

Research Article

Network Pharmacology-Based Strategy to Investigate Pharmacological Mechanisms of the Drug Pair Astragalus-Angelica for Treatment of Male Infertility

Feng Zhao ¹, Yingjun Deng,¹ Guanchao Du,¹ Shengjing Liu,¹ Jun Guo,¹ Hao Wang,¹ Yazhou Chen,¹ Fu Wang ¹ and Qiang Geng ²

¹Department of Andrology, Xiyuan Hospital of China Academy of Chinese Medical Sciences, No. 1 Xiyuan Playground, Zhongzhi Road, Beijing 100091, China

²Department of Andrology, The First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, No. 314 Anshan West Road, Tianjin 300193, China

Correspondence should be addressed to Fu Wang; fu311306@163.com and Qiang Geng; gengni@126.com

Received 29 May 2021; Accepted 15 September 2021; Published 16 October 2021

Academic Editor: Talha Bin Emran

Copyright © 2021 Feng Zhao 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.

Background. The traditional Chinese medicines *Astragalus* and *Angelica* are often combined to treat male infertility, but the specific therapeutic mechanism is not clear. Therefore, this study applies a network pharmacology approach to investigate the possible mechanism of action of the drug pair *Astragalus-Angelica* (PAA) in the treatment of male infertility. **Methods.** Relevant targets for PAA treatment of male infertility are obtained through databases. Protein-protein interactions (PPIs) are constructed through STRING database and screen core targets, and an enrichment analysis is conducted through the Metascape platform. Finally, molecular docking experiments were carried out to evaluate the affinity between the target protein and the ligand of PAA. **Results.** The active ingredients of 112 PAA, 980 corresponding targets, and 374 effective targets of PAA for the treatment of male infertility were obtained, which are related to PI3K-Akt signaling pathway, HIF-1 signaling pathway, AGE-RAGE signaling pathway, IL-17 signaling pathway, and thyroid hormone signaling pathway. **Conclusion.** In this study, using a network pharmacology method, we preliminarily analyzed the effective components and action targets of the PAA. We also explored the possible mechanism of action of PAA in treating male infertility. They also lay a foundation for expanding the clinical application of PAA and provide new ideas and directions for further research on the mechanisms of action of the PAA and its components for male infertility treatment.

1. Background

Male sterility, a common disease in andrology, is defined as male factor infertility in which a couple have attempted to conceive for more than one year without success. Approximately 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounters problems when attempting to conceive a first child, and one in six encounters problems when attempting to conceive a subsequent child. Three percent of women who are currently trying to conceive remain involuntarily childless, while 6% of parous women are not able to have as many children as

they would wish [1]. In 50% of involuntarily childless couples, a male infertility-associated factor is found, usually together with abnormal semen parameters [2]. The etiology of male infertility is complex. At present, the clear etiologies include urogenital diseases, varicoceles, endocrine disorders, gene abnormalities, and other systemic diseases. In addition, the European Association of Urology Guidelines (2020 Edition) states that approximately 30% of infertility cases are idiopathic male infertility [3]. The existing treatment measures include estrogen receptor modulators, sperm health-promoting agents, antibiotics, and other symptomatic treatments, but the efficacies are still unclear [4, 5].

The treatment of male infertility with traditional Chinese medicine (TCM) has a long history and is now gaining popularity in western countries [6, 7]. The goal of treatment is the balance between reproductive energy (QI), blood, and visceral Yin and Yang. Usually, traditional Chinese medicine is not used alone to treat diseases, but often in the form of multiple drugs cooperating with each other. *Astragalus* and *Angelica* are often used as a drug combination in the treatment of male infertility. *Astragalus* is the dried root of *Astragalus membranaceus* (Fisch.) Bge. or *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao. [8]. The efficacy is tonifying qi and strengthening the spleen. *Angelica* is the root of *Angelica sinensis* (Oliv.) [9]. The efficacy is tonifying the blood and activating blood circulation. The combination of the two can treat male sterility with deficiency of qi and blood [10, 11]. However, it is not clear what mechanism PAA is involved in the treatment of male infertility.

Network pharmacology is a new discipline that has been used in recent years to study the pharmacological mechanisms of traditional Chinese medicines. Through integration of current traditional Chinese medicine pharmacology methods, high-throughput bioinformatics, and high-end data analysis software programs, comprehensive analyses of the mechanisms of action of traditional Chinese medicines can be carried out [12]. It can predict the pharmacological mechanism of TCM “multicomponent, multitarget, and multipathway,” and provide better data and theoretical support for subsequent pharmacological experiments. The current study is comprised of three steps, including data collection, data processing, and network construction, enrichment analysis and mechanism prediction, and the flowchart of the technical strategy in this study is shown in Figure 1.

2. Materials and Methods

2.1. Identification of Active Components and Potential Targets of the Traditional Chinese Medicines. The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) [13] is a relatively classic database of traditional Chinese medicine ingredients with more than 500 traditional Chinese medicines and more than 30,000 compounds providing pharmacokinetic information corresponding to the compounds. It has become one of the databases most frequently used by scholars in network pharmacology research. The Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) [14] was the first online bioinformatics analysis tool designed specifically for studying the intrinsic molecular mechanisms of traditional Chinese medicines. Relevant active components of PAA were searched in the TCMSP and BATMAN-TCM databases with the following screening criteria, which were set in combination with the criteria of commonly used network pharmacology component screening methods and relevant literature records: an oral bioavailability (OB) $\geq 30\%$ and drug likeness (DL) ≥ 0.18 . Compounds that did not meet these screening criteria were excluded. And according to the literature, active

ingredients that are not eligible for screening and that have been experimentally proved to be effective are also incorporated into the alternative ingredients; thus, the obtained active ingredients and therapeutic targets of *Astragalus* and *Angelica* were typed into the UniProt database for each target <https://www.uniprot.org/> to obtain the standard number of targets, and the final results were counted into an Excel sheet.

2.2. Identification of Disease Targets. Online Mendelian Inheritance in Man (OMIM) (<https://omim.org/>) is a database of human genes and genetic phenotypes which focuses on molecular relationships between gene variation and dominant expression and contains information on more than 15,000 genes. GeneCards [15] (<https://www.genecards.org/>) contains a wealth of biomedical data on genes and their products, including genomic, proteomic, and gene function-related information. DisGeNET [16] is a multifunctional platform that can be used for the study of the molecular basis of human diseases and their complications, validation of disease candidate genes, and evaluation of the performance of text mining methods. The Genetic Association Database (GAD) [17] is a standardized tool for viewing the ever-growing data on human polymorphisms from case-control studies. Users can search for genes, diseases, polymorphisms, chromosome locations, and references to obtain relevant information. We input “male infertility” into the OMIM database, the GeneCards database, GAD (<https://geneticassociationdb.nih.gov/>), and DisGeNET (<http://www.disgenet.org>) to search for the targets related to male infertility and record them in an Excel sheet.

2.3. Identification of Therapeutic Targets and Construction of Protein-Protein Interactions (PPIs). The effective components, targets, and disease targets of the PAA were deduplicated and imported into a Venn diagram program (<http://bioinformatics.psb.ugent.be/web-tools/Venn/>). Finally, the targets at the intersection were considered the effective targets of PAA in the treatment of male infertility. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) can be used to judge the confidence of PPIs through different evidence scoring systems so that we will know which proteins play a major role in the treatment of diseases. The targets obtained after intersection analysis were input into the STRING to construct a PPI network, and the results were imported into Cytoscape software (version 3.7.2) [18]. According to the network analysis tool and the Molecular Complex Detection (MCODE) [19] plug-in, topology analysis was carried out to obtain the comprehensive data for each node. According to the betweenness centrality (BC), closeness centrality (CC), degree, and MCODE Score, the core targets of PAA in the treatment of male infertility were identified, and a PPI network was drawn.

2.4. Network Construction. The disease-drug-active ingredient-target network of PAA in the treatment of male

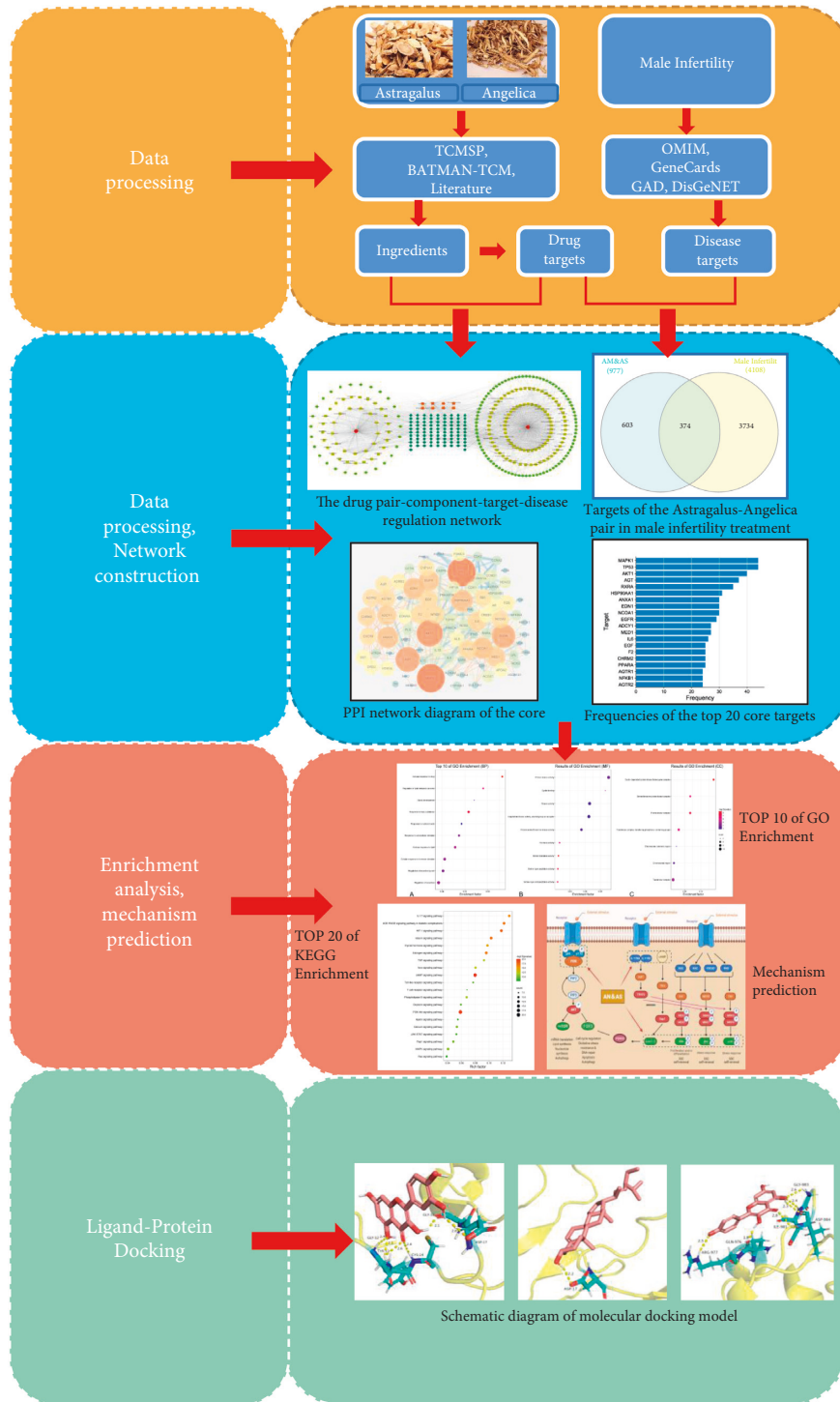


FIGURE 1: Technical strategy flow diagram.

infertility was visualized by using Cytoscape software. In the network, each node represents a data element, such as a disease, drug, active ingredient, or target. In the scale-free network, the index node degree reflects the participation of each node, and the size of the degree is directly proportional to the participation of the node. After importing the data, the Network Analyzer module was used to obtain the degree of each data element.

2.5. Functional Nodes for Core Targets. Metascape (<http://metascape.org/>) is a powerful tool for gene function annotation analysis that can help users apply the current popular bioinformatics analysis methods to batch gene and protein analysis in order to understand gene or protein functions. In this study, we used the Metascape database to perform gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG)

pathway enrichment. GO analysis was used to describe the functions of gene targets in terms of cellular component (CC), molecular function (MF), and biological process (BP). KEGG analysis was used to identify the signaling pathways associated with the gene targets, and the analysis results with statistical significance were selected ($P < 0.05$). A network of the interactions between the core targets and signaling pathway was drawn with Cytoscape software to obtain the comprehensive information for each node.

2.6. Construction of the Component-Target Pathway Network. The top 20 pathways from the results of KEGG analysis were filtered and combined with the file retrieval to obtain the possible pathways related to the treatment of male infertility and the targets that were enriched on the pathways. The targets connected with the effective components in the PAA pair were ultimately used to construct a component-target pathway network diagrams.

2.7. Ligand-Protein Docking. The information of six proteins (TP53, mapk1, IL-6, ANXA1, EGF, and EGFR) in the PPI network which were most closely related to male infertility was obtained through the RCSB PDB (<https://www.rcsb.org/>). The proteins selected as .pdb format were converted into .pdbqt format via AutoDock (<http://autodock.scripps.edu/>). Docking was performed in AutoDock with kaempferol, stigmaterol, and quercetin.

3. Results

3.1. Identification of Ingredients and Targets of the Traditional Chinese Medicine. The effective components of PAA were searched in the TCMSP and BATMAN-TCM, and the compounds were screened according to an $OB \geq 30\%$ and a $DL \geq 0.18$ and combined with the literature for screening [20–23]. Ultimately, 112 potential core compounds were obtained, and 977 corresponding targets were predicted.

The OMIM database, GeneCards database, GAD, and DisGeNET database were used to search for male infertility-related targets. A total of 4108 targets were obtained after deleting the duplicate items. Finally, 374 effective targets for the treatment of male infertility were obtained by mapping (Figure 2).

3.2. Construction of the PPI Network. A total of 374 common targets were imported into the STRING for PPI analysis, and the result with a combined score ≥ 0.900 was selected. A total of 1415 paired interaction relationships were obtained. The results were imported into Cytoscape, and after screening was performed on the basis of betweenness centrality, closeness centrality, degree, and MCODE score, 85 core targets were obtained. The PPI network was drawn with these targets, as shown in Figure 3(a). A bar chart was also created to show the frequency of occurrence of the top 20 targets, as shown in Figure 3(b) and Table 1.

3.3. Construction of the Drug Pair-Component-Target-Disease Regulation Network. The common target information and mapping relationships between the active components of the PAA pair and male infertility were imported into Cytoscape software. The topological structure of the network was assessed with the Network Analyzer function of Cytoscape, and the node importance was expressed in terms of the degree. Nodes with degrees greater than 20 for the active ingredients and 10 for the targets were selected, and a drug pair-component-target network diagram was established, as shown in Figure 4. The top five compounds with regard to degree were kaempferol, stigmaterol, beta sitosterol, quercetin, and canavanine. Among them, kaempferol, stigmaterol, and quercetin are closely related to male infertility.

3.4. GO Enrichment Analysis of Core Targets. GO target analysis yielded 398 significant results, including 382 BPs, 7 CCs, and 9 MFs. The top 10 BP, CC, and MF terms were used to create bubble charts, as shown in Figure 5. In the BP category, the main terms associated with the targets included the cellular response to drug, regulation of secretion, and response to nutrient levels terms. In the CC category, the enriched terms for the targets included the protein kinase complex, transferase complex, and chromosomal region terms. In the MF category, the targets were enriched for terms such as kinase activity, cyclin binding, and hormone activity.

3.5. KEGG Analysis of Core Targets. A total of 153 pathways were obtained through KEGG enrichment analysis. Combined with literature search, the top 20 pathways with high significance were selected for bubble mapping based on the size of P value. A literature search revealed that among the 20 pathways were important signaling pathways related to infertility, including the PI3K-Akt, HIF-1, AGE-RAGE, IL-17, and thyroid hormone signaling pathways. These pathways all involved targets such as TP53, mapk1, IL-6, ANXA1, EGF, and EGFR, and detailed information is available in Table 2. These results are shown in Figure 6.

3.6. Results of Docking. A total of 18 docking results were obtained, as shown in Table 3. The results of this study show that the binding energies of the ligands to the receptors are all negative, which indicates that there is binding activity between the compounds and the target protein, strong binding activity when the binding energy is less than -5 kJ/mol, and strong binding activity when it is less than -7 kJ/mol. The docking mode at which each ligand had the lowest binding energy was selected for display (see Figure 7).

4. Discussion

A previous report on the use of traditional Chinese medicines for the treatment of male infertility indicated that both *Astragalus* and *Angelica* are very frequently used in traditional Chinese medicines [24]. They are often used to treat

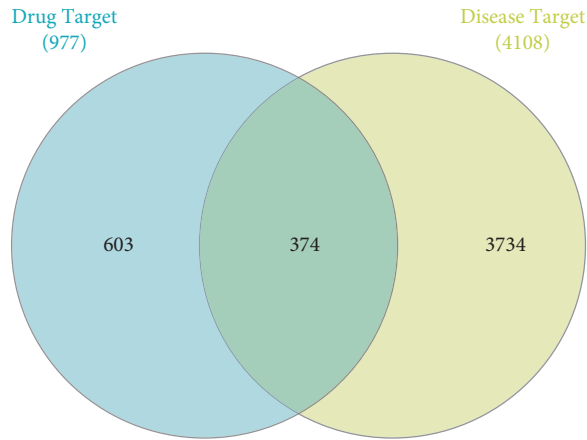


FIGURE 2: Targets of the PAA in male infertility treatment. The number in the blue circle is the unique target number of AS&AN, the number in the yellow circle is the unique target number of male infertility, and the middle number is the common target number common for both.

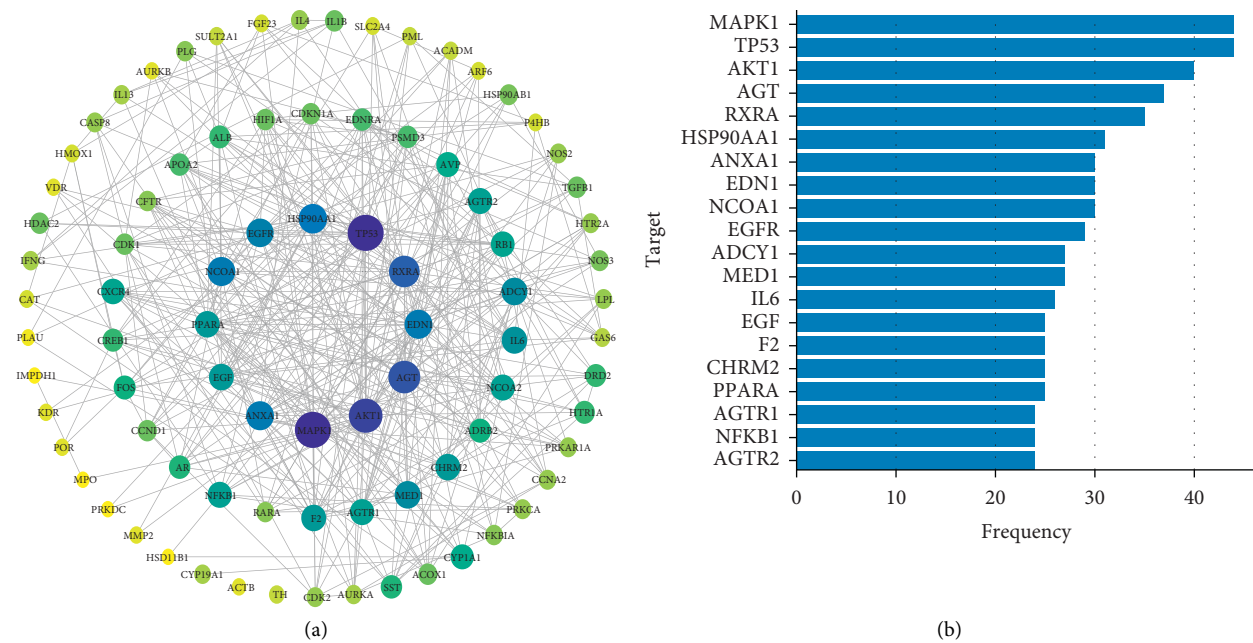


FIGURE 3: The PPI networks of AS&AN for male infertility. (a) PPI network diagram of the core. (b) Frequencies of the top 20 core targets (the y-axis represents targets, and the x-axis represents the frequency).

infertility due to deficiency of both qi and blood. According to the theory of traditional Chinese medicine, Qi can generate blood, and blood can carry Qi. Qi deficiency cannot generate blood, and blood deficiency cannot transform essence. *Astragalus* and *Angelica* can supplement both qi and blood and regulate Yin and Yang.

A total of 112 effective components, 980 corresponding targets, and 4108 disease targets of the PAA pair were screened from the component database, and 374 potential targets for male infertility were obtained after mapping. This finding shows that PAA has multicomponent and multi-target characteristics in the treatment of male infertility. The drug pair-component-target network diagram shows that kaempferol, stigmasterol, beta sitosterol, quercetin, and canavanine are the top active ingredients. The association of

these several components with male infertility is as follows. Kaempferol is a flavonoid that can significantly increase the levels of antioxidants such as SOD (superoxide dismutase), CAT (catalase), and GPX (glutathione peroxidase) in the sperm of diabetic rats; reduce the levels of inflammatory factors such as NF-κB (nuclear factor kappa B) and TNF-α (tumor necrosis factor alpha) in sperm; alleviate sperm damage [25]; and significantly improve sperm quality in mice with infertility induced by benzopyrene, a product of incomplete combustion of energy substances [26]. Quercetin and other flavonoids also have good anti-inflammatory effects [27]. Relevant studies have shown that after treatment with quercetin, the content of mtDNA (mitochondrial DNA) in patients' sperm significantly decreases, while the content of Cyt b (cytochrome b) and NADH 5 (nicotinamide

TABLE 1: Detailed data of the top 20 targets.

Target	Degree	Betweenness centrality	Closeness centrality	MCODE score
MAPK1	44	0.11909067	0.40682415	3.181818182
TP53	44	0.07271146	0.37349398	3.454545455
AKT1	40	0.10656178	0.4005168	4.169117647
Jun	39	0.04830527	0.38993711	2.759259259
AGT	37	0.07377641	0.3583815	17
RXRA	35	0.10861121	0.40207523	6.222222222
RELA	31	0.0222772	0.3708134	2.675324675
HSP90AA1	31	0.02770788	0.36470588	3.264705882
NCOA1	30	0.03957597	0.37575758	6.222222222
ANXA1	30	0.01792683	0.32460733	17
EDN1	30	0.03558045	0.37530266	11
EGFR	29	0.04140608	0.36172695	8
TNF	29	0.03633951	0.36299766	2.810526316
NR3C1	28	0.0253214	0.38130381	2.685714286
MED1	27	0.03261089	0.36172695	6.222222222
ADCY1	27	0.01606181	0.31155779	17
ESR1	26	0.04038295	0.37621359	2.947368421
IL6	26	0.02207884	0.35107588	6
PPARA	25	0.05492241	0.39340102	6.222222222
VEGFA	25	0.02270293	0.35227273	2.911764706

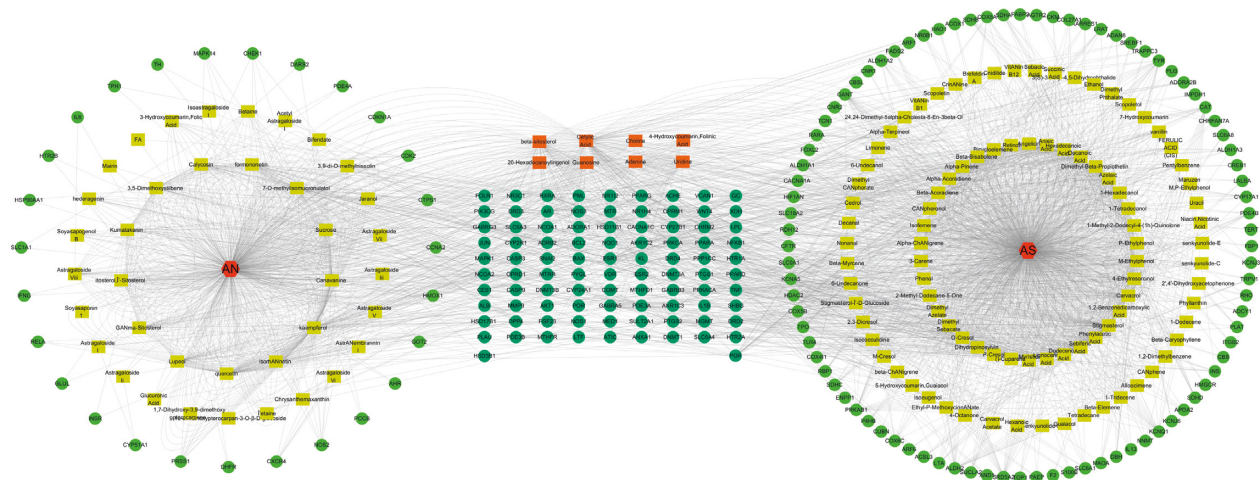


FIGURE 4: The drug pair-component-target-disease regulation network. The red node is the traditional Chinese medicine, the yellow node is the active ingredient, the green node is the target, the orange node between the two circles is the common active ingredient of *Astragalus* and *Angelica*, and the dark green node is the common target of *Astragalus* and *Angelica*.

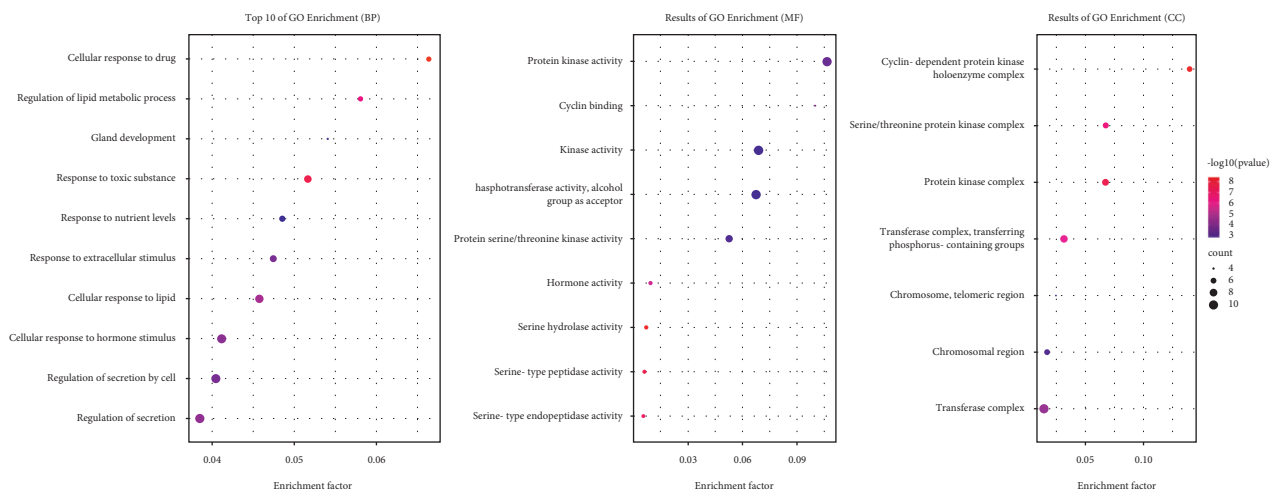


FIGURE 5: Top 10 of GO enrichment: (a) top 10 of GO enrichment (BP), (b) results of GO enrichment (MF), and (c) results of GO enrichment (CC) (the y -axis represents top 10 BP/MF/CC terms, and the x -axis represents the enrichment factors.).

TABLE 2: Male-infertility-related pathways and involved targets.

Description	Count	Targets
cAMP signaling pathway	18	ACOX1, ADCY1, ADRB2, AKT1, CFTR, CHRM2, CREB1, DRD2, EDN1, EDNRA, FOS, HTR1A, NFKB1, NFKBIA, PPARA, PRKCA, MAPK1, SST
PI3K-Akt signaling pathway	20	AKT1, CCND1, CDK2, CDKN1A, CHRM2, CREB1, EGF, EGFR, HSP90AA1, HSP90AB1, IL4, IL6, KDR, NFKB1, NOS3, PRKCA, MAPK1, RXRA, TP53, FGF23
IL-17 signaling pathway	12	CASP8, FOS, HSP90AA1, HSP90AB1, IFNG, IL1B, IL4, IL6, IL13, NFKB1, NFKBIA, MAPK1
FOXO signaling pathway	11	AKT1, CCND1, CAT, CDK2, CDKN1A, EGF, EGFR, IL6, MAPK1, SLC2A4, TGFB1
MAPK signaling pathway	13	AKT1, EGF, EGFR, FOS, IL1B, IL6, KDR, NFKB1, PRKCA, MAPK1, TGFB1, TP53, FGF23

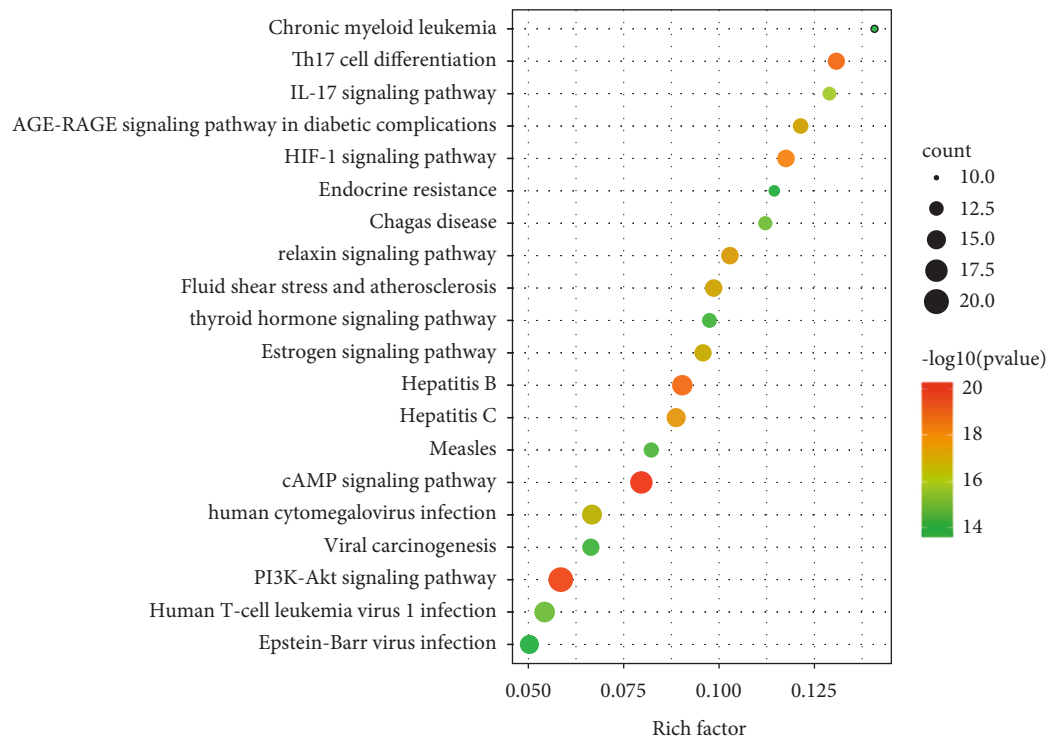


FIGURE 6: Top 20 of KEGG enrichment (the y-axis represents top 20 of KEGG terms, and the x-axis represents the rich factors).

TABLE 3: Docking results.

Ligand	Receptor (PDB ID)	Lowest binding energy (kJ/mol)
Stigmasterol	EGF (1nql)	-14.0
Stigmasterol	EGFR (5ug9)	-11.5
Quercetin	EGF (1nql)	-11.5
Stigmasterol	ANXA1 (1mcx)	-11.0
Stigmasterol	IL6 (4cni)	-10.0
Stigmasterol	EGFR (5ug9)	-9.3
Quercetin	EGF (1nql)	-9.0
Kaempferol	IL6 (4cni)	-8.8
Kaempferol	TP53 (3d06)	-8.7
Kaempferol	EGF (1nql)	-8.5
Stigmasterol	TP53 (3d06)	-8.3
Stigmasterol	MAPK1 (2waj)	-7.5
Kaempferol	ANXA1 (1mcx)	-7.2
Quercetin	TP53 (3d06)	-6.9
Quercetin	ANXA1 (1mcx)	-5.7
Kaempferol	MAPK1 (2waj)	-5.0
Quercetin	IL6 (4cni)	-3.8
Quercetin	MAPK1 (2waj)	-2.8

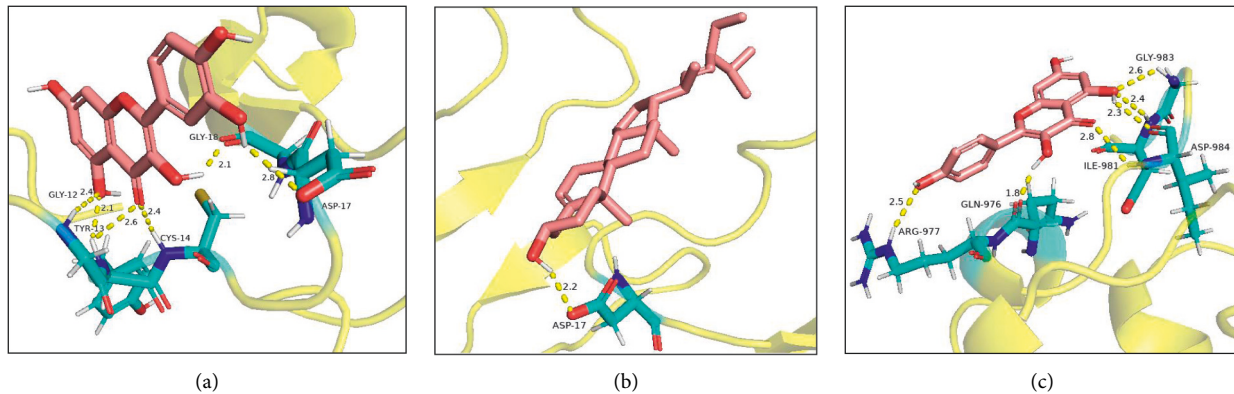


FIGURE 7: Schematic diagram of the molecular docking model. (a) Schematic diagram of docking between EGF and quercetin. (b) Schematic diagram of docking between EGF and stigmasterol. (c) Schematic diagram of docking between EGFR and kaempferol.

adenine dinucleotide 5) in sperm significantly increases, which plays a role in improving sperm hyperactivity and the acrosome reaction [28]. Stigmasterol belongs to the class of phytosterols. Studies have shown that dietary phytosterol supplementation can significantly increase the sperm number and sperm motility and reduce the sperm oxidative stress response [29]. Therefore, kaempferol, stigmasterol, quercetin, and other compounds may be the core compounds by which PAA alleviates male infertility.

The results showed that the potential targets of *Angelica* and *Astragalus* in the treatment of male infertility include TP53 (tumor protein P53), mapk1 (mitogen-activated protein kinase 1), IL-6 (interleukin-6 receptor), ANXA1 (annexin-A1), EGF (epidermal growth factor), and EGFR (epidermal growth factor receptor). Here, the relationship between these six targets and male infertility is described. The TP53 gene, an important tumor suppressor gene, can regulate cell growth, differentiation, aging, and the immune response. Meiosis is crucial in spermatogenesis, and TP53 mRNA and protein are expressed in primary spermatocytes and play important roles in spermatogenic cell apoptosis, suggesting that TP53 participates in meiosis [30, 31]. A growing number of studies on cancer have confirmed that the PI3K-Akt pathway can provide positive and negative regulation of p53 levels [32]. However, whether this regulation affects male fertility is not yet evidenced. MAPK (mitogen-activated protein kinase) is an important part of the MAPK signaling pathway. MAPK is closely related to proliferation, inflammation, differentiation, apoptosis, and other processes in male sperm [33]. ANXA1, an important member of the mammalian annexin family, is closely related to cell proliferation and membrane fusion [34]. Studies on human sperm have found that ANXA1 can bind and interact with F-actin in a Ca²⁺-dependent manner [35], and the actin cytoskeleton plays an important role in sperm capacitation and the acrosome reaction [36]. IL-6 is an inflammatory cytokine secreted by macrophages. Although it is mainly involved in the inflammatory reaction in vivo, its levels are significantly increased in the semen of infertile patients [37]. EGF is closely related to the reproductive activity of male animals. An appropriate dose of EGF can

specifically promote the proliferation of spermatogonial stem cells through EGFR in a time-dependent manner [38]. In addition, studies have shown that EGF can regulate the proliferation of Leydig cells and maintain the concentration of testosterone [39]. As shown in Table 2, these targets are upstream or downstream of PI3K-Akt, IL-17, camp, FOXO, MAPK, and other signal pathways. They may play a key role in the treatment of male infertility by PAA.

KEGG enrichment analysis showed that the target pathways of PAA in the treatment of male infertility included the PI3K-Akt, IL-17 (interleukin 17), cAMP, FOXO, MAPK, and other signaling pathways. A mechanism diagram is shown in Figure 8. How these signaling pathways relate to the development or treatment of male infertility is briefly described below. As shown in Figure 6, the PI3K-Akt signaling and cAMP signaling pathways involved the most targets. The PI3K/AKT pathway is an important signaling pathway in the human body. PI3K is a heterodimer that simultaneously exhibits serine/threonine kinase activity and phosphatidylinositol kinase activity. It is composed of the regulatory subunit P85 and the catalytic subunit p110, which is closely related to spermatogenesis and maturation; for example, it affects the proliferation and differentiation of spermatogonial stem cells and the meiosis of spermatocytes [40]. PI3K can be activated not only by tyrosine kinase (receptor tyrosine kinase, RTK) and ras proteins on the cell membrane but also by other proteins. AKT is the direct target gene of PI3K [41, 42]. After PI3K activation, Akt can be activated by phosphorylation of phosphatidylinositol 4-phosphate and phosphatidylinositol 4-diphosphate. Some studies have found that a PI3K-specific inhibitor (LY294002) can significantly improve human sperm motility and the percentage of forward sperm motility [43], and the effect is more obvious in people with asthenospermia [44]. This finding suggests that PI3K negatively regulates the motility of human sperm cells. In addition, PI3K is a negative regulator of autophagy, and autophagy can participate in the regulation of sperm survival and movement. Notably, aflatoxin can induce sperm cells autophagy by inhibiting the PI3K/AKT/mTOR pathway, thereby causing damage to male fertility [45]. FOXOs are located downstream of growth

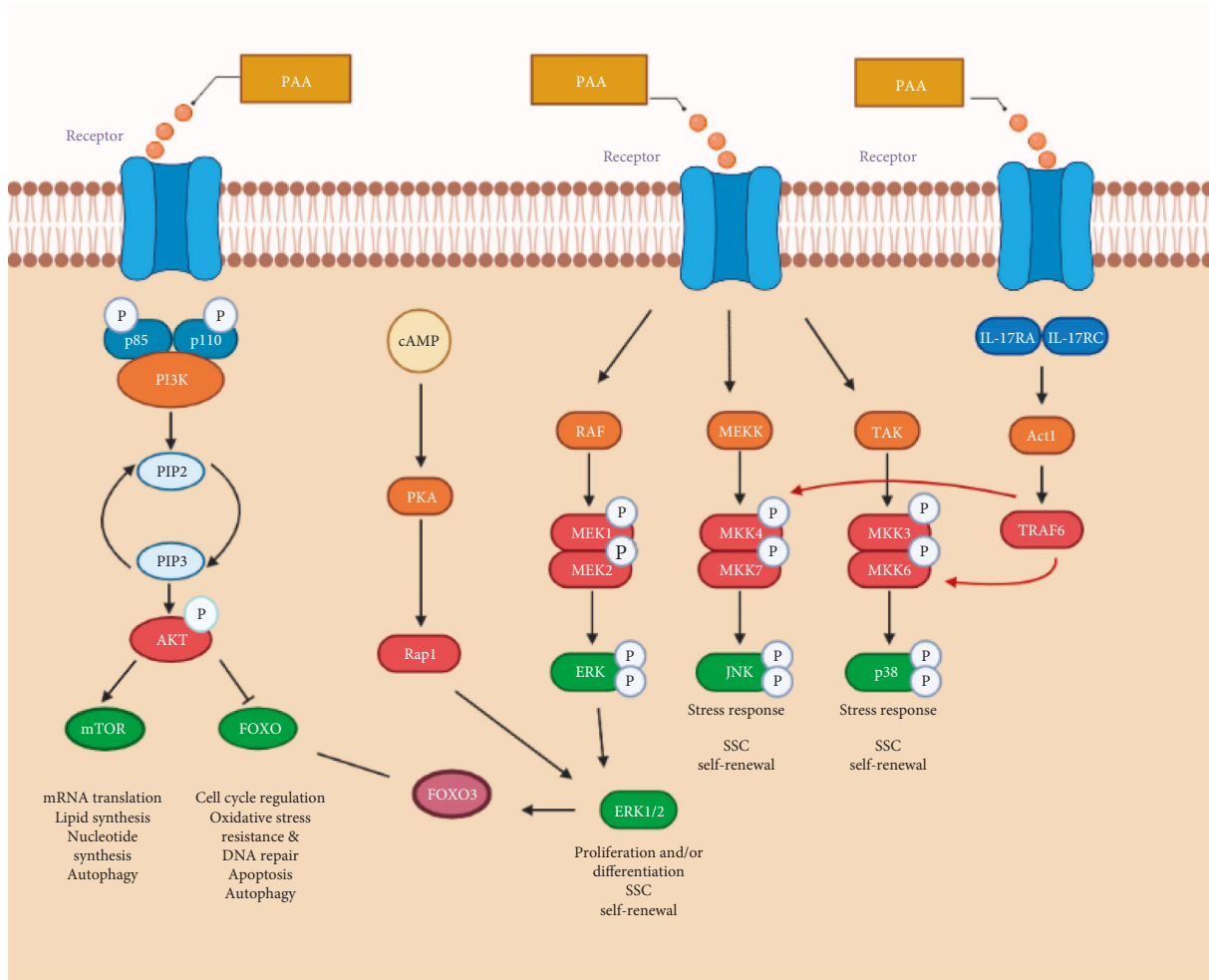


FIGURE 8: Diagram of signaling pathways associated with infertility.

factor and nutrient signaling. In mammals, the FOXOs include Foxo1, Foxo3, and Foxo4, which coordinate various responses, including cell cycle arrest and programmed cell death [46]. FOXO transcription factors are the key nodes at the intersections of many signaling pathways [46]. Previous studies have confirmed that FOXOs are located downstream of the PI3K/AKT signaling pathway and are regulated by Akt-dependent phosphorylation. When AKT is phosphorylated, FOXOs are inhibited [47]. In terms of reproduction, FOXO1 plays an important role in spermatogenesis [48, 49]. FOXO also plays an important role in the self-renewal and differentiation of spermatogonial stem cells (SSCs). When PI3K/AKT is activated, FOXO1 loses its activity, which further inhibits the self-renewal of SSCs [50], indicating that this FOXO plays an important regulatory role in the late stage of spermatogenesis. MAPK belongs to the serine/threonine kinase family. There are three main subfamilies: the ERK, c-Jun N-terminal kinase (JNK), and p38MAPK (MAPK14) subfamilies; this family is thought to be one important determinant of sperm development [51–54]. In rat testes, phosphorylated ERK1/2, JNK1/2, and p38MAPK are localized in SSCs, which play an important role in regulating nutritional supply,

maintaining cell connections and supporting mitosis and meiosis of germ cells [57]. The migration of germ cells and the release of sperm require adherens junctions (AJs) and tight junctions (TJs) between Sertoli cells (SC-SC junctions) and Sertoli cells (SC-GC junctions). This requirement makes normal spermatogenesis dependent on SSCs. Activation of the p38MAPK and ERK pathways interferes with the AJs between Sertoli cells, and activation of ERK pathways also affects the dynamics of these two connections, thus affecting the self-renewal of SSCs [55]. Many cells can respond to reproductive toxicants, and many of these responses are mediated by activation of the MAPK pathway; for example, bisphenol A can activate the ERK and JNK signaling pathways to induce apoptosis [56], and under the influence of di-n-butyl-phthalate (an endocrine disruptor), the rat testicular tissue is damaged, semen quality is decreased, and there is an elevated p-ERK1/2 and p-JNK expression in the MAPK pathway, but not p38 MAPK phosphorylation levels [57]. In other experiments, however, p38mpak was suggested to play a major role in Sertoli cell injury [58]. Although existing studies have reported inconsistent conclusions, it has become a fact that MAPKs have a close connection with male infertility, which

is in accordance with the findings of the present experiment. Camp is closely related to sperm motility [59]. Specifically, it plays an important role in the initiation, alteration, and maintenance of sperm motility [60]. In recent years, IL-17 has been shown to be a proinflammatory cytokine. Through specific binding with its receptor, it can promote inflammation, the immune response, hematopoiesis, and other processes. A cross-sectional study found that IL-17 levels in the semen of infertile patients were significantly higher than those in the semen of normal men [61].

5. Conclusion

In conclusion, this study uses a network pharmacology method to study the therapeutic effects of PAA on male infertility at multiple levels and finds that TP53, MAPK1, IL-6, ANXA1, EGF, EGFR, and other genes may be key targets. The PI3K-Akt, IL-17 cAMP, FOXO, and MAPK signaling pathways may be key pathways that mainly play anti-inflammatory and antioxidant roles and promote cell proliferation and cell ATP production to treat male infertility. A possible molecular mechanism by which this drug pair treats male infertility has been revealed. A limitation of this study is that the pharmacologically active components and targets discovered through network pharmacology are predictions; thus, the results should be verified experimentally. Subsequent research will verify the findings in animal experiments and clinical studies to improve the rationality and scientific basis of clinical PAA application.

Data Availability

Specific study data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by the Basic Scientific Research Service Fee Project of the Central Public Welfare Research Institute of the Chinese Academy of Traditional Chinese Medicine (ZZ15-XY-CT-02) and Beijing Science and Technology Development of TCM (JJ-2020-76).

Supplementary Materials

Table S1: available ingredient and target information of *Astragalus* collected in TCMSP database. Table S2: available ingredient and target information of *Astragalus* collected in BATMAN-TCM database. Table S3: available ingredient and target information of *Angelica* collected in TCMSP database. Table S4: available ingredient and target information of *Angelica* collected in BATMAN-TCM database. Table S5: The drug pair-component-node data of target-disease regulation network. (*Supplementary Materials*)

References

- [1] A. Agarwal, S. Baskaran, N. Parekh et al., "Male infertility," *The Lancet*, vol. 397, no. 10271, pp. 319–333, 2021.
- [2] A. Mehta, "Qualitative research in male Infertility," *Urologic Clinics of North America*, vol. 47, no. 2, pp. 205–210, 2020.
- [3] A. Salonia, C. Bettocchi, J. Carvalho et al., *EAU Guidelines on Sexual and Reproductive Health*, pp. 106–140, European Association of Urology, Arnhem, Netherlands, 2020.
- [4] R. Cannarella, R. A. Condorelli, L. M. Mongioi, F. Barbaggio, A. E. Calogero, and S. La Vignera, "Effects of the selective estrogen receptor modulators for the treatment of male infertility: a systematic review and meta-analysis," *Expert Opinion on Pharmacotherapy*, vol. 20, no. 12, pp. 1517–1525, 2019.
- [5] P. N. Schlegel, "Aromatase inhibitors for male infertility," *Fertility and Sterility*, vol. 98, no. 6, pp. 1359–1362, 2012.
- [6] D. F. Yao and J. N. Mills, "Male infertility: lifestyle factors and holistic, complementary, and alternative therapies," *Asian Journal of Andrology*, vol. 18, no. 3, pp. 410–418, 2016.
- [7] D. Jiang, A. Coscione, L. Li, and B.-Y. Zeng, "Effect of Chinese herbal medicine on male infertility," *International Review of Neurobiology*, vol. 135, pp. 297–311, 2017.
- [8] J. Fu, Z. Wang, L. Huang et al., "Review of the botanical characteristics, phytochemistry, and pharmacology of *Astragalus membranaceus* (Huangqi)," *Phytotherapy Research: PTR*, vol. 28, no. 9, pp. 1275–83, 2014.
- [9] W.-L. Wei, R. Zeng, C.-M. Gu, Y. Qu, and L.-F. Huang, "Angelica sinensis in China-A review of botanical profile, ethnopharmacology, phytochemistry and chemical analysis," *Journal of Ethnopharmacology*, vol. 190, pp. 116–141, 2016.
- [10] C.-Y. Zhou, G.-F. Ma, B. Wang, J.-X. Ma, and H.-S. Li, "Thoughts for distinguishing treatments from TCM in male infertility," *Journal of Traditional Chinese Medicine*, vol. 57, no. 13, pp. 1105–1108, 2016.
- [11] J. Guo, "Thoughts and approaches of integrated Chinese and western medicine for the treatment of oligo-astheno-teratozoospermia," *Chinese Journal of Integrative Medicine*, vol. 24, no. 7, pp. 483–486, 2018.
- [12] F. Zhao, S.-J. Liu, Q.-H. Gao et al., "Network pharmacology-based study of Chinese herbal qixiong formula in treating oligoasthenospermia," *World Journal of Traditional Chinese Medicine*, vol. 6, no. 4, pp. 481–488, 2020.
- [13] J. Ru, P. Li, J. Wang et al., "TCMSP: a database of systems pharmacology for drug discovery from herbal medicines," *Journal of Cheminformatics*, vol. 6, no. 13, p. 13, 2014.
- [14] Z. Liu, F. Guo, Y. Wang et al., "BATMAN-TCM: a bioinformatics analysis tool for molecular mechanism of traditional Chinese medicine," *Scientific Reports*, vol. 6, no. 1, p. 21146, 2016.
- [15] W. Zhang, A. Bojorquez-Gomez, D. O. Velez et al., "A global transcriptional network connecting noncoding mutations to changes in tumor gene expression," *Nature Genetics*, vol. 50, no. 4, pp. 613–620, 2018.
- [16] P. Janet, M. R.-A. Juan, S.-P. Josep et al., "The DisGeNET knowledge platform for disease genomics: 2019 update," *Nucleic Acids Research*, vol. 48, no. D1, pp. D845–D855, 2019.
- [17] N. Campbell, "Genetic association database," *Nature Reviews Genetics*, vol. 5, no. 2, p. 87, 2004.
- [18] P. Shannon, A. Markiel, O. Ozier et al., "Cytoscape: a software environment for integrated models of biomolecular interaction networks," *Genome Research*, vol. 13, no. 11, pp. 2498–2504, 2003.

- [19] G. D. Bader and C. W. Hogue, "An automated method for finding molecular complexes in large protein interaction networks," *BMC Bioinformatics*, vol. 4, p. 2, 2003.
- [20] A. Bumrungpert, S. Lilitchan, S. Tuntipopipat, N. Tirawanchai, and S. Komindr, "Ferulic acid supplementation improves lipid profiles, oxidative stress, and inflammatory status in hyperlipidemic subjects: a randomized, double-blind, placebo-controlled clinical trial," *Nutrients*, vol. 10, no. 6, p. 713, 2018.
- [21] X.-D. Li, W. Ding, F.-P. Miao, and N.-Y. Ji, "Halogenated chamigrane sesquiterpenes from *Laurencia okamurae*," *Magnetic Resonance in Chemistry*, vol. 50, no. 2, pp. 174–177, 2012.
- [22] M. R. Luderer, M. J. Mealy, and W. F. Bailey, "Asymmetric intramolecular carbolithiation of achiral substrates: synthesis of enantioenriched (R)-(+)-cuparene and (R)-(+)-herbertene," *Journal of Organic Chemistry*, vol. 79, no. 21, pp. 10722–10726, 2014.
- [23] A. Kundu, "Vanillin biosynthetic pathways in plants," *Planta*, vol. 245, no. 6, pp. 1069–1078, 2017.
- [24] W.-S. Fan, B.-Y. Song, Y.-S. Liu, K. Zhang, K.-X. Guo, and S.-S. Jing, "An analysis of the regularity of ancient Chinese medicines for the treatment of male infertility based on Data Mining," *Pharmacology and Clinics of Chinese Materia Medica (China)*, pp. 1–10, 2020.
- [25] M. M. Dobrzynska, A. Baumgartner, and D. Anderson, "Antioxidants modulate thyroid hormone- and noradrenaline-induced DNA damage in human sperm," *Mutagenesis*, vol. 19, no. 4, pp. 325–330, 2004.
- [26] J.-B. Xiao, "Dietary flavonoid aglycones and their glycosides: which show better biological significance?" *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 9, pp. 1874–1905, 2017.
- [27] R. Diao, H. Gan, F. Tian et al., "In vitro antioxidation effect of Quercetin on sperm function from the infertile patients with leukocytospermia," *American Journal of Reproductive Immunology*, vol. 82, no. 3, p. e13155, 2019.
- [28] Y. Zhang, M. Song, X. Rui, S. Pu, Y. Li, and C. Li, "Supplemental dietary phytosterin protects against 4-nitrophenol-induced oxidative stress and apoptosis in rat testes," *Toxicology Reports*, vol. 2, pp. 664–676, 2015.
- [29] E. Almon, N. Goldfinger, A. Kapon, D. Schwartz, A. J. Levine, and V. Rotter, "Testicular tissue-specific expression of the p53 suppressor gene," *Developmental Biology*, vol. 156, no. 1, pp. 107–116, 1993.
- [30] L. Yuan, J.-G. Liu, M.-R. Hoja, D. A. Lightfoot, and C. Höög, "The checkpoint monitoring chromosomal pairing in male meiotic cells is p53-independent," *Cell Death & Differentiation*, vol. 8, no. 3, pp. 316–317, 2001.
- [31] Q. Jin, B. Wang, J. Wang et al., "Association between TP53-gene Arg72Pro polymorphism and idiopathic infertility in southeast Chinese Han males," *Systems Biology in Reproductive Medicine*, vol. 59, no. 6, pp. 342–346, 2013.
- [32] A. G. Abraham and E. O'Neill, "PI3K/Akt-mediated regulation of p53 in cancer," *Biochemical Society Transactions*, vol. 42, no. 4, pp. 798–803, 2014.
- [33] L. Yu, X. Yang, B. Ma et al., "Abnormal arachidonic acid metabolic network may reduce sperm motility via P38 MAPK," *Open Biology*, vol. 9, no. 4, p. 180091, 2019.
- [34] S. Noh, A. Go, D. B. Kim, M. Park, H. W. Jeon, and B. Kim, "Role of antioxidant natural products in management of infertility: a review of their medicinal potential," *Antioxidants*, vol. 9, no. 10, p. 957, 2020.
- [35] E. Brener, S. Rubinstein, G. Cohen, K. Shternall, J. Rivlin, and H. Breitbart, "Remodeling of the actin cytoskeleton during mammalian sperm capacitation and acrosome Reaction1," *Biology of Reproduction*, vol. 68, no. 3, pp. 837–845, 2003.
- [36] G. Berruti, "Evidence for Ca-mediated F-actin/phospholipid binding of human sperm calpactin II," *Cell Biology International Reports*, vol. 15, no. 10, pp. 917–927, 1991.
- [37] A. Sharma, S. V. Lagah, D. Nagoorvali et al., "Supplementation of glial cell line-derived neurotrophic factor, fibroblast growth factor 2, and epidermal growth factor promotes self-renewal of putative buffalo (*Bubalus bubalis*) spermatogonial stem cells by upregulating the expression of miR-20b, miR-21, and miR-106a," *Cellular Reprogramming*, vol. 21, no. 1, pp. 11–17, 2019.
- [38] C. Foresta, A. Bettella, M. Merico, A. Garolla, A. Ferlin, and M. Rossato, "Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility," *Fertility and Sterility*, vol. 77, no. 2, pp. 238–244, 2002.
- [39] E. M. Eldamhoury, G. A. Elatrash, H. M. Rashwan, and A. I. El-Sakka, "Association between leukocytospermia and semen interleukin-6 and tumor necrosis factor-alpha in infertile men," *Andrology*, vol. 6, no. 5, pp. 775–780, 2018.
- [40] H. Xu, L. Shen, X. Chen et al., "mTOR/P70S6K promotes spermatogonia proliferation and spermatogenesis in Sprague Dawley rats," *Reproductive BioMedicine Online*, vol. 32, no. 2, pp. 207–217, 2016.
- [41] J. T. Busada, B. A. Niedenberger, E. K. Velte, B. D. Keiper, and C. B. Geyer, "Mammalian target of rapamycin complex 1 (mTORC1) is required for mouse spermatogonial differentiation in vivo," *Developmental Biology*, vol. 407, no. 1, pp. 90–102, 2015.
- [42] L.-X. Feng, N. Ravindranath, and M. Dym, "Stem cell factor/c-kit up-regulates cyclin D3 and promotes cell cycle progression via the phosphoinositide 3-Kinase/p70 S6 kinase pathway in spermatogonia," *Journal of Biological Chemistry*, vol. 275, no. 33, pp. 25572–25576, 2000.
- [43] M. Luconi, L. Bonaccorsi, G. Forti, and E. Baldi, "Effects of estrogenic compounds on human spermatozoa: evidence for interaction with a nongenomic receptor for estrogen on human sperm membrane," *Molecular and Cellular Endocrinology*, vol. 178, no. 1–2, pp. 39–45, 2001.
- [44] S. S. du Plessis, D. R. Franken, E. Baldi, and M. Luconi, "Phosphatidylinositol 3-kinase inhibition enhances human sperm motility and sperm-zona pellucida binding," *International Journal of Andrology*, vol. 27, no. 1, pp. 19–26, 2004.
- [45] W. Huang, Z. Cao, J. Zhang, Q. Ji, and Y. Li, "Aflatoxin B1 promotes autophagy associated with oxidative stress-related PI3K/AKT/mTOR signaling pathway in mice testis," *Environmental Pollution*, vol. 255, no. Pt 2, p. 113317, 2019.
- [46] D. R. Calnan and A. Brunet, "The FoxO code," *Oncogene*, vol. 27, no. 16, pp. 2276–2288, 2008.
- [47] P. Huang, Z. Zhou, F. Shi et al., "Effects of the IGF-1/PTEN/Akt/FoxO signaling pathway on male reproduction in rats subjected to water immersion and restraint stress," *Molecular Medicine Reports*, vol. 14, no. 6, pp. 5116–5124, 2016.
- [48] J.-H. Paik, R. Kollipara, G. Chu et al., "FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis," *Cell*, vol. 128, no. 2, pp. 309–323, 2007.
- [49] T. Hosaka 3rd, W. H. Biggs, D. Tieu et al., "Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 9, pp. 2975–2980, 2004.
- [50] M. J. Goertz, Z. Wu, T. D. Gallardo, F. K. Hamra, and D. H. Castrillon, "Foxo1 is required in mouse spermatogonial

- stem cells for their maintenance and the initiation of spermatogenesis," *Journal of Clinical Investigation*, vol. 121, no. 9, pp. 3456–3466, 2011.
- [51] T. Almog and Z. Naor, "Mitogen activated protein kinases (MAPKs) as regulators of spermatogenesis and spermatozoa functions," *Molecular and Cellular Endocrinology*, vol. 282, no. 1-2, pp. 39–44, 2008.
- [52] X. Guo, P. Bian, J. Liang et al., "Synergistic effects induced by a low dose of diesel particulate extract and ultraviolet-A in *Caenorhabditis elegans*: DNA damage-triggered germ cell apoptosis," *Chemical Research in Toxicology*, vol. 27, no. 6, pp. 990–1001, 2014.
- [53] B. B. Friday and A. A. Adjei, "Advances in targeting the Ras/Raf/MEK/Erk mitogen-activated protein kinase cascade with MEK inhibitors for cancer therapy," *Clinical Cancer Research*, vol. 14, no. 2, pp. 342–346, 2008.
- [54] P.-B. Sun, Y.-Y. Wang, T. Gao et al., "Hsp90 modulates human sperm capacitation via the Erk1/2 and p38 MAPK signaling pathways," *Reproductive Biology and Endocrinology*, vol. 19, no. 1, 2021.
- [55] F.-D. Ni, S.-L. Hao, and W.-X. Yang, "Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis," *Cell Death & Disease*, vol. 10, no. 8, p. 541, 2019.
- [56] J. Peretz, L. Vrooman, W. A. Ricke et al., "Bisphenol A and reproductive health: update of experimental and human evidence, 2007-2013," *Environmental Health Perspectives*, vol. 122, no. 8, pp. 775–786, 2014.
- [57] H. Wang, W. Zhou, J. Zhang, and H. Li, "Role of JNK and ERK1/2 MAPK signaling pathway in testicular injury of rats induced by di-N-butyl-phthalate (DBP)," *Biological Research*, vol. 52, no. 1, p. 41, 2019.
- [58] Y. Song, Y. Shi, H. Yu, Y. Hu, Y. Wang, and K. Yang, "p,p'-Dichlorodiphenoxydichloroethylene induced apoptosis of Sertoli cells through oxidative stress-mediated p38 MAPK and mitochondrial pathway," *Toxicology Letters*, vol. 202, no. 1, pp. 55–60, 2011.
- [59] M. Balbach, V. Beckert, J. N. Hansen, and D. Wachten, "Shedding light on the role of cAMP in mammalian sperm physiology," *Molecular and Cellular Endocrinology*, vol. 468, pp. 111–120, 2018.
- [60] C. J. Brokaw, "Regulation of sperm flagellar motility by calcium and cAMP-dependent phosphorylation," *Journal of Cellular Biochemistry*, vol. 35, no. 2, pp. 175–184, 1987.
- [61] A. P. Mary, H. Nandeesha, D. Papa, T. Chitra, R. N. Ganesh, and V. Menon, "Matrix metalloproteinase-9 is elevated and related to interleukin-17 and psychological stress in male infertility: a cross-sectional study," *Int J Reprod Biomed*, vol. 19, no. 4, pp. 333–338, 2021.