Hindawi
Andrologia
Volume 2023, Article ID 2820367, 12 pages
https://doi.org/10.1155/2023/2820367

Research Article

Investigation of the Underlying Mechanism of Depression on Male Infertility: A Bioinformatics and Experimental Research Study

Qi Zhao, Fei Yan, Chuan Ma, Junlong Feng, Sheng Deng, Cong Zhao, Huanan Zhang, Haisong Li, Bin Wang, and Jisheng Wang

Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, Beijing, China

Correspondence should be addressed to Haisong Li; lihs369@sina.cn, Bin Wang; dayiwangbln@sina.com, and Jisheng Wang; houdejisheng@sina.com

Received 5 January 2023; Revised 22 March 2023; Accepted 28 March 2023; Published 20 April 2023

Academic Editor: Yong-Gang Duan

The impact of depression on spermatogenic function and protein expression was investigated by reference to a bioinformatics database for the prediction of key targets and pathways. Experimental validation was performed using a rat model established using the chronic restraint stress method. Organ coefficients and semen parameters were measured. Hematoxylin and eosin (HE) staining was performed to compare testicular tissue between model and control rats. Western blotting and RT–qPCR were conducted to evaluate protein and mRNA expression. As a result, depression and male infertility (MI) were found to share 81 common targets, and the PI3K/Akt/FOXO1 signaling pathway was involved in both conditions. Animal experiments showed testis and epididymis coefficients to be lower in the model group. HE staining showed damage to the seminiferous tubules of model rats. PI3K and p-Akt mRNA and protein were present at lower levels and FOXO1 at higher levels in the model group. Depression led to reduced spermatogenic function in rats and might be associated with differential expression of the PI3K/Akt/FOXO1 signaling pathway.

1. Introduction

Depression may be defined as a continuous and prolonged period of low mood, accompanied by symptoms such as loss of appetite, insomnia, severe fatigue, and a lack of sense of self-worth [1–3]. Epidemiological surveys have found that the lifetime prevalence of depression is 15%-18% [4], and 350 million people worldwide may suffer from the condition according to the World Health Organization (WHO) [5]. Indeed, depression is predicted to be a leading contributor to global disease burden by 2030 [6] and has been associated with diabetes, hypertension, and male infertility [7–9].

Male infertility (MI) refers to the inability of a couple to conceive naturally due to factors associated with the male partner after having regular sex for more than one year without the use of any contraception [10]. The global prevalence of infertility has been estimated at 7% with male factors accounting for up to 40% of cases [11]. 42.9% of male patients seeking fertility treatment have been diagnosed with depression [12]. And male patients are significantly more likely to experience depression after the failure of assisted reproductive technology (ART) than beforehand [13]. Men diagnosed with depression also had significantly lower semen volume, sperm count, and total motility than healthy men [14].

Many studies have sought to identify mechanisms which would explain the association between MI and depression. Chronic depression impaired the reproductive system through interactions between the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes [15]. Moreover, depression could reduce sexual function in men, which in turn damage male fertility and exacerbate negative moods in males [16]. Although the use of antidepressants may improve depressive symptoms, the harmful effects on male reproductive function had gradually been discovered.

Antidepressants have been reported to reduce sperm quality [17], induce sperm DNA fragmentation [18], and lower testosterone levels [19]. In summary, patients with MI are at higher risk of depression, but underlying mechanisms remain unclear.

Vast quantities of information relating to human health and biodiversity are available from biological databases and may be utilized to aid the understanding of the underlying biological mechanisms [20]. Therefore, the current study used bioinformatics analysis to predict the protein targets associated with depression and MI. Depression may affect the expression of the PI3K/Akt/FOXO1 pathway and related proteins, and these may also be associated with MI. Then, a rat model of depression was established, and spermatogenic function in testicular tissues and changes in signaling pathway proteins (PI3K, Akt, and FOXO1) were evaluated. The flow chart of the study design is shown in Figure 1.

2. Materials and Methods

2.1. Experimental Animals. Twenty male SPF Sprague–Dawley (SD) rats (aged 4-5 weeks; 200-220 g) were purchased from Beijing Vitonliol Laboratory Animal Technology Co., Ltd. (Animal License No. SCXK (Beijing) 2020-0033). Rats were housed in the SPF animal house of Beijing University of Chinese Medicine with 10-12 h ambient sunlight, 55-60% indoor humidity, and a temperature of 21-25°C. Animals were fed a solid diet and deionized water for 7 days of adaptation. Ethical approval was granted by the Experimental Animal Ethics Committee of Beijing University of Chinese Medicine (ethics number: BUCM-4-2020122303-4150), and guidelines for the ethical review of laboratory animal welfare under People's Republic of China National Standard GB/T 35892-2018 were followed.

2.2. Drugs and Reagents. All reagents were purchased from GenePool (Beijing, China), including a sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) Gel Kit (GPP1816), SDS–PAGE Loading Buffer (5x) (GPP1820), Protein Extraction Kit (GPP1815), Tris–Glycine Running Buffer (5x) (GPP1821), Total RNA Extraction Kit (DNase I) (GPQ1801), mRNA cDNA Synthesis Kit (GPQ1803), mRNA/lncRNA qPCR Kit (GPQ1808), and RNA Loading Buffer (5x) (GPQ181). Anti-PI3K antibody (1: 1000 dilution, Abcam, AB191606, Cambridge, UK) and GAPDH (1: 5000 dilution, AB181602) were purchased from Abcam; anti-p-Akt antibody (1: 1000 dilution, Bioss, bs-2720R, Beijing, China) and anti-FOXO1 antibody (1: 1000 dilution, Bioss, bs-2537R) were purchased from Beijing Bioss Biotechnology Co., Ltd. An electrophoresis instrument (CAVOY, PP-1150), spectrophotometer (Nano-Drop 2000, Thermo Scientific), real-time PCR instrument (LineGene 9600 Plus, Bioer Technology), and dual vertical electrophoresis tank (CAVOY, MP-8001) were also used.

2.3. Bioinformatics Analysis

2.3.1. Identification of Molecular Targets. Targets related to depression and MI were identified from the GeneCards database (https://www.genecards.org/), and a Venn diagram was constructed to indicate common targets. Network construction and analysis were performed based on the common targets.

2.3.2. Construction of the Protein–Protein Interaction (PPI) Network. Common targets were imported into the STRING database (https://string-db.org/, ver. 11.0), and a minimum interaction score value of 0.700 was selected as the screening criterion. Then, the PPI results were imported into Cytoscape software for visualization. The top ten proteins with the highest core degrees were analyzed by the CytoHubba plug-in and regarded as potential key targets.

2.3.3. GO and KEGG Pathway Enrichment Analyses. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on common target proteins using the DAVID database (https://david.ncifcrf.gov/) [21]. GO functional analysis was described in terms of biological processes (BPs), cellular components (CCs), and molecular functions (MFs). KEGG enrichment analysis revealed the signaling pathways enriched by the above targets.

2.4. In Vivo Experiments

2.4.1. Establishment of the Animal Model. Twenty SD rats were randomly divided by a random number table into control ($n = 10$) and depression model ($n = 10$) groups. Chronic restraint stress was used to establish the model of depression [22]. Model rats were placed inside a thin wire mesh to restrict movement on experimental day 1, and care was taken to adjust the mesh to ensure that rats could breathe freely. Restraint was imposed from 9:00 to 15:00 daily, during which time rats were not allowed food or water. At the end of the restraint period, the rats were returned to a single cage with food and water *ad libitum*. The modeling process lasted for 28 days. Control animals were reared normally with food and water *ad libitum*.

2.4.2. Behavioral Testing. The sucrose preference and open field tests were conducted for all animals, and significant differences between control and model groups ($p < 0.05$) were considered to show the successful establishment of the model [23].

(1) Sucrose Preference Test (SPT). Rats were trained to drink sugary water prior to the experiment, and two bottles of 1% sucrose solution were given for the first 24 h. Thereafter, one bottle was replaced with sterile water. After adaptation, rats were deprived of water and food for 24 hours before being given access to 2 bottles of water: one of 1% sucrose and one of sterile water. Bottles were randomly placed on the left and right and weighed after 1 h. The sucrose preference ratio was determined using the following formula: sucrose preference ratio (%) = sucrose intake (ml) × 100%/[sucrose intake (ml) + water intake (ml)].

(2) Open Field Test (OFT). The OFT device included a field reaction box (80 cm × 80 cm × 40 cm) with the bottom divided into 25 equal compartments, and experiments were

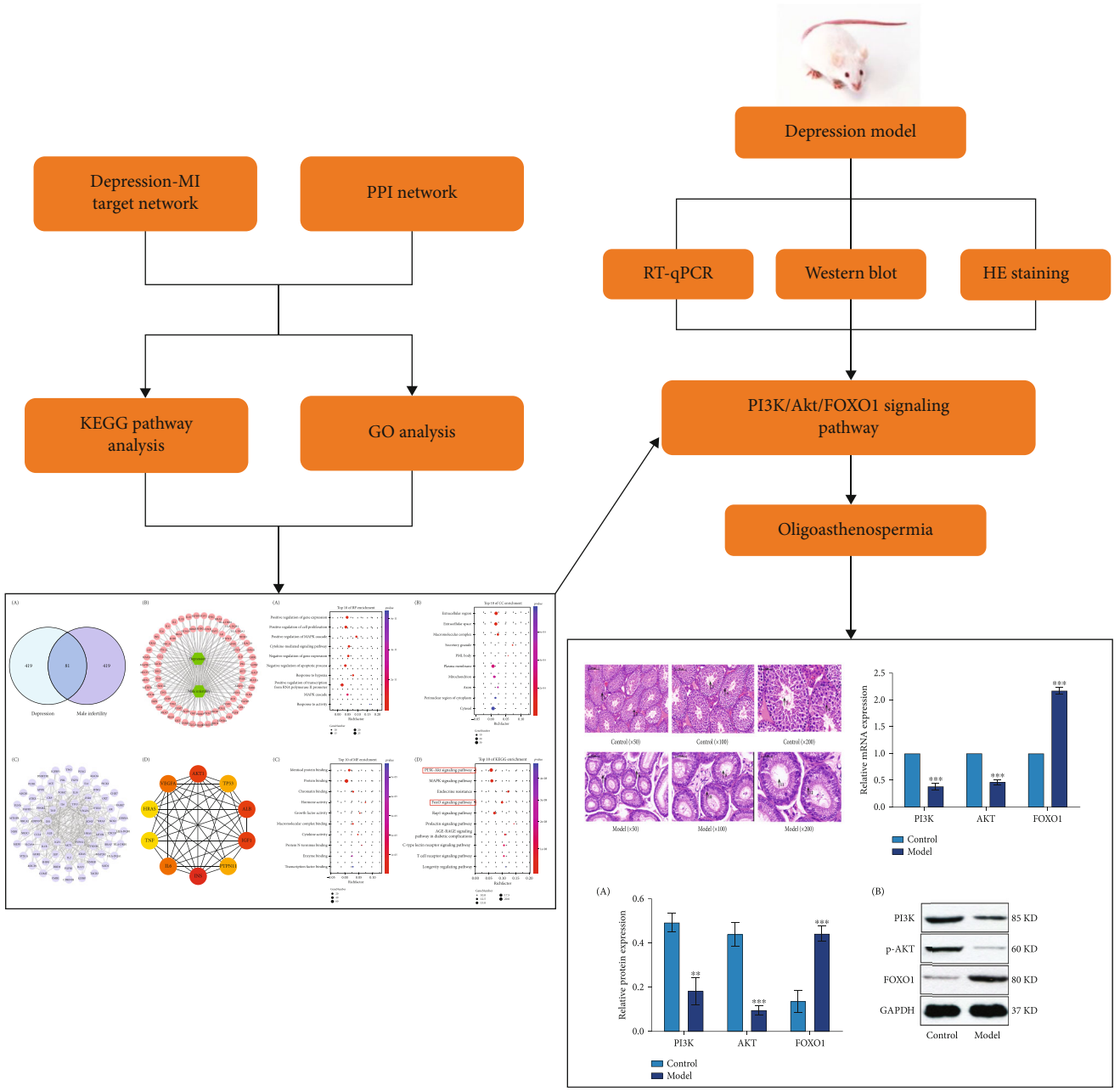

Figure 1: Study design flow chart. Proteins and pathways associated with depression and MI were identified from the DAVID database, and mRNA and proteins were measured by RT–qPCR and Western blotting. The results of *in vivo* experiments were consistent with the bioinformatics prediction results. The decline of semen quality in a rat model of depression was associated with the PI3K/Akt/FOXO1 signaling pathway.

conducted in a dark, quiet room. Rats were placed in the reaction box, and the number of times they crossed the bottom (number of squares into which all four paws entered) was used to score horizontal movements. The number of times they stood upright (1 point for both feet leaving the bottom until they dropped) was used to score vertical movements. The experimental area was cleaned with 75% ethanol after each rat completed the test to remove odors. Two trained observers assessed rat locomotion over a period of 3 min before modeling and 1 day before sampling to avoid subjective errors.

2.4.3. Preparation of Tissue Samples. Rats were weighed and anesthetized by intraperitoneal injection of 50 mg/kg 1% sodium pentobarbital before sacrifice. Testes and epididymal tissues were removed and weighed using a precision electronic balance. Organ coefficients (testis or epididymis) were calculated as follows: organ coefficient = organ weight (g)/body weight (g) × 100%.

The epididymis was transferred to prewarmed 0.9% saline, tissues minced with fine scissors, and incubated to assess semen quality (sperm concentration and viability). The right testes were used for Western blotting (WB) and

reverse transcription polymerase chain reaction (RT–qPCR). The left testes were used for morphological observations by hematoxylin and eosin (HE) staining.

2.4.4. Analyses of Semen Quality. The epididymis was incubated in 0.9% saline at 37°C for 5 min, and sperm quality was analyzed by the Weili Sperm Quality Testing System (Beijing Xing Rong Technology Co., Ltd., China, No. WLJY-9000) at 37°C. Aliquots (5 μL) of semen were dropped onto the counting plate and covered with a coverslip for microscopic observations according to WHO standards [24]; 10 fields of view were randomly selected, and the number of spermatozoa in 10 large squares was counted. Mean values are presented as $\times 10^6$ sperm per ml.

2.4.5. Histological Analysis. The left testis ($n = 10$) was removed and cut along the largest surface. Half the sample was fixed with 4% paraformaldehyde for 24 h, trimmed and cut along the transverse axis, and fixed for 12 h. The sample was rinsed with running tap water for 24 h with deionized water for 2 h and dehumidified with absorbent paper before dehydration and degreasing with alcohol and xylene. Samples were embedded in paraffin, and 4-5 μm sections were cut. Three slices were taken from each testis, 10 fields of view were randomly selected for each slice, HE staining was performed, and histopathological changes were observed by electron microscopy (BK-FL4, OPTEC; Chongqing, China).

2.4.6. Western Blotting Assay. A portion of tissue from the right testis was lysed with RIPA lysis buffer to extract total proteins which were measured and separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked in 5% skimmed milk for 1 h at room temperature and incubated with anti-PI3K, anti-p-Akt, anti-FOXO1, or anti-GAPDH antibodies overnight at 4°C. Membranes were washed three times with PBS, incubated with goat anti-rabbit secondary antibody for 1 h, and rewashed. Positive binding was visualized using an ECL developer, and the optical density of bands was analyzed using Quantity One software v.4.6.2 (Bio-Rad, Hercules, California, USA).

2.4.7. RT–qPCR Assay. 100 mg testis tissues were homogenized and centrifuged, and 250 μL trichloromethane was added for 3 min before a second centrifugation. The supernatant was left at -20°C for 15 min and centrifuged at 4°C for 10 min. 20 μL RNase-free water was added to the precipitate to resuspend the RNA, and reverse transcription was performed by the cDNA reverse transcription kit according to the manufacturer's instructions. PCR amplification was performed as follows: 35-45 cycles of predenaturation at 95°C for 2 min, denaturation at 95°C for 15 s, extension at 55-68°C for 30 s, and annealing at 45°C for 20 s. Primer sequences are shown in Table 1. Relative quantification (RQ) was performed by the $RQ = 2^{-Ct\Delta\Delta}$ method to determine differences in gene expression by comparing the ploidy of the target gene relative to the reference gene.

Table 1: The primer sequences for qPCR.

Primer		Primer sequence (5′ to 3′)
PI3K	Upstream	GAGAACCTATTGCGAGGGAAAC
	Downstream	GAC ATTGAGGGAGTATTGTGC
AKT	Upstream	CTTCTTCCACCTGTCTCGTGAGC
	Downstream	ACAGCCCGAAGTCCGTTATCTT
FOXO1	Upstream	CAGCCAGGCACCTCATAACA
	Downstream	GGACACCCATCCTACCATAGC
GADPH	Upstream	ACGGCAAGTTCAACGGCACAG
	Downstream	GAAGACGCCAGTAGACTCCACGAC

2.5. Statistical Analysis. All analyses were performed using SPSS software (version 26.0, Armonk, NY, USA). Continuous data are presented as mean ± SD. All data met the normal distribution and Chi-square requirement, and Student's t test was used to compare two groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Target Screening. The top 500 depression targets and the top 500 MI targets were screened separately from the GeneCards database. The Venn diagram (Figure 2(a)) showed 81 common targets associated with depression and MI.

3.2. PPI Network Construction. Common targets for depression and MI were uploaded into the STRING database, and a network featuring the two diseases was constructed (Figure 2(b)) with a PPI network for visualization (Figure 2(c)) using Cytoscape software. The CytoHubba plug-in was used to analyze the PPI network and identify the top 10 targets, AKT1, VEGFA, IL6, TNF, IGF1, INS, TP53, ALB, PTPN11, and HRAS, according to their degree (Figure 2(d)).

3.3. GO and KEGG Enrichment Analyses. GO and KEGG enrichment analyses of the common targets were conducted via the DAVID database. The targets were involved in 410 BPs, 55 CCs, 54 MFs, and 138 signaling pathways. KEGG enrichment analysis revealed a possible association of the PI3K/Akt/FOXO1 signaling pathway with depression and MI. GO terms and KEGG pathways were visualized by the Omicshare website (https://www.omicshare.com/) according to their p values (Figure 3).

3.4. Validation of a Rat Model of Depression. Model rats were sluggish, slow in movement, had an increased respiratory rate, reduced diet, and decreased body weight compared with controls. The SPT showed no significant differences in sugar-water consumption or sugar-water preference between the two groups on day 1 (Figure 4(a)). However, the sugar-water consumption and sucrose preference ratio were significantly lower in the model than in the control group by day 28 ($p < 0.01$). OFT scores for horizontal and vertical movements did not differ between the two groups on day 1, but scores were significantly lower for the model

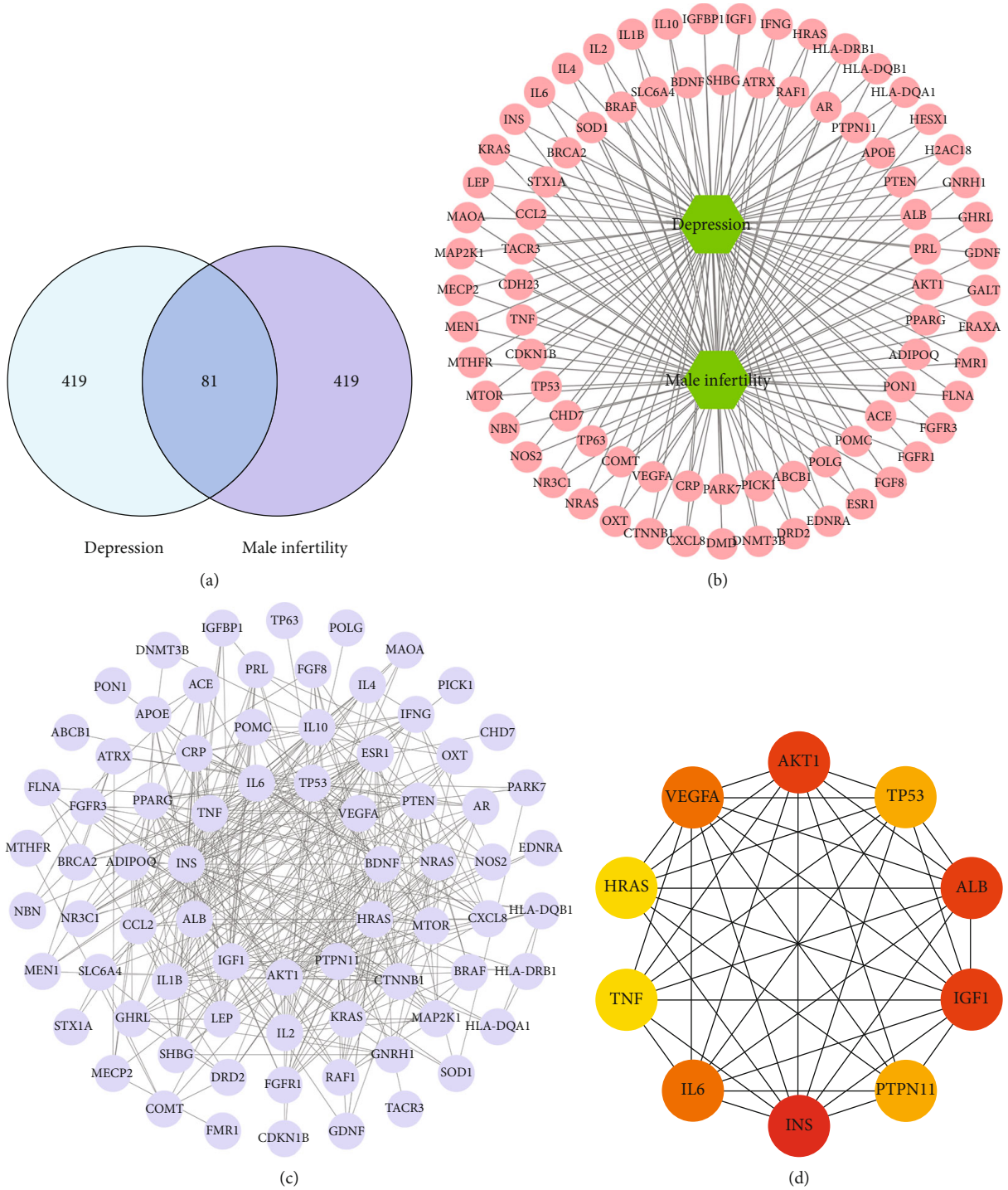

Figure 2: Bioinformatics analysis. (a) Intersection of targets of depression and MI. (b) Common target-disease network built by Cytoscape (3.7.1). (c) protein–protein interaction (PPI) network built by Cytoscape (3.7.1). (d) PPI network processed by Cytoscape (3.7.1) plug-in (CytoHubba).

group than for controls by day 28 ($p < 0.01$) (Figures 4(b) and 4(c)).

3.5. Organ Coefficients and Semen Quality. Testis and epididymis coefficients were reduced in model rats compared with controls ($p < 0.001$, $p < 0.05$) (Figures 5(a) and 5(b)). Sperm concentration and motility were both lower in the model group ($p < 0.001$) (Figures 5(c) and 5(d)), showing poorer semen quality in depressed rats than in controls.

3.6. HE Staining of Testis Tissues. Spermatozoa in the testes of the control group appeared normal in the structure under the light microscope with tightly arranged varicose seminiferous tubules and a large number of normal spermatozoa at

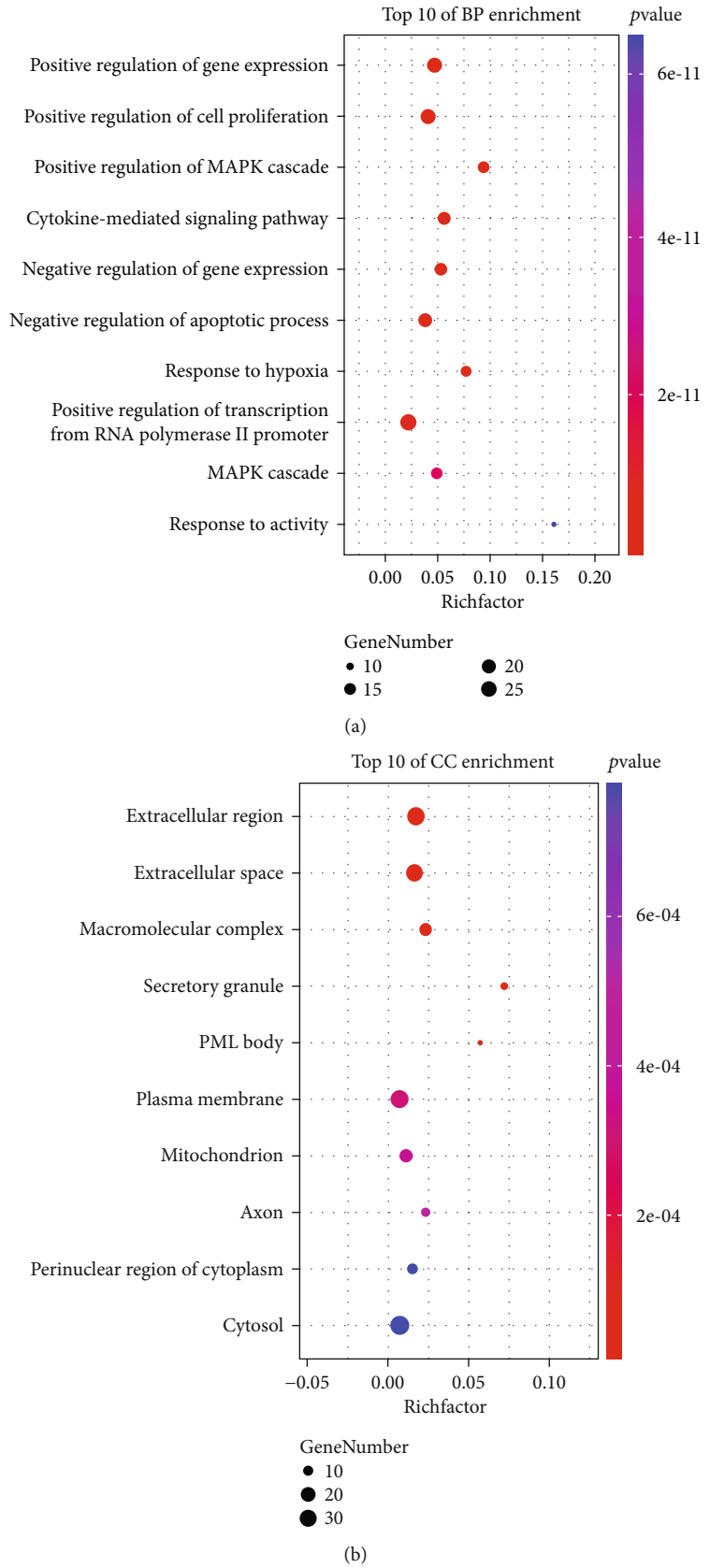

Figure 3: Continued.

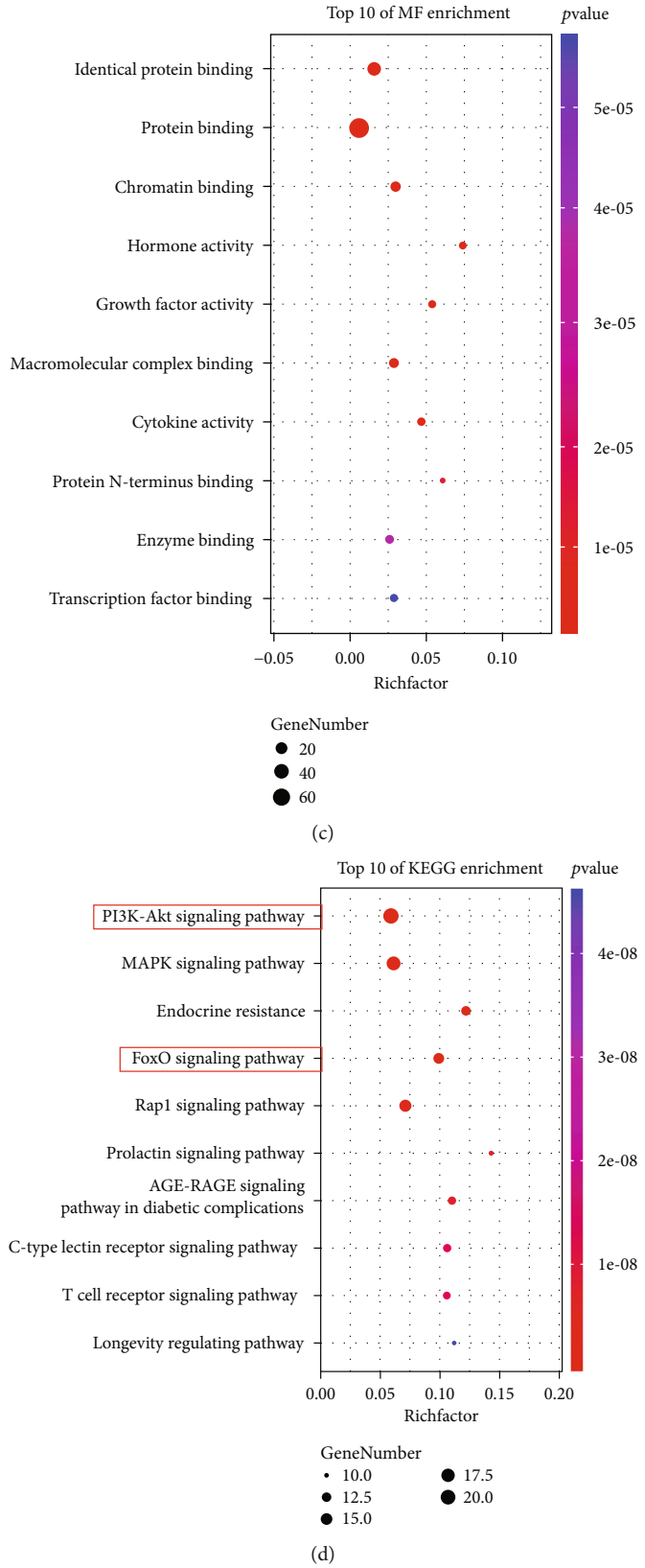

Figure 3: Analyses of GO and KEGG enrichment. (a) Biological process, (b) cell component, (c) molecular function, and (d) KEGG pathway. The size of the dots corresponds to the number of genes annotated in the entry, and the color of the dots corresponds to the corrected p value. Abbreviations: GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: biological process; CC: cell component; MF: molecular function.

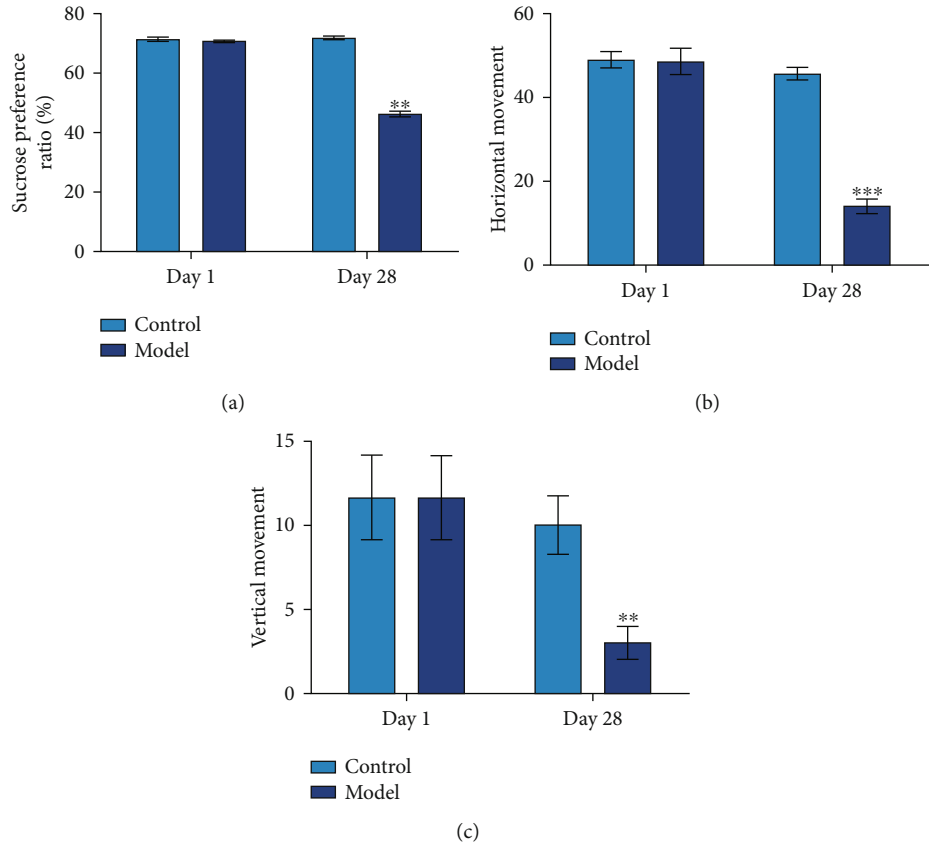

Figure 4: Validation of a rat model of depression. (a) Sucrose preference test. (b) Horizontal movements in the open field test. (c) Vertical movements in the open field test. The model group was compared with the control group, $^{**}p < 0.01$ and $^{***}p < 0.001$.

all levels in the tubular lumen. Model spermatozoa showed an increased occurrence of abnormal structures of the seminiferous tubules. The arrangement of the seminiferous tubules was irregular, the lumen was reduced, and a large number of vacuolated structures were seen. In addition, an increase was seen in the proportion of abnormal spermatozoa and spermatocytes in tubules. Spermatogenic cells at all levels in the seminiferous tubules were reduced, the number of layers was disordered, and many spermatogenic cells were shed (Figure 6).

3.7. Protein Expression of PI3K, p-Akt, and FOXO1. By analyzing the gray value of the expression, the PI3K protein level in the control group was significantly higher than that in the model group ($p < 0.01$). Compared with the control group, the expression level of p-Akt in the model group was significantly reduced ($p < 0.001$). Compared with the control group, the expression levels of FOXO1 in the model group were significantly increased ($p < 0.001$). The results showed that the PI3K/Akt/FoxO1 pathway proteins were significantly changed in the samples from the rat depression model group (Figure 7).

3.8. Expression of PI3K, Akt, and FOXO1 mRNA. Compared to the control group, the expression level of PI3K mRNA in the model group was significantly reduced ($p < 0.001$). Compared to the control group, the expression level of Akt mRNA in the model group was significantly reduced ($p < 0.001$). The expression level of FOXO1 mRNA in the model group was significantly increased ($p < 0.001$) (Figure 8).

4. Discussion

Infertile patients often feel low self-esteem, mistrust, lack of self-confidence, insecurity, and lack of faith [25, 26] with 25% to 60% reporting psychiatric symptoms and having higher depression levels than fertile couples [27]. Furthermore, depression tended to be more severe in patients with primary infertility than in those with secondary infertility [28]. Depression has a measurable impact on MI. Men with higher depression scores had significantly lower sperm concentrations and total sperm counts than nondepressed men [29]. Depression and MI thus form a vicious cycle, and disease progression is exacerbated.

The phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) signaling pathway is widespread and regulates such as proliferation, differentiation, apoptosis, and glucose transport [30]. PI3K/Akt has been closely associated with depression and is the target of antidepressants [31, 32]. PI3K/Akt signaling was considered to have the potential to improve depressive symptoms by regulating downstream targets, such as Bcl-2, mTOR, and FOXO1 [33, 34]. Akt knockout mice exhibited a depression-like phenotype [35], and levels

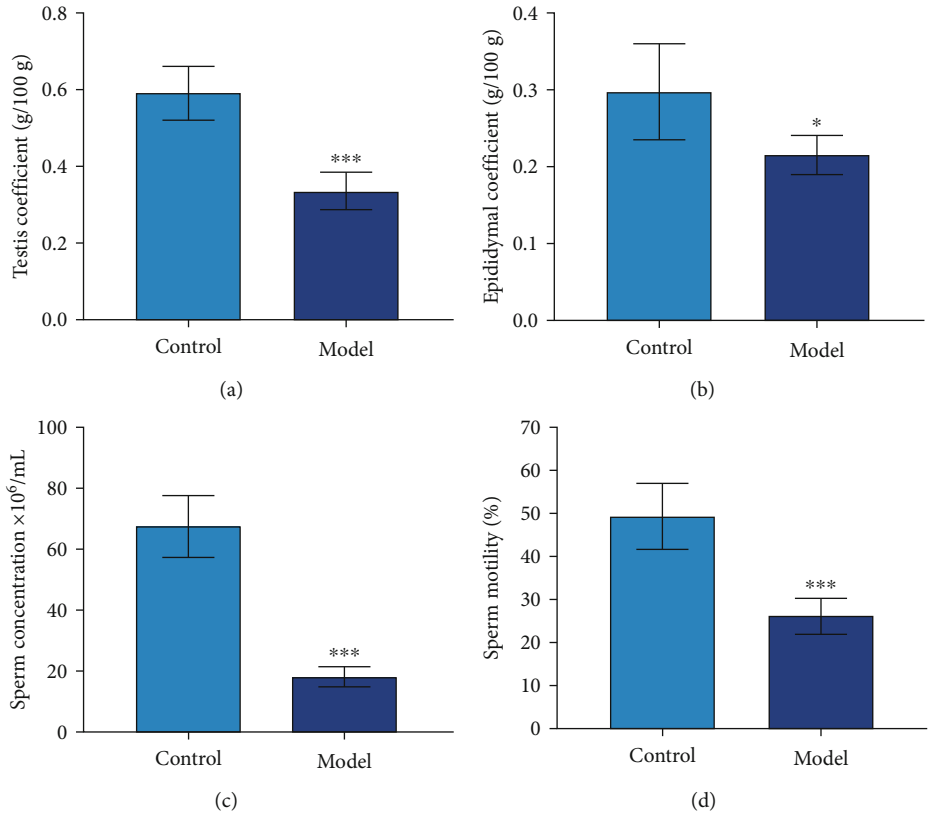

Figure 5: Organ coefficients and semen quality. (a) Testis coefficient, (b) epididymis coefficient, (c) sperm concentration, and (d) sperm motility. The model group was compared with the control group, $^{*}p < 0.05$ and $^{***}p < 0.001$.

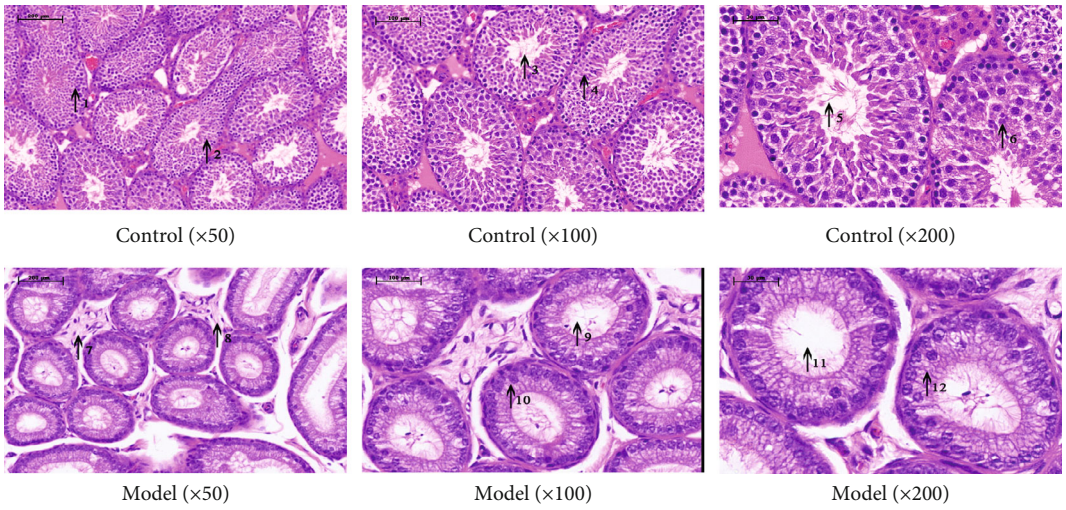

Figure 6: HE staining of testis tissues. Seminiferous tubules were closely arranged in control tissues, and the basement membrane and interstitium were clearly and uniformly demarcated. The arrangement of spermatogonia and spermatocytes at all levels was ordered (Arrows 1-6). The tubules were sparsely arranged, and the interstitium was edematous in model tissues. Tubule epithelium was separated from the interstitium, and tubules had a cavernous structure. All levels of spermatogonia were reduced in number, and the interstitial cells were hyperplastic. (Arrows 7-12).

of phosphorylated Akt in the ventral tegmental area were reduced in a mouse model of stress-induced depression, and fluoxetine reversed this effect [36]. The PI3k/Akt pathway inhibited glycogen synthase kinase-3β (GSK3β) [37] to enhance the synaptic transmission and antidepressant effects of ketamine [38]. FOXO1 is a member of the FOX family of transcription factors and is involved in antistress, apoptosis, cell proliferation, differentiation, and metabolism

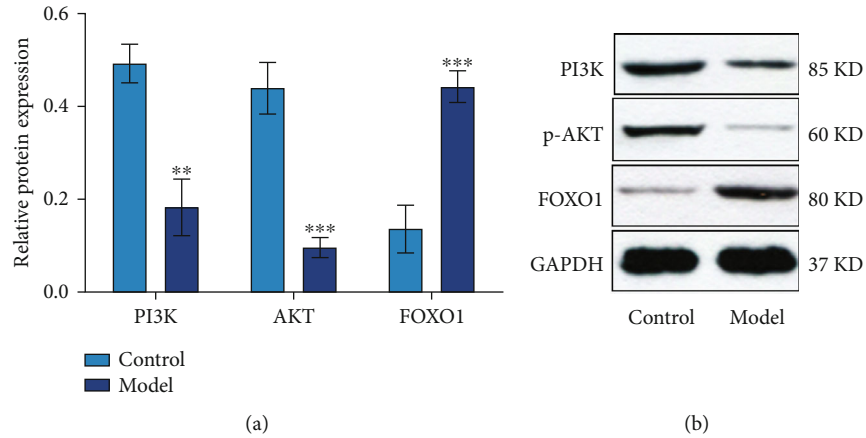

Figure 7: Expression of PI3K, p-AKT, and FOXO1 proteins. Values are the mean ± SEM ($n = 10$ animals per group). Student's t test was used. The model group was compared with the control group, $^{**}p < 0.01$ and $^{***}p < 0.001$. GAPDH was the loading control.

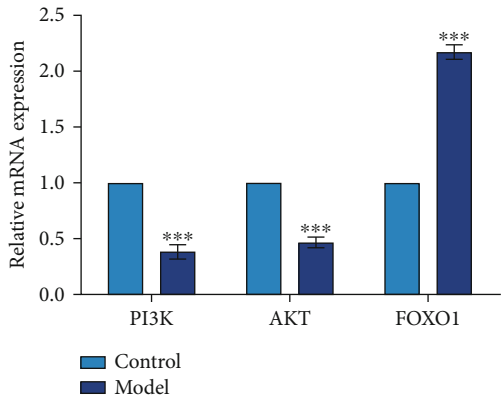

Figure 8: Expression of the mRNA of PI3K, AKT, and FOXO1. Values are the mean ± SEM ($n = 10$ animals per group). Student's t test was used. The model group was compared with the control group, $^{***}p < 0.001$.

[39]. FOXO proteins were widely distributed in brain regions associated with emotional and stress regulation [40]. FOXO1 was mainly found in the striatum and hippocampus, and attenuation of its expression increased the differentiation of adult hippocampal neuronal progenitors [40]. Brain-derived neurotrophic factor (BDNF) is thought to be involved in the pathogenesis of mental disorders and regulates FOXO protein [41]. BDNF reduced FOXO transcriptional activity by stimulating tyrosine receptor kinase B (TrkB) and downstream kinases [42]. Activation of PI3K/AKT signaling promoted FOXO1 phosphorylation and inactivation with antidepressant effects [43]. Indeed, the FOXO pathway is the target for several antidepressant drugs, such as fluoxetine and venlafaxine, which regulate FOXO protein [44].

The PI3K/Akt pathway also regulates the spermatogenic function and promotes sperm proliferation and inhibits apoptosis. Activation of the downstream target proteins, mTOR, and p70S6K improved cyclin D3 expression in sperm cells, accelerating cell proliferation and differentiation [45]. FSH expression in supporting cells promoted PI3K/Akt activation and participated in mammalian spermatocyte proliferation, and PI3K inhibitors reduced supporting cell proliferation [46]. Moreover, cyclophosphamide inhibited the PI3K/Akt pathway to regulate FOXO1 expression and inhibit germ cell apoptosis [47]. Therefore, abnormal expression of the PI3K/Akt/FOXO1 pathway has been closely associated with depression and MI.

The current bioinformatic analysis and literature review have indicated the involvement of the PI3K/Akt/FOXO1 signaling pathway in the link between depression and MI. Organ coefficients and semen parameters were significantly decreased in depressed rats, and testicular spermatogenesis was impaired, indicating that depression had a negative effect on MI. PI3K and p-Akt protein and mRNA were decreased, while FOXO1 was increased, consistent with the findings of other scholars. Therefore, abnormal expression of the PI3K/Akt/FOXO1 signaling pathway has been implicated in the reduced spermatogenic function in the rat model of depression.

A potential mechanism to link depression and MI is presented as a basis for future experimental and clinical studies. However, we acknowledge some limitations of the present study. Many depression-MI-related pathways were predicted, and the PI3K/Akt/FOXO1 pathway was chosen for further study. Previous work has suggested this pathway to be involved with the two diseases. Future experiments concerning the effects of depression on reproductive hormones and the roles of apoptosis and autophagy on depression and MI are planned. The impact of depression on spermatogenesis in rats is in the experimental exploration stage, and refinements to experimental design and drug intervention investigations are required.

5. Conclusion

Bioinformatics analysis showed that depression and MI were correlated with the PI3K/Akt/FOXO1 signaling pathway. Spermatogenic function was decreased in a rat model of depression. Protein and mRNA levels of PI3K and p-Akt were reduced, and FOXO1 was increased. Depression may thus reduce spermatogenic function in rats, perhaps through

a mechanism involving differential expression of PI3K/Akt/FOXO1 pathway proteins.

Data Availability

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

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Qi Zhao, Fei Yan, and Chuan Ma contributed in conception and designed of the manuscript. Junlong Feng and Sheng Deng contributed in animal experiments and data collection. Cong Zhao and Huanan Zhang contributed in data analysis and manuscript modification. Haisong Li, Bin Wang, and Jisheng Wang oversaw the study. All authors read and approved the final manuscript. Qi Zhao, Fei Yan, and Chuan Ma contributed equally to this work and share first authorship.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (no. 81973647) and the Capital Health Development Research Special Project (no. 2020-2-4191). The author sincerely thanked Beijing University of Chinese Medicine for the assistance and site support provided during this period.

Supplementary Materials

Figure S1: uncropped western blots. *(Supplementary Materials)*

References

[1] R. Huang, K. Wang, and J. Hu, "Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials," *Nutrients*, vol. 8, no. 8, p. 483, 2016.

[2] L. T. Park and C. A. Zarate Jr., "Depression in the primary care setting," *The New England Journal of Medicine*, vol. 380, no. 6, pp. 559–568, 2019.

[3] K. Smith, "Mental health: a world of depression," *Nature*, vol. 515, no. 7526, pp. 180-181, 2014.

[4] C.-M. D. Collaborators, "Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic," *Lancet*, vol. 398, no. 10312, pp. 1700–1712, 2021.

[5] R. Belliveau, S. Horton, C. Hereford, L. Ridpath, R. Foster, and E. Boothe, "Pro-inflammatory diet and depressive symptoms in the healthcare setting," *BMC Psychiatry*, vol. 22, no. 1, p. 125, 2022.

[6] J. B. Dwyer, A. Aftab, R. Radhakrishnan et al., "Hormonal treatments for major depressive disorder: state of the art," *The American Journal of Psychiatry*, vol. 177, no. 8, pp. 686–705, 2020.

[7] A. T. Rantanen, J. J. A. Korkeila, E. S. Loyttyniemi, U. K. M. Saxen, and P. E. Korhonen, "Awareness of hypertension and depressive symptoms: a cross-sectional study in a primary care population," *Scandinavian Journal of Primary Health Care*, vol. 36, no. 3, pp. 323–328, 2018.

[8] N. Sartorius, "Depression and diabetes," *Dialogues in Clinical Neuroscience*, vol. 20, no. 1, pp. 47–52, 2018.

[9] J. J. Yland, M. L. Eisenberg, E. E. Hatch et al., "A North American prospective study of depression, psychotropic medication use, and semen quality," *Fertility and Sterility*, vol. 116, no. 3, pp. 833–842, 2021.

[10] D. P. Patel, T. G. Jenkins, K. I. Aston et al., "Harnessing the full potential of reproductive genetics and epigenetics for male infertility in the era of "big data"," *Fertility and Sterility*, vol. 113, no. 3, pp. 478–488, 2020.

[11] A. L. Falsig, C. S. Gleerup, and U. B. Knudsen, "The influence of omega-3 fatty acids on semen quality markers: a systematic PRISMA review," *Andrology*, vol. 7, no. 6, pp. 794–803, 2019.

[12] H. Ahmadi, L. Montaser-Kouhsari, M. R. Nowroozi, and S. Bazargan-Hejazi, "Male infertility and depression: a neglected problem in the Middle East," *The Journal of Sexual Medicine*, vol. 8, no. 3, pp. 824–830, 2011.

[13] A. Milazzo, G. Mnatzaganian, A. G. Elshaug, S. A. Hemphill, J. E. Hiller, and on behalf of The Astute Health Study Group, "Depression and anxiety outcomes associated with failed assisted reproductive technologies: a systematic review and meta-analysis," *PLoS One*, vol. 11, no. 11, article e0165805, 2016.

[14] Y. X. Ye, H. G. Chen, B. Sun et al., "Associations between depression, oxidative stress, and semen quality among 1,000 healthy men screened as potential sperm donors," *Fertility and Sterility*, vol. 117, no. 1, pp. 86–94, 2022.

[15] M. B. Bhongade, S. Prasad, R. C. Jiloha, P. C. Ray, S. Mohapatra, and B. C. Koner, "Effect of psychological stress on fertility hormones and seminal quality in male partners of infertile couples," *Andrologia*, vol. 47, no. 3, pp. 336–342, 2015.

[16] B. Ozkan, E. Orhan, N. Aktas, and E. R. Coskuner, "Depression and sexual dysfunction in Turkish men diagnosed with infertility," *Urology*, vol. 85, no. 6, pp. 1389–1393, 2015.

[17] L. Nørr, B. Bennedsen, J. Fedder, and E. R. Larsen, "Use of selective serotonin reuptake inhibitors reduces fertility in men," *Andrology*, vol. 4, no. 3, pp. 389–394, 2016.

[18] C. Tanrikut, A. S. Feldman, M. Altemus, D. A. Paduch, and P. N. Schlegel, "Adverse effect of paroxetine on sperm," *Fertility and Sterility*, vol. 94, no. 3, pp. 1021–1026, 2010.

[19] M. R. Safarinejad, "Evaluation of endocrine profile and hypothalamic-pituitary-testis axis in selective serotonin reuptake inhibitor-induced male sexual dysfunction," *Journal of Clinical Psychopharmacology*, vol. 28, no. 4, pp. 418–423, 2008.

[20] J. Gauthier, A. T. Vincent, S. J. Charette, and N. Derome, "A brief history of bioinformatics," *Briefings in Bioinformatics*, vol. 20, no. 6, pp. 1981–1996, 2019.

[21] D. W. Huang, B. T. Sherman, Q. Tan et al., "DAVID bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists," *Nucleic Acids Research*, vol. 35, no. Web Server issue, pp. W169–W175, 2007.

[22] S. Chiba, T. Numakawa, M. Ninomiya, M. C. Richards, C. Wakabayashi, and H. Kunugi, "Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the

prefrontal cortex," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 39, no. 1, pp. 112–119, 2012.

[23] M. Moretti, I. Werle, P. B. da Rosa et al., "A single coadministration of subeffective doses of ascorbic acid and ketamine reverses the depressive-like behavior induced by chronic unpredictable stress in mice," *Pharmacology, Biochemistry, and Behavior*, vol. 187, article 172800, 2019.

[24] W. C. Ford, "Comments on the release of the 5th edition of the WHO Laboratory manual for the examination and processing of human semen," *Asian Journal of Andrology*, vol. 12, no. 1, pp. 59–63, 2010.

[25] A. Drosdzol and V. Skrzypulec, "Depression and anxiety among Polish infertile couples–an evaluative prevalence study," *Journal of Psychosomatic Obstetrics and Gynaecology*, vol. 30, no. 1, pp. 11–20, 2009.

[26] R. A. Sherrod, "Understanding the emotional aspects of infertility: implications for nursing practice," *Journal of Psychosocial Nursing and Mental Health Services*, vol. 42, no. 3, pp. 40–47, 2004.

[27] D. De Berardis, M. Mazza, S. Marini et al., "Psychopathology, emotional aspects and psychological counselling in infertility: a review," *La Clinica Terapeutica*, vol. 165, no. 3, pp. 163–169, 2014.

[28] T. Yoldemir, M. Yassa, and K. Atasayan, "Comparison of depression between primary and secondary infertile couples," *Gynecological Endocrinology*, vol. 36, no. 12, pp. 1131–1135, 2020.

[29] P. Zou, X. Wang, L. Sun et al., "Semen quality in Chinese college students: associations with depression and physical activity in a cross-sectional study," *Psychosomatic Medicine*, vol. 80, no. 6, pp. 564–572, 2018.

[30] D. A. Fruman, H. Chiu, B. D. Hopkins, S. Bagrodia, L. C. Cantley, and R. T. Abraham, "The PI3K pathway in human disease," *Cell*, vol. 170, no. 4, pp. 605–635, 2017.

[31] L. H. Cao, J. Y. Qiao, H. Y. Huang et al., "PI3K-AKT signaling activation and icariin: the potential effects on the Perimenopausal depression-like rat model," *Molecules*, vol. 24, no. 20, p. 3700, 2019.

[32] I. V. A. Lima, A. F. Almeida-Santos, T. H. Ferreira-Vieira et al., "Antidepressant-like effect of valproic acid–possible involvement of PI3K/Akt/mTOR pathway," *Behavioural Brain Research*, vol. 329, pp. 166–171, 2017.

[33] J. Li, Y. Li, W. Duan et al., "Shugan granule contributes to the improvement of depression-like behaviors in chronic restraint stress-stimulated rats by altering gut microbiota," *CNS Neuroscience & Therapeutics*, vol. 28, no. 9, pp. 1409–1424, 2022.

[34] Z. Liu, H. Guo, X. Cao et al., "A combined study of GSK3 β polymorphisms and brain network topological metrics in major depressive disorder," *Psychiatry Research*, vol. 223, no. 3, pp. 210–217, 2014.

[35] C. Leibrock, T. F. Ackermann, M. Hierlmeier, F. Lang, S. Borgwardt, and U. E. Lang, "Akt2 deficiency is associated with anxiety and depressive behavior in mice," *Cellular Physiology and Biochemistry*, vol. 32, no. 3, pp. 766–777, 2013.

[36] V. Krishnan, M. H. Han, M. Mazei-Robison et al., "AKT signaling within the ventral tegmental area regulates cellular and behavioral responses to stressful stimuli," *Biological Psychiatry*, vol. 64, no. 8, pp. 691–700, 2008.

[37] S. Frame, P. Cohen, and R. M. Biondi, "A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation," *Molecular Cell*, vol. 7, no. 6, pp. 1321–1327, 2001.

[38] R. J. Liu, M. Fuchikami, J. M. Dwyer, A. E. Lepack, R. S. Duman, and G. K. Aghajanian, "GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine," *Neuropsychopharmacology*, vol. 38, no. 11, pp. 2268–2277, 2013.

[39] S. Lee and H. H. Dong, "FoxO integration of insulin signaling with glucose and lipid metabolism," *The Journal of Endocrinology*, vol. 233, no. 2, pp. R67–r79, 2017.

[40] D. Y. Kim, I. Hwang, F. L. Muller, and J. H. Paik, "Functional regulation of FoxO1 in neural stem cell differentiation," *Cell Death and Differentiation*, vol. 22, no. 12, pp. 2034–2045, 2015.

[41] H. Wang, R. Quirion, P. J. Little et al., "Forkhead box O transcription factors as possible mediators in the development of major depression," *Neuropharmacology*, vol. 99, pp. 527–537, 2015.

[42] E. S. Emamian, D. Hall, M. J. Birnbaum, M. Karayiorgou, and J. A. Gogos, "Convergent evidence for impaired AKT1-GSK3β signaling in schizophrenia," *Nature Genetics*, vol. 36, no. 2, pp. 131–137, 2004.

[43] S. N. Mediwala, H. Sun, A. T. Szafran et al., "The activity of the androgen receptor variant AR-V7 is regulated by FOXO1 in a PTEN-PI3K-AKT-dependent way," *Prostate*, vol. 73, no. 3, pp. 267–277, 2013.

[44] T. Rana, T. Behl, A. Sehgal et al., "Elucidating the possible role of FoxO in depression," *Neurochemical Research*, vol. 46, no. 11, pp. 2761–2775, 2021.

[45] S. B. Meroni, M. N. Galardo, G. Rindone, A. Gorga, M. F. Riera, and S. B. Cigorraga, "Molecular mechanisms and signaling pathways involved in Sertoli cell proliferation," *Frontiers in Endocrinology*, vol. 10, p. 224, 2019.

[46] M. F. Riera, M. Regueira, M. N. Galardo, E. H. Pellizzari, S. B. Meroni, and S. B. Cigorraga, "Signal transduction pathways in FSH regulation of rat Sertoli cell proliferation," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 302, no. 8, pp. E914–E923, 2012.

[47] P. Dong, L. Xia, L. Hu, K. Yang, H. Wang, and P. Ye, "Runjing decoction alleviated cyclophosphamide-induced oligoasthenospermia rats by inhibiting cell apoptosis via RXFP1/AKT/FOXO1 pathway," *Andrologia*, vol. 53, no. 11, article e14216, 2021.