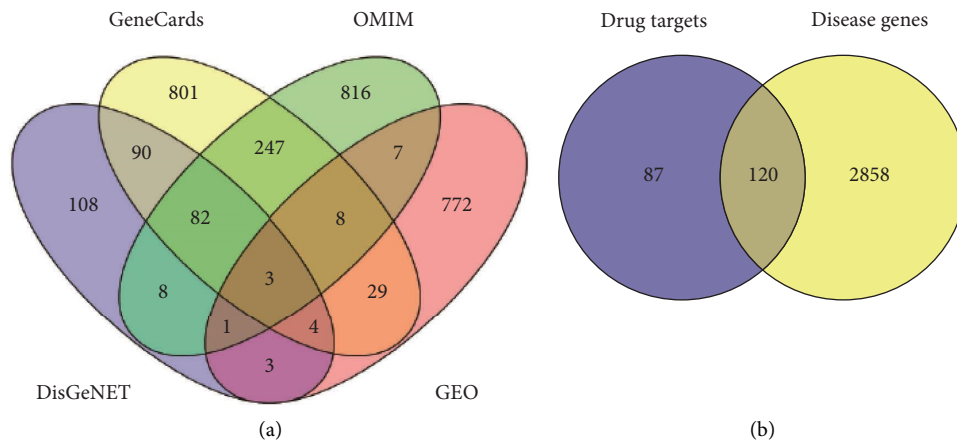


FIGURE 4: Targets related to KTCs in POI treatment. (a) The Venn diagram of POI therapeutic targets in the three disease databases and GSE135697 dataset. (b) The Venn diagram of the targets in disease databases in (a) and the therapeutic targets of KTCs.

5. Discussion

The mammalian ovary is the primary female reproductive organ involved in oocyte maturation and in the synthesis and secretion of sex steroid hormones, estrogens, and progesterone, which are crucial for female fertility. The cortex region of the human ovary possesses a finite pool of

primordial follicles, whose number is set before birth [11]. A variety of factors, such as genetic, immune, iatrogenic, and environmental ones, lead to damage or premature depletion of primordial follicles, which is often accompanied by a decline in follicle quality, and ultimately lead to POI [12].

Patients with POI are young and may have fertility requirements. But so far, there is no recognized effective way

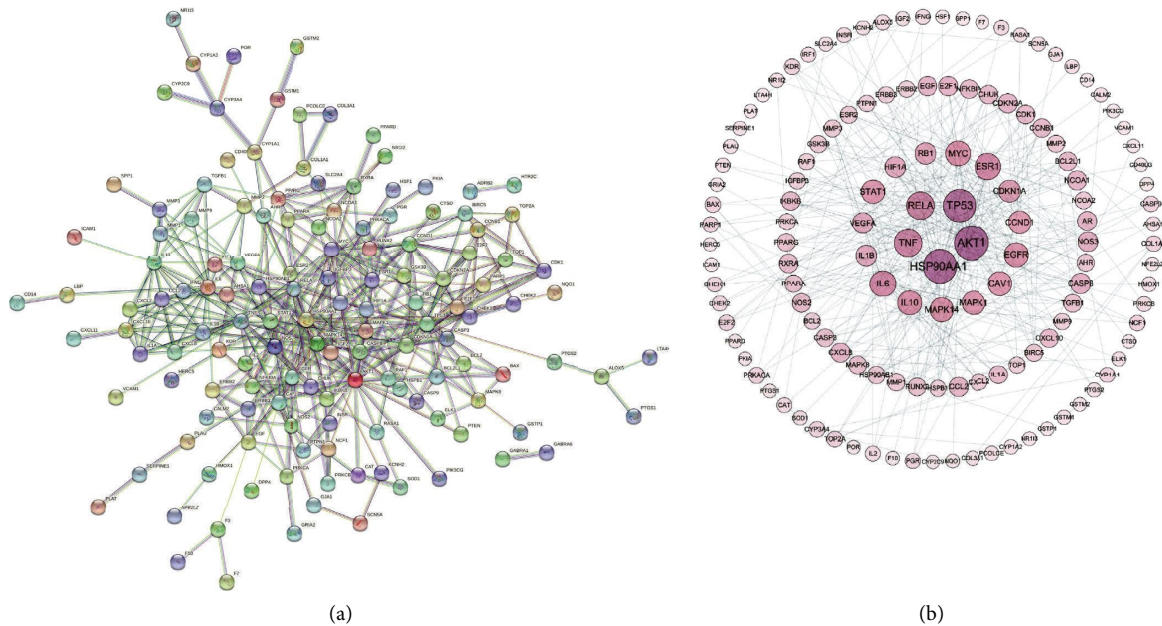


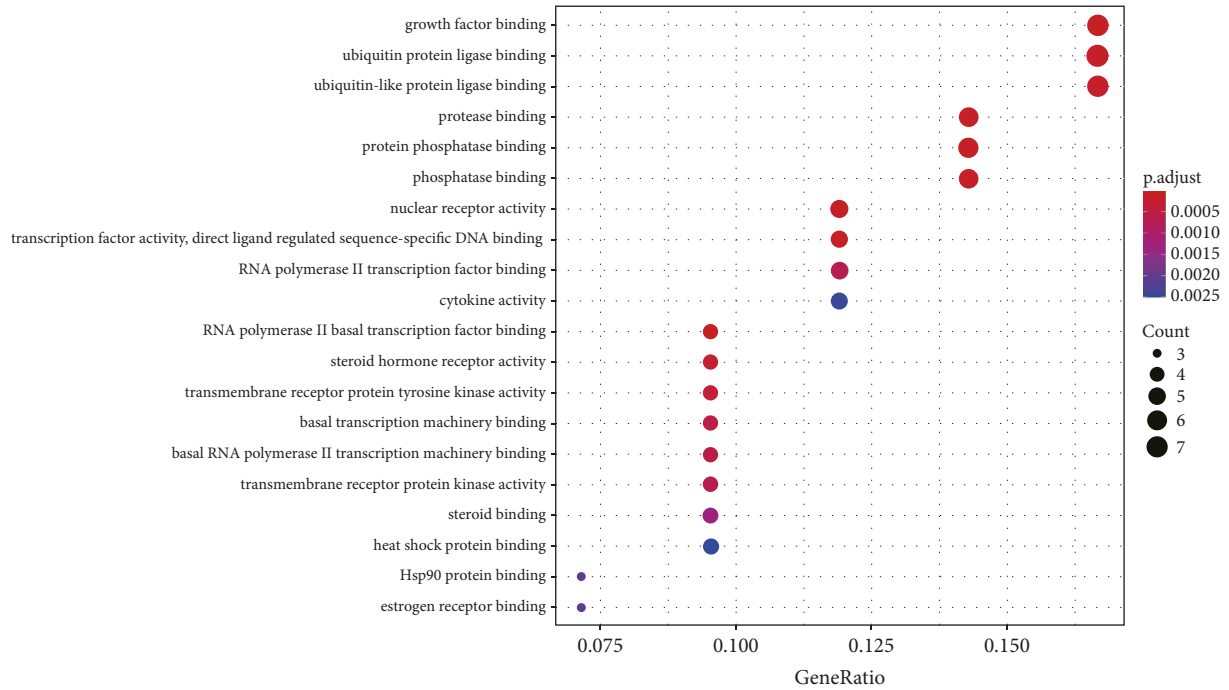
FIGURE 5: PPI network of the intersection genes from STRING database (a) and PPI network drawn using Cytoscape software (b) showing cellular interactions in the treatment of POI with KTCs.

for these patients undergoing IVF-ET to obtain ideal pregnancy rate by autologous eggs. In order to improve the outcomes of assisted reproduction, adding adjuvant drugs for pretreatment is considered to be a common method. These drugs, including DHEA, oral contraceptives, growth hormone, coenzyme Q10, and immunomodulator, are either expensive, ineffective, or have serious adverse effects [13, 14]. Several studies have shown that TCM has several advantages in improving ovarian function. KTC is a Chinese patent medicine for the treatment of perimenopausal syndrome. Recent studies have reported that KTCs can improve ovarian stimulation and pregnancy outcomes in this group, but the mechanism remains unestablished [15–18].

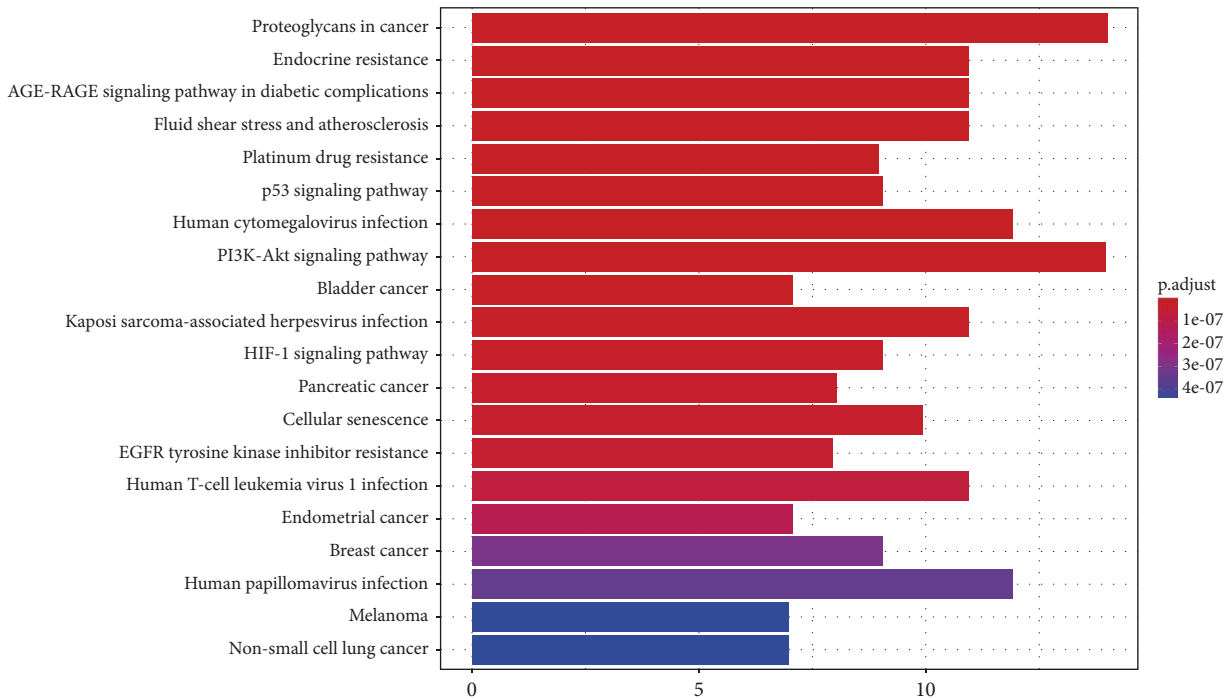
By intersecting the therapeutic targets of KTCs and POI in the common databases, 120 potential therapeutic targets of KTCs for POI were finally obtained in our research. Then, they were uploaded to the STRING database to construct a PPI network, and finally 5 core targets were found, including TP53, AKT1, TNF, HSP90AA1, and RELA. TP53, a transcription factor, is involved in cell cycle regulation that acts to negatively regulate cell division by controlling a set of genes required for this process. Research studies have shown that cytotoxic drugs could induce cytotoxicity in the maturing oocyte by activating autophagy and apoptosis in a caspase-dependent manner and could induce oxidative stress by generating reactive oxygen species that elevated the mutated ovarian TP53 protein [19]. As an important regulator of ovarian function, AKT1 is one of the serine/threonine-protein kinases and regulates many processes including metabolism, proliferation, cell survival, growth, and angiogenesis through serine and/or threonine phosphorylation of a range of downstream substrates [20]. TNF is a cytokine secreted by macrophages with a wide range of biological activities. TNF- α is related to the uptake of glucose

in tissues, which may lead to the decline of female fertility [21]. Heat shock protein 90AA1 (HSP90AA1) is a molecular chaperone that aids in protein folding. Functional HSP90 operates as dimer and has intrinsic ATPase activity. Interestingly, HSP90AA1 has been implicated in cytokine production and is involved in normal reproductive processes, including estrogen receptor α regulation and luteolysis [22, 23]. RELA (v-rel avian reticuloendotheliosis viral oncogene homolog A), formerly known as nuclear factor kappa light chain polypeptide gene enhancer, is a member of the NF- κ B family and is involved in many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis.

Our research findings indicate that KTCs influence the fluid shear stress, PI3K-Akt, and p53 signaling pathways based on GO and KEGG enrichment analysis. Previous studies have found that both oocytes and granulosa cells have PI3K/Akt signalling pathways. These are involved in regulating oocyte growth and primordial follicle development and play an important role in follicular recruitment [24, 25]. Follicle-stimulating hormones bind to specific receptors on the membrane of ovarian granulosa cells in order to activate upstream protein kinase A (PKA), which then activates the downstream PI3K/Akt pathway, thereby inducing aromatase expression, regulating the secretion of estrogen and progesterone, and promoting the maturation of ovarian granulosa cells [26, 27]. Abnormal regulation of the PI3K/Akt pathway can lead to premature recruitment of follicles, depleting the follicle pool [28] and resulting in premature ovarian failure. The activity of the p53 tumor suppressor protein can induce cell cycle arrest or apoptosis and is related to the apoptosis of granulosa cells. Several experiments have confirmed the close relationship between the p53 pathway and granulosa cell death [29]. Therefore, KTCs may regulate



(a)



(b)

FIGURE 6: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of the 120 potential targets involved in the treatment of POI with KTCs.

the recruitment, growth, and development of follicles by regulating PI3K/AKT and p53 signalling pathways, inhibit granulosa cell atresia and apoptosis, promote follicle maturation, regulate follicle sensitivity to follicle-stimulating hormone, and thus affect ovarian function.

By molecular docking, we found that quercetin-TNF, Kaempferol-TNF, stigmasterol-AKT1, and stigmasterol-TP53 have low docking energies which are less than -7 kcal/mol. The binding was better at binding energy ≤ -5.0 kcal mol⁻¹ and very good at binding energy

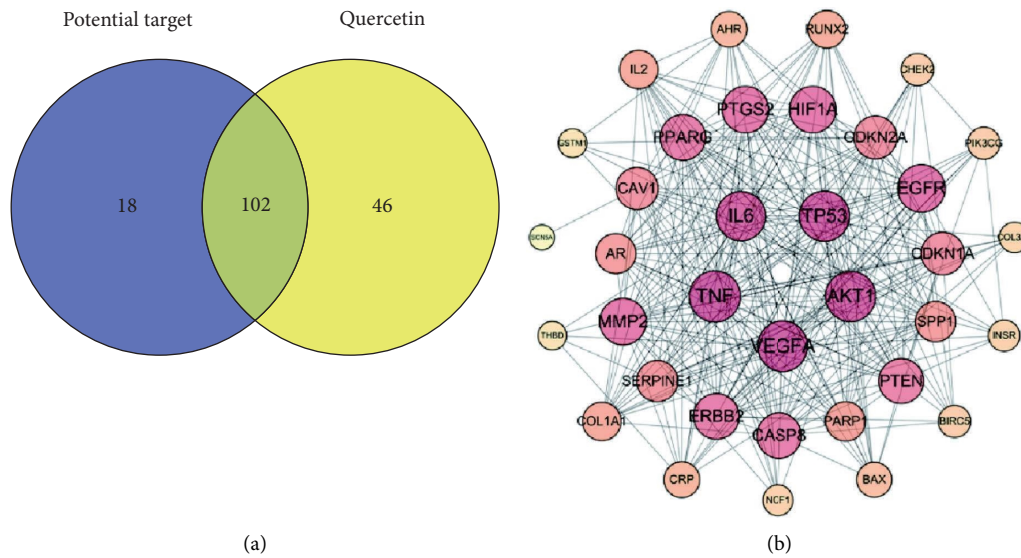


FIGURE 7: The Venn diagram of intersection therapeutic targets between KTCs and quercetin (a) and PPI network of quercetin corresponding targets (b).

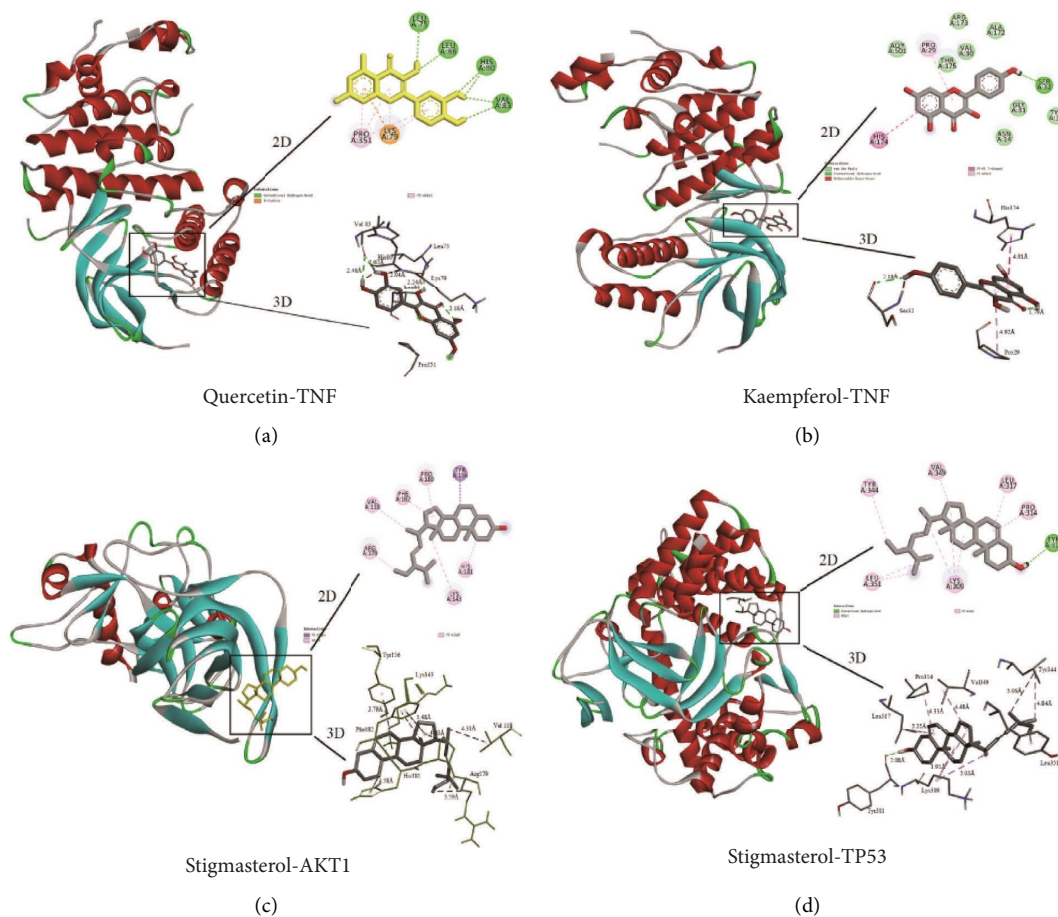


FIGURE 8: The results of molecular docking between the core ingredients of KTCs and the core therapeutic targets of KTCs in POI.

$\leq -7.5 \text{ kcal mol}^{-1}$. Quercetin and kaempferol are flavonoids widely present in fruits, vegetables, and medicinal plants. They have attracted much attention due to their antioxidant,

anti-inflammatory, and anticancer properties [30]. Quercetin works as an antioxidant by lowering free radical generation, preventing lipid peroxidation, and altering

antioxidants, so as to protect the residual follicles of POI patients from oxidative stress damage and improve their ovarian function [31].

6. Conclusion

Using network pharmacology and molecular docking, the mechanism by which KTCs improve ovarian function in patients with POI was discovered. The core active ingredients of KTCs for POI were discovered to be quercetin, kaempferol, and stigmasterol, and its main therapeutic targets were AKT1, TP53, and TNF. Possible pathways related to endocrine resistance include the PI3K/AKT and p53 signaling pathways. They probably act by regulating the recruitment of follicles, reducing the apoptosis of granulosa cells, promoting their growth and development, reducing oxidative stress damage, and improving the sensitivity of follicles to FSH. However, there are some limitations. First, we only conducted bioinformatics mining, and our results should be verified in clinical samples. The second limitation is that only a small number of patients were included in the dataset GSE135697; we look forward to the release of more POI microarray data to refine our study.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The data used in our study were obtained from public database; therefore, ethical approval was not required. No animals/humans were used in the studies that are the basis of this research.

Disclosure

The authors declare that all data were generated in-house and that no paper mill was used.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ruihong Cai, Hailiang Wang, and Jinhua Zhang conceived and designed research. Jintuo Zhou edited images. Qiuping Lin analysed data. Ruihong Cai and Hailiang Wang wrote the manuscript. All authors have read and approved the manuscript.

Acknowledgments

The authors would like to thank <https://www.enago.cn> for providing manuscript insurance service.

Supplementary Materials

Supplementary Table 1: pharmacokinetic characteristics of active ingredients of KTCs. Supplementary Table 2: therapeutic targets corresponding to effective ingredients. Supplementary Table 3: therapeutic targets of POI disease database and GEO database. Supplementary Table 4: the DEGs related to POI in GSE135697. Supplementary Table 5: the intersection of KTCs' therapeutic targets and POI-related databases. Supplementary Table 6: analysis of PPI network, GO, and KEGG of KTCs' potential therapeutic targets. Supplementary Table 7: molecular docking results of TP53, AKT1, and TNF and their corresponding effective ingredients. (*Supplementary Materials*)

References

- [1] L. Webber, M. Davies, R. Anderson et al., "ESHRE Guideline: management of women with premature ovarian insufficiency," *Human Reproduction*, vol. 31, no. 5, pp. 926–937, 2016.
- [2] J. J. Zhang, L. Fang, L. Y. Shi et al., "Protective effects and mechanisms investigation of Kuntai capsule on the ovarian function of a novel model with accelerated aging ovaries," *Journal of Ethnopharmacology*, vol. 195, pp. 173–181, 2017.
- [3] S. X. Luan, P. P. Sun, Y. H. Zhang, W. J. Zhao, and H. G. Ma, "Effect of Kuntai capsule on poor ovarian response patients undergoing in-vitro fertilization-embryo transfer," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 37, no. 12, pp. 1439–1442, 2017.
- [4] H. Zhang, F. F. Qin, A. L. Liu et al., "RETRACTED: Kuntai capsule attenuates premature ovarian failure through the PI3K/AKT/mTOR pathway," *Journal of Ethnopharmacology*, vol. 239, Article ID 111885, 2019.
- [5] F. Q. Liang, W. W. Cao, Y. T. Huang et al., "Isoflavone biochanin A, a novel nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element activator, protects against oxidative damage in HepG2 cells," *BioFactors*, vol. 45, no. 4, pp. 563–574, 2019.
- [6] X. S. Li, J. X. Ma, L. Guo et al., "Identification of bioactive compounds and potential mechanisms of kuntai capsule in the treatment of polycystic ovary syndrome by integrating network pharmacology and bioinformatics," *Oxidative Medicine and Cellular Longevity*, vol. 2022, Article ID 3145938, 11 pages, 2022.
- [7] X. Y. Wang, X. Y. Zhang, Y. J. Dang et al., "Long noncoding RNA HCP5 participates in premature ovarian insufficiency by transcriptionally regulating MSH5 and DNA damage repair via YB1," *Nucleic Acids Research*, vol. 48, no. 8, pp. 4480–4491, 2020.
- [8] S. Elbe and G. Buckland-Merrett, "Data, disease and diplomacy: GISAIID's innovative contribution to global health," *Global Challenges*, vol. 1, no. 1, pp. 33–46, 2017.
- [9] T. N. Hasan, S. S. Naqvi, M. U. Rehman et al., "Ginger ring compounds as an inhibitor of spike binding protein of alpha, beta, gamma and delta variants of SARS-CoV-2: an in silico study," *Narrative*, vol. 3, no. 1, p. e98, 2023.
- [10] M. Abinaya and M. Gayathri, "Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-Trihydroxyflavone from *alstonia scholaris* leaf against *P. aeruginosa*," *Bioorganic Chemistry*, vol. 87, pp. 291–301, 2019.
- [11] S. Gonfloni, C. Jodice, B. Gustavino, and E. Valentini, "DNA damage stress response and follicle activation: signaling routes

- of mammalian ovarian reserve,” *International Journal of Molecular Sciences*, vol. 23, no. 22, pp. 14379–14388, 2022.
- [12] X. C. Ding, X. W. Gong, Y. Y. Fan et al., “DNA double-strand break genetic variants in patients with premature ovarian insufficiency,” *Journal of Ovarian Research*, vol. 16, no. 1, p. 135, 2023.
- [13] Q. H. Y. Wong, T. W. Y. Yeung, S. S. F. Yung, J. K. Y. Ko, H. W. R. Li, and E. H. Y. Ng, “The effect of 12-month dehydroepiandrosterone supplementation on the menstrual pattern, ovarian reserve markers, and safety profile in women with premature ovarian insufficiency,” *Journal of Assisted Reproduction and Genetics*, vol. 35, no. 5, pp. 857–862, 2018.
- [14] P. H. Feng, Q. Xie, Z. Liu, Z. X. Guo, R. Y. Tang, and Q. Yu, “Study on the reparative effect of PEGylated growth hormone on ovarian parameters and mitochondrial function of oocytes from rats with premature ovarian insufficiency,” *Frontiers in Cell and Developmental Biology*, vol. 9, Article ID 649005, 2021.
- [15] Y. Y. Luo, S. C. Li, and L. Z. Xu, “The efficacy and safety of traditional chinese herbal medicine kantai in treatment of premature ovarian insufficiency: a meta-analysis,” *Chinese Journal of Evidence-Based Medicine*, vol. 19, pp. 953–959, 2019.
- [16] H. Huang, J. P. Li, M. Chen, and S. F. He, “Study on kantai capsules combined with moxibustion in the treatment of early ovarian insufficiency,” *Heilongjiang Medical Journal*, vol. 44, pp. 478–481, 2020.
- [17] F. Lian and X. Y. Jiang, “Effect of Kuntai capsules on the number of retrieved oocytes, high-quality oocytes and embryos in in vitro fertilization of poor ovarian response patients,” *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 34, no. 8, pp. 917–921, 2014.
- [18] B. Zhang, N. Chu, X. M. Qiu et al., “Effects of heyuan kantai capsule on follicular development and oocyte cohesin levels in aged mice,” *Chinese Journal of Integrative Medicine*, vol. 24, no. 10, pp. 768–776, 2018.
- [19] S. A. Mawed, C. Marini, M. Alagawany et al., “Zinc oxide nanoparticles (ZnO-NPs) suppress fertility by activating autophagy, apoptosis, and oxidative stress in the developing oocytes of female zebrafish,” *Antioxidants*, vol. 11, no. 8, Article ID 11081567, 2022.
- [20] A. Makker, M. M. Goel, and A. A. Mahdi, “PI3K/PTEN/Akt and TSC/mTOR signaling pathways, ovarian dysfunction, and infertility: an update,” *Journal of Molecular Endocrinology*, vol. 53, no. 3, pp. R103–R118, 2014.
- [21] S. S. Mei, C. Q. Yu, J. Ding, and W. Cheng, “A network pharmacology study on the similarities and differences in the mechanisms of Zuo Gui Wan/You Gui Wan for the treatment of premature ovarian failure,” *Combinatorial Chemistry and High Throughput Screening*, vol. 25, 2022.
- [22] A. E. Dhamad, Z. Q. Zhou, J. H. Zhou, and Y. C. Du, “Systematic proteomic identification of the heat shock proteins (hsp) that interact with estrogen receptor alpha (ER α) and biochemical characterization of the era-hsp70 interaction,” *PLoS One*, vol. 11, no. 8, Article ID e0160312, 2016.
- [23] J. T. Seibert, M. K. Adur, R. B. Schultz et al., “Differentiating between the effects of heat stress and lipopolysaccharide on the porcine ovarian heat shock protein response,” *Journal of Animal Science*, vol. 97, no. 12, pp. 4965–4973, 2019.
- [24] A. J. W. Hsueh, K. Kawamura, Y. Cheng, and B. C. J. M. Fauser, “Intraovarian control of early folliculogenesis,” *Endocrine Reviews*, vol. 36, no. 1, pp. 1–24, 2015.
- [25] J. Yang, J. L. Liang, and J. J. Qin, “Research progress on the correlation between PI3K/Akt signalling pathway and premature ovarian failure,” *Progres en Obstetrique et Gynecologie*, vol. 25, pp. 156–158, 2016.
- [26] X. W. Li, L. Lang, Y. B. Ji, B. B. Wang, and C. L. Zhao, “Study on process of the signaling pathway of follicle stimulating hormone to promote the ovarian granulosa cell proliferation and differentiation,” *J Beijing Union Univers*, vol. 2, pp. 46–50, 2012.
- [27] E. Nilsson, B. Zhang, and M. K. Skinner, “Gene bionetworks that regulate ovarian primordial follicle assembly,” *BMC Genomics*, vol. 14, no. 1, p. 496, 2013.
- [28] Y. Ma, Y. Y. Zhong, X. Chen et al., “Hyperbaric oxygen treatment ameliorates the decline in oocyte quality and improves the fertility of aged female mice,” *Reproductive Sciences*, vol. 30, no. 6, pp. 1834–1840, 2022.
- [29] X. D. Huang and Q. Yu, “Bioinformatic analysis confirms differences in circular RNA expression profiles of cumulus cells between patients with ovarian and peritoneal endometriosis associated infertility,” *Frontiers in Endocrinology*, vol. 14, Article ID 1137235, 2023.
- [30] D. L. Liu, S. J. Liu, S. Q. Hu, Y. C. Chen, and J. Guo, “Probing the potential mechanism of quercetin and kaempferol against heat stress-induced sertoli cell injury: through integrating network pharmacology and experimental validation,” *International Journal of Molecular Sciences*, vol. 23, no. 19, Article ID 231911163, 2022.
- [31] Y. Chen, Y. Zhao, C. Y. Miao et al., “Quercetin alleviates cyclophosphamide-induced premature ovarian insufficiency in mice by reducing mitochondrial oxidative stress and pyroptosis in granulosa cells,” *Journal of Ovarian Research*, vol. 15, no. 1, p. 138, 2022.