Therapeutic Effects and Mechanism of *Panax ginseng* in Improving Spermatogenesis: Evidence from Network Pharmacology and Molecular Docking

Tran Nhat Minh[1,#], Hoang Thi Ai Phuong[2,#], Dang Ngoc Phuc[2,3], Nguyen Thanh Tung[2,*]

(1) Faculty of Traditional Medicine, Hue University of Medicine and Pharmacy, Hue University

(2) Regenerative Medicine Group, Faculty of Basic Science, University of Medicine and Pharmacy, Hue University

(3) Faculty of Medicine, Dong A University

Abstract

Background: Spermatogenesis is a complex process involving mitotic cell division, meiosis, and spermiogenesis. This study aimed to examine the therapeutic effects and mechanisms of *Panax ginseng* in improving spermatogenesis, using a systematic network pharmacology approach and molecular docking. **Methods:** Traditional Chinese Medicine Systems Pharmacology (TCMSP) and Herbal Ingredients' Targets (HIT) databases were used to screen for bioactive compounds in *Panax ginseng*. The SwissTargetPrediction, BATMAN-TCM, HIT, and TCMSP databases were used to identify and obtain the targets. The OMIM database and GeneCards Version 5.20 were searched to obtain targets related to spermatogenesis. The protein-protein interaction (PPI) network was constructed using common targets from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. The DAVID tool was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. AutoDock Vina software was used for molecular docking analysis. **Results:** A total of 250 overlapping target genes were identified in *Panax ginseng* and during spermatogenesis. PPI network analysis revealed that tumor protein P53, heat shock protein 90, Alpha Family Class A Member 1, AKT Serine/Threonine Kinase 1, Jun Proto-Oncogene, AP-1 Transcription Factor Subunit, Signal Transducer and Activator of Transcription 3, and Mitogen-Activated Protein Kinase 1 were the top ten most relevant targets. The results of the GO and KEGG analyses showed that the common targets of *Panax ginseng* and spermatogenesis were mainly involved in pathways related to cancer, p53, MAPK, lipid and atherosclerosis, and the human T-cell leukemia virus 1 infection signaling pathway. Molecular docking analysis suggested that potential targets for *Panax ginseng*, including quercetin, stigmasterol, inermin, ginsenoside Rg5 had the lowest docking energy for STAT3 and HSP90AA1. **Conclusion:** The present study identified the active components and probable molecular therapeutic mechanisms of *Panax ginseng* in enhancing spermatogenesis, providing a foundation for the widespread use of *Panax ginseng* in the male reproductive system.

Keywords: *Panax ginseng, spermatogenesis, network pharmacology, TP53, MAPK, quercetin, molecular docking*.

1. INTRODUCTION

Human sperm production, also known as spermatogenesis, is distinct from processes observed in most other mammals in terms of both quality and quantity [1]. The development of sperm cells from stem cells in the testes is a complex process involving multiple cell types, hormones, genes, and epigenetic regulators [2]. The existence of diverse cell types presents a challenge when attempting to gather detailed information the development of germ and somatic cells. As a result, there is a lack of information that has limited our ability to comprehend the process of sperm production and apply findings from model organisms to humans [3].

Panax ginseng is a highly regarded herb in Eastern traditional medicine with a long history of use in the treatment of various diseases. This herb is effective in a range of conditions, including diabetes [4], anti-aging treatments, and neurological deficits resulting from cerebral ischemia [5]. It has also been reported to be effective in treating cancer, Alzheimer's disease, hypertension, acquired immune deficiency syndrome, and reproductive disorders [6]. Studies have shown that *Panax ginseng* exerts a range of physiological effects on the cardiovascular, immune, and neuronal systems [7]. Additionally, it has been traditionally used to boost libido and treat infertility in men, and it can improve sexual performance,

[#] These authors contributed equally to this work

Corresponding author: Nguyen Thanh Tung;
Email: nguyenthanhtung@hueuni.edu.vn or nttung@huemed-univ.edu.vn
Received: 12/4/2024; Accepted: 18/6/2024; Published: 25/6/2024

DOI: 10.34071/jmp.2024.4.13

promote spermatogenesis, and act directly on sperm via steroid receptors to preserve male fertility during disease states [7].

Previous research has indicated that *Panax ginseng* is effective in protecting against testicular damage caused by doxorubicin and aging, and in improving spermatogenesis in models of testicular dysfunction [8], [9]. The herb contains various pharmaceutical components, including ginsenosides, polyacetylenes, polyphenolic compounds, and acidic polysaccharides [10]. Studies have shown that ginseng can improve sperm motility and count [11]. It also protects muscles from exercise-induced oxidative stress and enhances erectile function by improving parameters such as penile rigidity, girth, duration, libido, and patient satisfaction [12]. Although medications, lifestyle changes, and natural or alternative treatments can help restore normal sexual function, there is a growing preference for natural remedies, such as herbal supplements, as they are safer than synthetic drugs and assisted reproductive technologies, which have diverse side effects [8]. However, the molecular mechanisms underlying these benefits are not well understood or have not been extensively studied.

Here, meta-analysis studies were done to evaluate the efficacy of *Panax ginseng* on the male reproductive system. A network pharmacology approach was used to explore the potential pharmacological mechanisms of ginseng on the male reproductive system. Molecular docking was conducted to determine the binding efficiency of putative ginseng compound-target pairs.

2. MATERIALS AND METHODS

2.1. Screening Of The Bioactive Compounds Found In *Panax ginseng*

The compounds found in *Panax ginseng* were sourced from Traditional Chinese Medicine Systems Pharmacology (TCMSP) and Herbal Ingredients' Targets (HIT) databases. These compounds were selected based on their pharmacokinetic properties, with only those meeting the screening criteria of an oral bioavailability (OB) greater than 30% and a drug-likeness (DL) value of at least 0.18, retained for further research.

2.2. Screening Of The Target prediction of *Panax ginseng*

The targets of *Panax ginseng* were determined using three public databases, including TCMSP, HIT, BATMAN-TCM (with a score cutoff of 20 and an adjusted P-value of 0.05), and the SwissTargetPrediction webtool (<http://www.swisstargetprediction.ch/>, Prob value >0). In addition, the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) was used to obtain the compounds' structures for molecular docking by entering their corresponding names, PubChem compound IDs, and Chemical Abstracts Service (CAS) numbers.

2.3. Screening Predicted Targets Of The Spermatogenesis Process

The targets related to the spermatogenesis process were obtained by searching the OMIM database (<https://omim.org/search/advanced/>), and GeneCards Version 5.20 (Updated: Apr 1, 2024) (<https://www.genecards.org/>), using the keywords "spermatogenesis," "spermatogenic," "oligozoospermia," "cryptozoospermia," and "azoospermia." The GeneCards Database provides information on all the known human genes, including their genomic, proteomic, transcriptional, hereditary, and functional characteristics. Targets with scores greater than the median were selected. The two sets of targets from OMIM and GeneCards were merged, duplicates were removed, and the resulting targets were used in subsequent studies.

2.4. Retrieval Of Venn Diagram

The common targets between Panax ginseng and Spermatogenesis Process were visualized by Venn diagram using VENNY 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>).

2.5. Construction Of Protein-Protein Interaction (PPI) Network

The PPI network of *Panax ginseng* in regulating the spermatogenesis process was assembled using STRING (<https://string-db.org/>, version 12.0), a database of known and predicted PPI that utilizes bioinformatic techniques to gather information. In this study, we restricted the species to H. sapiens and selected the proteins with the highest confidence level of 0.9. The unconnected proteins were removed [13]. Cytoscape is a network biology visualization and analysis software that visualizes molecular interactions and biological processes [14]. Potential active components and matching targets were imported into Cytoscape 3.10.2, which can graphically display and analyze the networks. In the compound-target network, each component or target is represented by a node, and the connection between the component and the target is represented by a line.

2.6. Core Gene Analysis

The Cytoscape 3.9.0 software was used to

construct a topology network in which the degree of a node was determined, which was defined as the number of connections that it had to other nodes. The core targets were chosen based on nodes with degree values above three times of the average [15].

2.7. Gene Ontology Functional and Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analysis

The Gene Ontology (GO) analysis is employed to identify biological processes (BP), cellular components (CC), and molecular functions (MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed significant signaling pathways involved in crucial biological processes. In our study, we utilized the Database for Annotation, Visualization, and Integrated Discovery (DAVID), which offers a comprehensive set of functional annotation tools for investigators to comprehend the biological significance behind extensive lists of genes (version 7.0) with a corrected value of less than 0.01 (using Benjamini's correction) for processing GO and KEGG [16], [17].

2.8. Molecular docking

Molecular docking is widely employed in drug design because of its ability to predict the binding capacity of ligands and proteins as well as the specific location of this interaction. Using molecular docking, we identified the top five core targets and their corresponding bioactive compounds. The molecular docking process involved the following steps. The 3D structure of the target protein was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and Protein Data Bank (https://www.rcsb.org/) [18]. For molecular docking, the compound network and the target proteins with the highest degree in the core network were selected using AutoDock 1.5. Vina was used for hydrogenation, charge calculation, and nonpolar hydrogen combination. AutoDock Vina 1.1.2 was utilized to calculate the docking energy [19]. AutoDock Vina uses CMD command characters for molecular docking and relies on PyMOL 2.5.2 software for visualization of the results [18]. Typically, a binding capacity exists between the target and compound if the docking energy between the receptor and ligand is less than −5 kcal/mol. Network visualization and construction were performed using PyMOL and LigPLOS software, respectively [20].

3. RESULT

3.1 Bioactive Components of *Panax ginseng*

The Pharmacokinetic parameters of the components, oral bioavailability (OB), and drug-likeness (DL) were used as the screening conditions in this study as follows: OB ≥ 40% and DL ≥ 0.18 [21], from which we selected 25 active ingredients that satisfied these conditions (Table 1).

Table 1. Basic information on the main active ingredients of *Panax ginseng*

Mol ID	Molecule Name	PubChem CID	OB (%)	Caco-2	DL
TCMSP					
MOL000358	Beta-sitosterol	222284	36.91	1.32	0.75
MOL000422	Kaempferol	5280863	41.88	0.26	0.24
MOL000449	Stigmasterol	5280794	43.83	1.44	0.76
MOL000787	Fumarine	4970	59.26	0.56	0.83
MOL002879	Diop	395120	43.59	0.79	0.39
MOL003648	Inermin	91510	65.83	0.91	0.54
MOL004492	Chrysanthemaxanthin	21160900	38.72	0.51	0.58
MOL005308	Aposiopolamine	5319581	66.65	0.66	0.22
MOL005314	Celabenzine	442847	101.88	0.77	0.49
MOL005317	Deoxyharringtonine	285342	39.27	0.19	0.81
MOL005318	Dianthramine	441562	40.45	-0.23	0.2
MOL005320	Arachidonate	444899	45.57	1.27	0.2
MOL005321	Frutinone A	441965	65.9	0.89	0.34

MOL005344	Ginsenoside rh2	119307	36.32	-0.51	0.56
MOL005348	Ginsenoside-Rh4_qt	21599928	31.11	0.5	0.78
MOL005356	Girinimbin	96943	61.22	1.72	0.31
MOL005357	Gomisin B	6438572	31.99	0.6	0.83
MOL005360	Malkangunin	90473155	57.71	0.22	0.63
MOL005376	Panaxadiol	73498	33.09	0.82	0.79
MOL005384	Suchilactone	10915582	57.52	0.82	0.56
MOL005399	Alexandrin_qt	5742590	36.91	1.3	0.75
MOL005401	Ginsenoside Rg5_qt	11550001	39.56	0.88	0.79
HIT2.0					
C0104	Ginsenoside Rh2	119307	36.32	-0.51	0.56
C0159	Isovitexin	162350	31.29	-1.24	0.72
C0164	Kaempferol	5280863	41.88	0.26	0.24
C0352	Quercetin	5280343	46.43	0.05	0.28
C0513	Squalene	638072	33.55	2.08	0.42
C0749	Stigmasterol	5280794	43.83	1.44	0.76
C1178	Beta-Sitosterol	222284	36.91	1.32	0.75

3.2. Target Prediction of *Panax ginseng*

To predict the candidate targets of the active components of *Panax ginseng*, the present study employed different databases for screening. We selected common prediction as a potential and vital target for further analysis using the TCMSP, HIT 2.0, BATMAN-TCM and SwissTargetPrediction databases. Following UniProt standardization and deduplication, 1216 targets were identified and are presented in Table 2. Among them, quercetin was the most common predictor and had the highest degree values from the scores listed in the prediction.

Table 2. The number of target predictions of *Panax ginseng*

***Panax ginseng* gene**	**TCMSP**	**BATMAN**	**SWISS (SWISS > 0.2)**	**HIT2.0**
Alexandrin_qt	1	0	26 (1)	0
Aposiopolamine	8	6	100	0
Arachidonate	4	172	100 (7)	55
Beta-sitosterol	37	96	44 (10)	10
Celabenzine	0	0	100	0
Chrysanthemaxanthin	0	5	0	0
Deoxyharringtonine	2	2	100	0
Dianthramine	3	77	14	0
Diop	3	27	5	0
Frutinone A	16	0	33	0
Fumarine	27	19	40	0
Gomisin B	0	0	100	0
Ginsenoside Rg5_qt	0	2	48	0
Ginsenoside rh2	12	1	20	5
Ginsenoside-Rh4_qt	2	2	94	0

Girinimbin	10	0	100	0
Inermin	17	10	79	1
Isovitexin	6	0	3 (1)	4
Kaempferol	61	5	100 (99)	44
Malkangunin	0	0	0	0
Panaxadiol	1	2	78	2
Quercetin	151	89	100 (99)	201
Squalene	0	98	5	2
Stigmasterol	31	74	41 (12)	1
Suchilactone	15	0	100	0

3.3. Predicted Targets of The Spermatogenesis Process

The OMIM and GeneCards databases were searched using the search term spermatogenesis. These are comprehensive disease-related data platforms, and there is a wide range of data related to complex diseases, including data literature and experimental verification. Search results from the three platforms identified 548 and 2105 target genes that play a role in spermatogenesis from the OMIM and Gencard databases, respectively (Table 3). The merging of these two sets of targets, removing duplicates, and retaining the remaining genes, we obtained targets that will be used in our next study.

3.4. *Panax ginseng* and Spermatogenesis Process Overlapping Targets

After obtaining the gene list from different omics, Venn diagrams are often used to display shared or unique genes among these gene lists. Venn diagrams are commonly used to visually represent the relationships between multiple datasets, including unions, intersections, and distinctions. There are numerous programs available for generating Venn diagrams in various research fields [22]. We used VENNY 2.1 on the 1216 genes of *Panax ginseng's* active components and 2200 genes involved in the spermatogenesis process, as depicted in Figure 1.

Table 3. The Number of Predicted Targets of The Spermatogenesis Process

Database	Spermatogenesis	Spermatogenic	Oligozoospermia	Cryptozoospermia	Azoospermia
OMIM	399	197	38	12	134
Genecard	1761	586	201	11	733

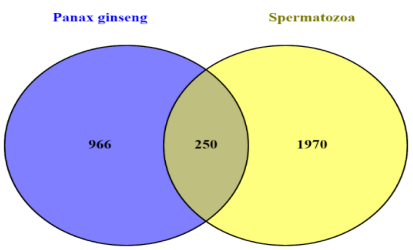

Figure 1. Overlapping targets of *Panax ginseng* and Spermatogenesis Process

3.5. Protein-Protein Interaction Network

An analysis of 250 overlapping target genes of *Panax ginseng* and the Spermatogenesis Process was conducted using a PPI network constructed using the STRING database (Figure 2). The results of the STRING analysis were imported into Cytoscape 3.10.2, where the network analysis plug-in was used to count the nodes in the network graph and examine their connectivity based on the node degree. Node degree indicates

the number of biological functions a node has in the network, with a higher degree indicating greater connectivity. The PPI network of targets generated using STRING analysis was imported into Cytoscape 3.10.2 software. Nodes represent proteins.

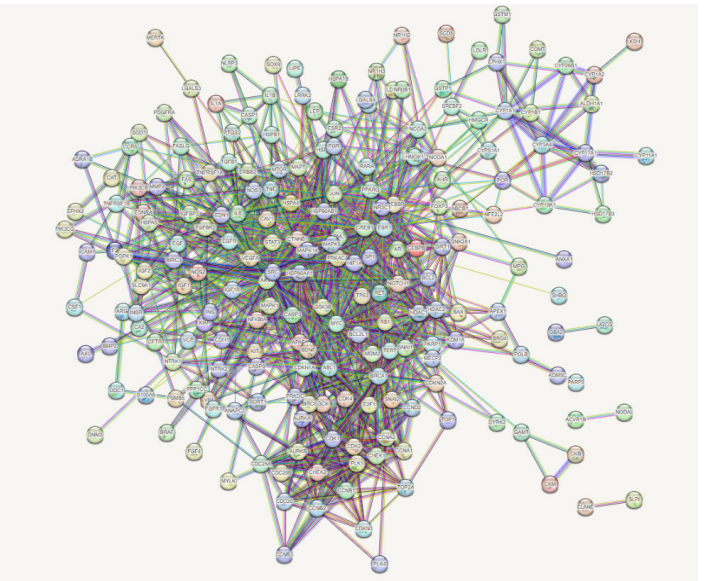

Figure 2. Protein-protein interaction network of overlapping targets

3.6. Core Gene Analysis

According to topological studies, 10 core targets were chosen based on their node degree value being greater than three times the average, including Tumor Protein P53 (TP53), Heat Shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1), AKT Serine/Threonine Kinase 1 (AKT1), Jun Proto-Oncogene, AP-1 Transcription Factor Subunit (JUN), Signal Transducer And Activator Of Transcription 3 (STAT3), Mitogen-Activated Protein Kinase 1 (MAPK1), Proto-Oncogene, Non-Receptor Tyrosine Kinase (SRC), Histone Deacetylase 1 (HDAC1), Estrogen Receptor 1 (ESR1), CREB Binding Protein (CREBBP), and Catenin Beta 1 (CTNNB1). A network diagram of the 10 core targets, as illustrated in Figure 3, was constructed to represent their interconnections.

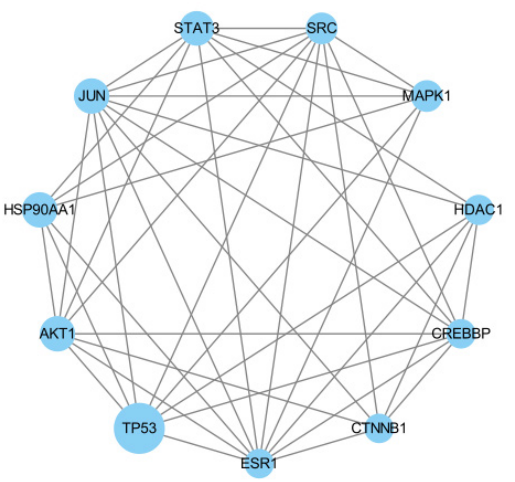

Figure 3. Ten core targets were selected with a node degree value larger than the threefold average

3.7. Gene Ontology Functional Analysis

Metascape data were utilized for enrichment analysis of 250 overlapping targets of *Panax ginseng* in the regulation of spermatogenesis. The results were visualized using online biological tools, and the 15 most significantly enriched biological process (BP) terms (p <0.01) were selected for analysis. This study revealed that BPs are primarily involved in the mechanism of *Panax ginseng* regulation of the spermatogenic process, including positive regulation of transcription from RNA polymerase II promoter (GO0045944), positive regulation of gene expression (GO:0010628), positive regulation of transcription, DNA-templated (GO:0045893), protein phosphorylation (GO:000468), positive regulation of cell proliferation (GO:0008284), and negative regulation of apoptotic process (GO:0043066). Similarly, the 15 most significantly enriched molecular function (MF) terms (p<0.01) were selected for analysis, revealing that the intersecting genes were mainly enriched for protein binding (GO:0005515), identical protein binding (GO:0042802), ATP (GO:0005524), enzyme binding (GO:0019899), and protein homodimerization activity (GO:0042803). Finally, the 15 most significantly enriched cellular component (CC) terms (P<0.01) were selected for analysis, showing that the intersecting genes were mainly enriched in the cytosol (GO:0005829), nucleus (GO:0005634), cytoplasm (GO:0005737), nucleoplasm (GO:0005654), and plasma membrane (GO:0005886). The top 15 GO terms for the three categories are shown in Fig. 4.

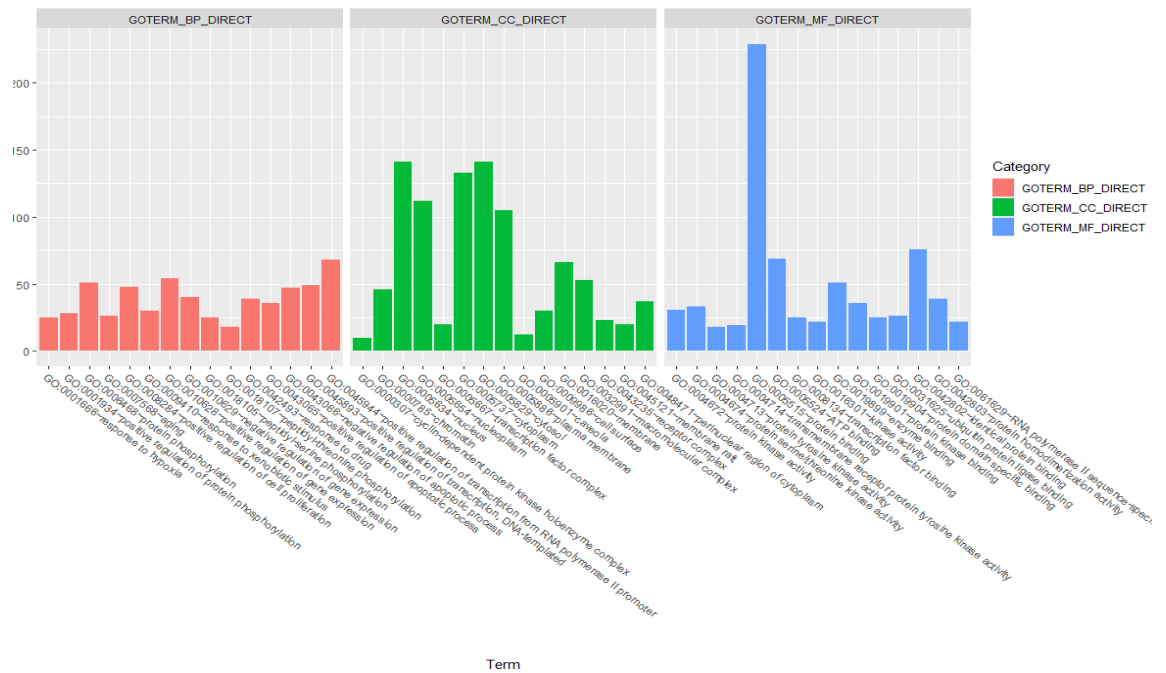

Figure 4. Gene ontology functional enrichment analysis of overlapping targets. Top 15 GO enrichment terms for the three categories

3.8. KEGG pathway enrichment analysis

To understand how *Panax ginseng* affects sperm production, we analyzed 250 genes that intersected using DAVID. This analysis revealed 125 signaling pathways (p < 0.01) that were significantly involved, including pathways related to cancer (hsa052000), the p53/signaling pathway (hsa04115), the MAPK/signaling pathway (hsa04010), Lipid and atherosclerosis (hsa05417), and human T-cell leukemia virus 1 infection (hsa05166) and human cytomegalovirus infection (hsa05163). The top 25 pathways are shown in Figure 5 and the core genes involved in sperm production are listed in Table 4.

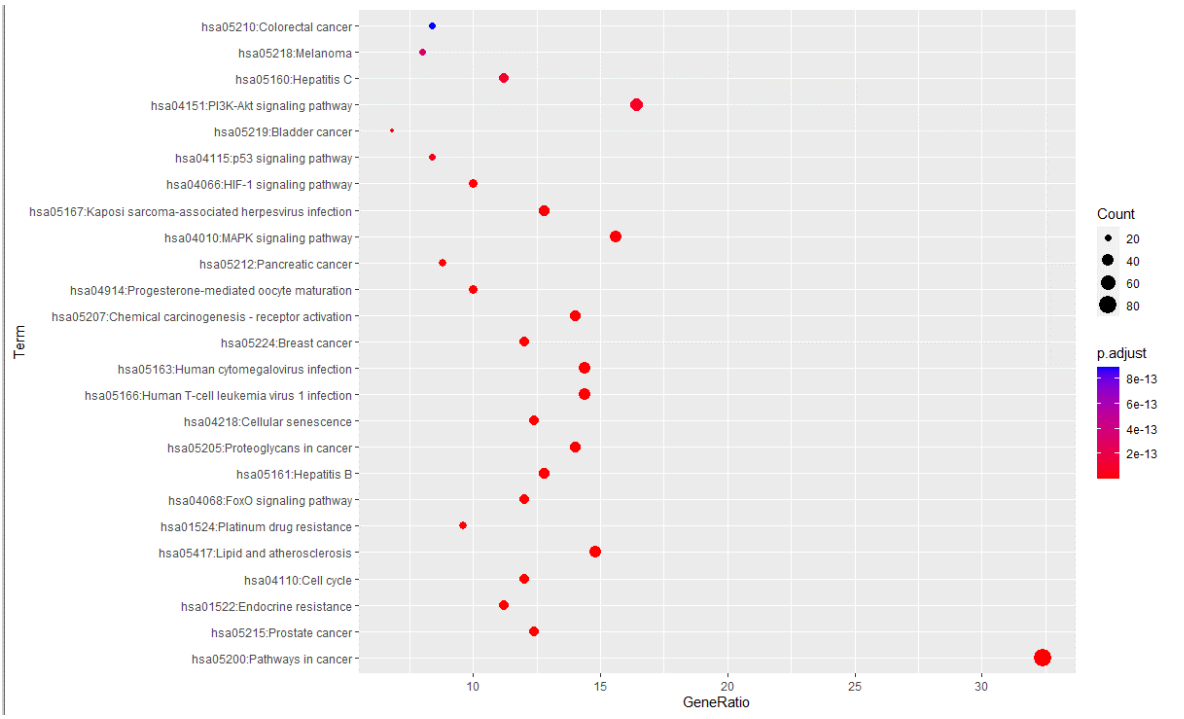

Figure 5. The top 25 KEGG pathway enrichment terms

Table 4. Function information on the Core Gene in Spermatogenic Function

Symbol	Description	Biological and Spermatogenic Function
TP53	Tumor Protein P53	- Spermatogonial cell proliferation [23] - Contribute to the pathogenesis of male infertility with spermatogenetic failure [24]S- Regulating necrosis and homeostasis during spermatogenesis [25] - Sperm cell development and male reproduction [26]
HSP90AA1	Heat shock protein 90 alpha family class A member 1	- Stabilizes HIF-1α in support of the spermatogenesis [27] - Stabilize and activate the testis-specific Serine/Threonine kinases for the spermatid development [28] - Regulates the division of germ cells, formation of sperm microtubes, protection of sperm from oxidative stress, and inducement of acrosomal reaction [29]
AKT1	AKT Serine/ Threonine Kinase 1	- Facilitate the proliferation and anti-apoptosis of immature Sertoli cells and spermatogenic cells [30] - Proliferation and differentiation of spermatogonia and somatic cells, and the regulation of sperm autophagy and testicular endocrine function [31]
JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit	- Considered as the potential key transcription factor in undifferentiated spermatogonia [32].

STAT3	Signal Transducer And Activator Of Transcription 3	- STAT3 phosphorylation in the Sertoli cells regulates the mammalian spermatogenesis [33] - Regulates the nuclear elongation, chromatin condensation, and acrosome formation of sperm [34] - Regulates spermatogonial stem cell differentiation [35]
MAPK1	Mitogen-Activated Protein Kinase 1	- Involved in spermatogenesis, sperm maturation, activation, capacitation, and acrosome reaction [36] - Regulate cell junction dynamics, Sertoli cells and germ cells proliferation, and lactate supply for spermatids [37]
SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase	- Promoting the release of immature spermatids [38] - Src kinases are necessary for spermatogonial stem cell growth [39] - Spermatogonial stem cells are dependent on Src family kinase signaling [40]
HDAC1	Histone Deacetylase 1	- HDAC1 were colocalized in nuclei of spermatogonia [41] - HDAC1 plays a role in transcriptional repression during spermatogenesis [42] - Key protein for nuclear rearrangement during spermatogenesis[43]
ESR1	Estrogen Receptor 1	- The male gonad is partially controlled by an androgen-estrogen balance, with aromatase acting as a modulator [44] - Signaling pathways support processes essential for male fertility in the testis and reproductive tract [45] - Play an important function in the last stages of spermatogenesis [46] - The disruption of ESR1 leads to a temporary increase in testicular weight, tubular dilation, and atrophy of the seminiferous tubules [47]
CREBBP	CREB Binding Protein	- Differential histone acetylation levels between progenitor spermatogonia and stem cells are mediated by the SRCAP-CREBBP/EP300 complex [48] - The function of CREM in spermiogenesis during the initial stage of the process [49] - Role in the regulation of male germ cell spontaneous apoptosis and sperm number [50]
CTNNB1	Catenin Beta 1	- Participates in the proliferation of primordial germ cells and their differentiation into female germ cells capable of meiosis [51] - CTNNB1 participates in the regulation of Sertoli cell activity in spermatogenic stem cells through WNT4 [52]

3.9. Molecular Docking Results

Protein Data Bank (PDB) (http://www.rcsb.org/) was used to collect structures of targets that were evaluated to explore the binding modes, including TP53 (PDB ID: 2PCX) , HSP90AA1 (PDB ID: 5NYI), AKT1 (PDB ID: 3O96), JUN (PDB ID: 1JNM), STAT3 (PDB ID: 6NJS) and five components Quercetin (CID: 52803343), Inermin (CID: 161298), Stigmasterol (CID: 5280794), Ginsenoside Rg5 (CID: 11550001). Protein structures were processed by adding hydrogen atoms, and the Sitemap module was used to explore and define binding sites. The ligand-docking module was used to simulate the molecular docking of the compounds and proteins, and the docking score was evaluated using the docking score function. The results of molecular docking of the top five core genes with these compounds are shown in Table 5. A score <5 points indicated that the compound had excellent docking activity with the target. Docking scores of quercetin with TP53 (−6.6), HSP90AA1 (−7.9), AKT1 (−9.7), JUN (-5.5) and STATA3 (−8) were the highest among the selected components. Supplement (+)-stigmasterol had the lowest docking energy scores at AKT1; Inermin had the lowest docking energy scores at HSP90AA1, whereas ginsenoside Rg5 had the lowest docking energy at STAT3 and HSP90AA1. The molecular docking results for the remaining active ingredients and target genes are shown in Figure 6.

Table 5. Molecular docking of top 5 core genes (TP53, HSP90AA1, AKT1, JUN, STAT3) with compounds

CID	Compound	Biding energy (kcal/mol)				
		TP53	HSP90AA1	AKT1	JUN	STAT3
		2PCX	5NYI	3O96	1JNM	6NJS
5742590	Alexandrin_qt					-7.8
5319581	Aposiopolamine			-8.6		
5312542	Arachidonate			7.2		
222284	Beta-sitosterol			-10.5		
442847	Celabenzine		-7.4			
285342	Deoxyharringtonine		-6.5			
441965	Frutinone A		-8.7			
11550001	Ginsenoside Rg5_qt		-7.3			-8.1
119307	Ginsenoside rh2		-6.6			-7.8
21599928	Ginsenoside-Rh4_qt		-7.9			-8
96943	Girinimbin			-11.0		
161298	Inermin		-9.3			
5280863	Kaempferol			-9.4	-5.4	
5280343	Quercetin	-6.6	-7.9	-9.7	-5.5	-8
5280794	Stigmasterol			-11.9		

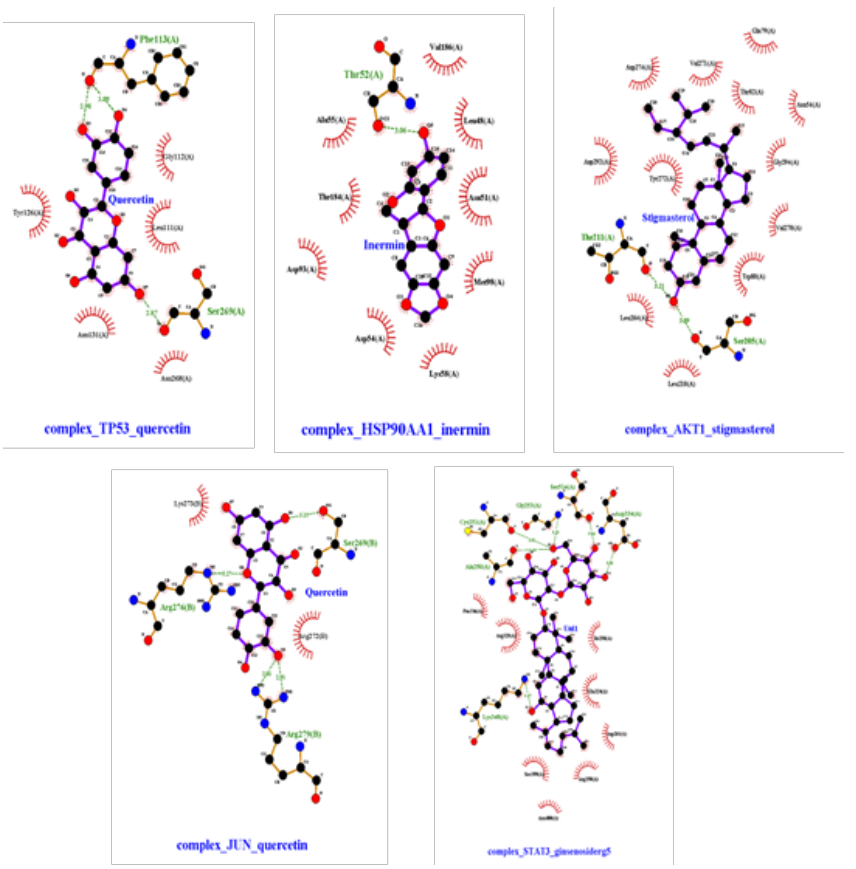

Figure 6. Ligplot+ results showing the interactions of most active compounds of top 5 core genes (TP53,

HSP90AA1, AKT1, JUN, STAT3) with compounds

4. DISCUSSION

The testes, the primary male reproductive organ, are located inside the scrotum and produce sperm cells as well as the primary male hormone testosterone [53]. Spermatogenesis is the process by which sperm cell production occurs and germ cells give rise to haploid spermatozoa. The complex process of spermatogenesis involves three steps [54]. The first step involves mitotic cell division. The second step involves meiosis, in which diploid cells form haploid cells. The final stage of spermatogenesis includes spermatozoa production and mature and motile sperm cells from round spermatids through a process called spermiogenesis. Diminished fertility or infertility may result from a decrease in sperm count, changes in morphology, or impaired motility [55]. Under severe conditions, complete absence of spermatozoa can lead to infertility.

The central concept of Network pharmacology is to uncover the mechanism of drug action and guide the design of drug molecules using multidimensional, multi-pathway, and multi-target points of the interaction network of genes, proteins, and metabolites [56]*=µN . With the rapid development of network pharmacology, the mechanisms of traditional Chinese medicine in the treatment of many serious diseases have been successfully predicted, and the multitarget integrated prevention and treatment approach has been applied to cancer [57], arthritis [58], diabetes, and other diseases with promising results [59].

The mechanisms of action of *Panax ginseng* in improving spermatogenesis are complex and involve multiple components and targets. When the pathogenesis of spermatogenesis is unclear, it becomes more difficult to analyze the mechanism of action of *Panax ginseng* [7] [11] [12] [8].

In the present study, *Panax ginseng* active component–target network analysis revealed that TP53, HSP90AA1, AKT1, JUN, STAT3, MAPK1, and other active ingredients can act on multiple targets in the network. This finding suggests that these components may be important for the therapeutic effect of *Panax ginseng* in improving spermatogenesis and warrant further exploration TP53 has the most potential targets, followed by AKT1.

As previously reported, TP53 is considered to add an extra degree of stringency to other spermatogenic "quality control" mechanisms, as they are essential genes in the apoptosis pathway. Moreover, TP53 contributes to the efficiency of DNA repair during the post-mitotic stages of spermatogenesis. If either the TP53 or MDM2 pathway is abnormal in its function, for example, some SNPs that affect their functions, infertility may occur. Loss of functional p53 protein leads to disruption of apoptosis. Cong Huang and others report that polymorphisms of TP53 may be associated with germ cell apoptosis and male infertility [60]. In particular, research observations may correlate with the p53 protein's function in aneuploidy in cancer, its involvement in spermatogenesis, and the occurrence of two cases of Turner syndrome in families where germline TP53 mutations have been documented in the literature [61].

To further explore the potential mechanism of *Panax ginseng* in improving spermatogenesis, we conducted a KEGG analysis of 250 potential targets. Looking at the pathways in the testes, we found that the PI3K/AKT1 signaling pathway is also involved in spermatogenesis. This is consistent with other research targeting this pathway, which may be able to control oxidative stress, apoptosis, and sperm motility [62]. Several studies have reported that Akt plays a major role in the expansion and maturation of testicular tissue, and is essential for controlling cell division, growth, and apoptosis [63]. Akt is a serine/threonine protein kinase activated by insulin, and various growth factors are critical to many cellular processes, such as glucose metabolism, transcription, cell proliferation, apoptosis, migration, cell growth, and tumorigenesis. In vitro fertilization (IVF) can improve sperm characteristics, fertilization, and the success of the embryo/pregnancy process by directly stimulating the Akt pathway or by improving insulin sensitivity and glucose absorption in male diabetics [64].

To learn more about the possible molecular pathway through which *Panax ginseng* encourages spermatogenesis, we conducted molecular docking studies of five targets closely related according to KEGG-based screening, using the experimentally validated key components of quercetin ligands. Quercetin has garnered much interest because of its antibacterial, anticancer, anti-inflammatory, antioxidant, and neuroprotective properties. Sertoli cells play crucial roles in spermatogenesis, providing support, nutrition, and protection, as the only cells in direct contact with germ cells [65]. Ranawat reported that quercetin exhibits pro-oxidant and antioxidant characteristics in human and rodent biological systems [66]. According to cell experiment

results, quercetin can lower expression levels in Sertoli cells, preserve cell structure, and stop the reduction in cell viability caused by heat stress [65]. In the present study, quercetin and its ability to treat spermatogenic disorders were investigated using network pharmacology to identify signals and their targets.

This study has certain limitations. We only examined the network pharmacology level of *Panax ginseng's* impact on spermatogenesis. However, there is still room for improvement in the accuracy of database data and real-time updates, and present network information technology is not all-inclusive. As a result, pharmacodynamic validation of the current study's findings is necessary, and mechanistic investigations are required to clarify the intricate multitarget, multipathway, and synergistic interactions.

5. CONCLUSION

The results showed that 25 bioactive compounds of *Panax ginseng* and 2220 corresponding targets significantly contributed to improving spermatogenesis through complex-related signaling pathways and biological processes, such as pathways related to cancer, the p53 signaling pathway, the MAPK signaling pathway, and the PI3K/AKT1 signaling pathway. In addition, molecular docking effectively verified the binding behavior of the top five core targets and their corresponding bioactive substances.

Funding

This study was supported by The Vietnamese Ministry of Education and Training's Research Projects in Science and Technology (Grant number B2023-DHH-11).

Acknowledgments

The authors also acknowledge the partially supported by Hue University, Vietnam, under the Core Research Program, Research Group on Regenerative Medicine (Research Group on Regenerative Medicine, NCTB. DHH.2024.02).

REFERENCE

1. Schlegel, P.N., *Human Spermatogenesis: Insights From the Clinical Care of Men With Infertility.* Frontiers in Endocrinology, 2022. **13**.
2. Roosen-Runge, E.C., *The process of spermatogenesis in animals*. Vol. 5. 1977: CUP Archive.
3. Rabbani, M., et al., *Decoding the Spermatogenesis Program: New Insights from Transcriptomic Analyses.* Annual Review of Genetics, 2022. **56**: p. 339-368.
4. Tran, M.N. and S. Lee, *The Molecular Mechanisms of Panax ginseng in Treating Type 2 Diabetes Mellitus: Network Pharmacology Analysis and Molecular Docking Validation.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**: p. 3082109.
5. Zheng, G.-q., et al., *Ginseng total saponins enhance neurogenesis after focal cerebral ischemia.* Journal of ethnopharmacology, 2011. **133**(2): p. 724-728.
6. Kopalli, S.R., et al., *Korean Red Ginseng (Panax ginseng Meyer) with enriched Rg3 ameliorates chronic intermittent heat stress–induced testicular damage in rats via multifunctional approach.* Journal of Ginseng Research, 2019. **43**(1): p. 135-142.
7. Leung, K.W. and A.S. Wong, *Ginseng and male reproductive function.* Spermatogenesis, 2013. **3**(3): p. e26391.
8. Kopalli, S.R., et al., *Korean red ginseng extract rejuvenates testicular ineffectiveness and sperm maturation process in aged rats by regulating redox proteins and oxidative defense mechanisms.* Experimental gerontology, 2015. **69**: p. 94-102.
9. Park, J.S., et al., *The therapeutic effect of tissue cultured root of wild Panax ginseng CA Mayer on spermatogenetic disorder.* Archives of pharmacal research, 2006. **29**: p. 800-807.
10. Kim, M.K., et al., *Microbial conversion of major ginsenoside $ Rb_1 $ to pharmaceutically active minor ginsenoside Rd.* Journal of Microbiology, 2005. **43**(5): p. 456-462.
11. Park, W.S., et al., *Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM).* Fertility and sterility, 2007. **88**(4): p. 1000-1002.
12. MacKay, D., *Nutrients and botanicals for erectile dysfunction: examining the evidence.* Alternative Medicine Review, 2004. **9**(1).
13. Szklarczyk, D., et al., *The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets.* Nucleic acids research, 2021. **49**(D1): p. D605-D612.
14. Shannon, P., et al., *Cytoscape: a software environment for integrated models of biomolecular interaction networks.* Genome research, 2003. **13**(11): p. 2498-2504.
15. Chin, C.-H., et al., *cytoHubba: identifying hub objects and sub-networks from complex interactome.* BMC systems biology, 2014. **8**: p. 1-7.
16. Huang, D.W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists*

using DAVID bioinformatics resources. Nature protocols, 2009. **4**(1): p. 44-57.

17. Roncaglia, P., et al., *The Gene Ontology (GO) cellular component ontology: integration with SAO (Subcellular Anatomy Ontology) and other recent developments.* Journal of biomedical semantics, 2013. **4**: p. 1-11.

18. DeLano, W.L., *Pymol: An open-source molecular graphics tool.* CCP4 Newsl. Protein Crystallogr, 2002. **40**(1): p. 82-92.

19. Trott, O. and A.J. Olson, *AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading.* Journal of computational chemistry, 2010. **31**(2): p. 455-461.

20. Wallace, A.C., R.A. Laskowski, and J.M. Thornton, *LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions.* Protein engineering, design and selection, 1995. **8**(2): p. 127-134.

21. Tran, M.N. and S. Lee, *The molecular mechanisms of panax ginseng in treating type 2 diabetes mellitus: Network pharmacology analysis and molecular docking validation.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**.

22. Jia, A., L. Xu, and Y. Wang, *Venn diagrams in bioinformatics.* Briefings in bioinformatics, 2021. **22**(5): p. bbab108.

23. Beumer, T.L., et al., *The role of the tumor suppressor p53 in spermatogenesis.* Cell Death Differ, 1998. **5**(8): p. 669-77.

24. Ebrahim Abadi, Z., et al., *The frequency of TP53 R72P and MDM2 309T>G polymorphisms in Iranian infertile men with spermatogenetic failure: A case-control study.* Int J Reprod Biomed, 2018. **16**(8): p. 491-496.

25. Napoletano, F., et al., *p53-dependent programmed necrosis controls germ cell homeostasis during spermatogenesis.* PLoS Genet, 2017. **13**(9): p. e1007024.

26. Gao, X., et al., *The REGgamma-Proteasome Regulates Spermatogenesis Partially by P53-PLZF Signaling.* Stem Cell Reports, 2019. **13**(3): p. 559-571.

27. Tang, X., et al., *Heat shock protein-90alpha (Hsp90alpha) stabilizes hypoxia-inducible factor-1alpha (HIF-1alpha) in support of spermatogenesis and tumorigenesis.* Cancer Gene Ther, 2021. **28**(9): p. 1058-1070.

28. Jha, K.N., et al., *Heat shock protein 90 functions to stabilize and activate the testis-specific serine/threonine kinases, a family of kinases essential for male fertility.* J Biol Chem, 2013. **288**(23): p. 16308-16320.

29. Zhou, L.L., et al., *[Heat shock protein 90 in male reproduction].* Zhonghua Nan Ke Xue, 2021. **27**(4): p. 351-355.

30. Chen, K.Q., et al., *The PI3K/AKT signaling pathway: How does it regulate development of Sertoli cells and spermatogenic cells?* Histol Histopathol, 2022. **37**(7): p. 621-636.

31. Deng, C.Y., et al., *The Role of the PI3K/AKT/mTOR Signalling Pathway in Male Reproduction.* Curr Mol Med, 2021. **21**(7): p. 539-548.

32. Zhu, Z., et al., *Dynamics of the Transcriptome during Human Spermatogenesis: Predicting the Potential Key Genes Regulating Male Gametes Generation.* Sci Rep, 2016. **6**: p. 19069.

33. Nagasawa, K., et al., *Regionally distinct patterns of STAT3 phosphorylation in the seminiferous epithelia of mouse testes.* Mol Reprod Dev, 2018. **85**(3): p. 262-270.

34. Chen, X., et al., *Cyclin-dependent kinase 7 is essential for spermatogenesis by regulating retinoic acid signaling pathways and the STAT3 molecular pathway.* IUBMB Life, 2021. **73**(12): p. 1446-1459.

35. Oatley, J.M., et al., *Regulation of mouse spermatogonial stem cell differentiation by STAT3 signaling.* Biol Reprod, 2010. **83**(3): p. 427-33.

36. Li, M.W., D.D. Mruk, and C.Y. Cheng, *Mitogen-activated protein kinases in male reproductive function.* Trends Mol Med, 2009. **15**(4): p. 159-68.

37. Ni, F.-D., S.-L. Hao, and W.-X. Yang, *Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis.* Cell Death & Disease, 2019. **10**(8): p. 541.

38. Xiao, X., et al., *c-Src and c-Yes are two unlikely partners of spermatogenesis and their roles in blood-testis barrier dynamics.* Biology and Regulation of Blood-Tissue Barriers, 2013: p. 295-317.

39. Braydich-Stolle, L., et al., *Role of Src family kinases and N-Myc in spermatogonial stem cell proliferation.* Developmental biology, 2007. **304**(1): p. 34-45.

40. Oatley, J.M., M.R. Avarbock, and R.L. Brinster, *Glial cell line-derived neurotrophic factor regulation of genes essential for self-renewal of mouse spermatogonial stem cells is dependent on Src family kinase signaling.* Journal of Biological Chemistry, 2007. **282**(35): p. 25842-25851.

41. Omisanjo, O.A., et al., *DNMT1 and HDAC1 gene expression in impaired spermatogenesis and testicular cancer.* Histochemistry and cell biology, 2007. **127**: p. 175-181.

42. Wang, L. and D.J. Wolgemuth, *BET protein BRDT complexes with HDAC1, PRMT5, and TRIM28 and functions in transcriptional repression during spermatogenesis.* Journal of cellular biochemistry, 2016. **117**(6): p. 1429-1438.

43. Choi, E., et al., *A novel germ cell-specific protein, SHIP1, forms a complex with chromatin remodeling activity during spermatogenesis.* Journal of Biological Chemistry, 2008. **283**(50): p. 35283-35294.

44. Carreau, S. and R.A. Hess, *Oestrogens and spermatogenesis.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2010. **365**(1546): p. 1517-1535.

45. Cooke, P.S. and W.H. Walker. *Nonclassical androgen and estrogen signaling is essential for normal spermatogenesis*. in *Seminars in Cell & Developmental Biology*. 2022. Elsevier.

46. Morini, M., et al., *The expression of nuclear and membrane estrogen receptors in the European eel throughout spermatogenesis.* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2017. **203**: p. 91-99.

47. Hess, R.A., *Disruption of estrogen receptor signaling and similar pathways in the efferent ductules and initial segment of the epididymis.* Spermatogenesis, 2014. **4**(2): p. e979103.

48. Lord, T., et al., *A novel high throughput screen to identify candidate molecular networks that regulate spermatogenic stem cell functions.* Biology of Reproduction, 2022. **106**(6): p. 1175-1190.

49. Sassone-Corsi, P., *Coupling gene expression to cAMP signalling: role of CREB and CREM.* The international journal of biochemistry & cell biology, 1998. **30**(1): p. 27-38.

50. Wang, H., et al., *Functional role of GKAP1 in the regulation of male germ cell spontaneous apoptosis and sperm number.* Molecular Reproduction and Development, 2019. **86**(9): p. 1199-1209.

51. Chassot, A.-A., et al., *Constitutive WNT/CTNNB1 activation triggers spermatogonial stem cell proliferation and germ cell depletion.* Developmental biology, 2017. **426**(1): p. 17-27.

52. Boyer, A., et al., *CTNNB1 signaling in sertoli cells downregulates spermatogonial stem cell activity via WNT4.* PloS one, 2012. **7**(1): p. e29764.

53. Suede, S.H., A. Malik, and A. Sapra, *Histology, spermatogenesis.* 2020.

54. Nishimura, H. and S.W. L'Hernault, *Spermatogenesis.* Current Biology, 2017. **27**(18): p. R988-R994.

55. Holstein, A.-F., W. Schulze, and M. Davidoff, *Understanding spermatogenesis is a prerequisite for treatment.* Reproductive Biology and Endocrinology, 2003. **1**(1): p. 1-16.

56. Shao-jie, H., et al., *Potential mechanism study of herbal pair Schizonepetae herba and Saposhnikoviae radix against coronavirus pneumonia via network pharmacology and molecular docking.* Natural Product Research and Development, 2020. **32**(7): p. 1087.

57. Lee, H.-S., et al., *A network pharmacology study on the molecular mechanisms of FDY003 for breast cancer treatment.* Evidence-based Complementary and Alternative Medicine, 2021. **2021**.

58. Xie, B., et al., *Targets of hydroxychloroquine in the treatment of rheumatoid arthritis. A network pharmacology study.* Joint Bone Spine, 2021. **88**(2): p. 105099.

59. Zhou, J., et al., *Network pharmacology analysis of traditional Chinese medicine formula Xiao Ke Yin Shui treating type 2 diabetes mellitus.* Evidence-based Complementary and Alternative Medicine, 2019. **2019**.

60. Huang, C., et al., *Genetic variants in TP53 and MDM2 associated with male infertility in Chinese population.* Asian journal of andrology, 2012. **14**(5): p. 691.

61. Zhang, X., et al., *Melatonin protects spermatogonia from the stress of chemotherapy and oxidation via eliminating reactive oxidative species.* Free Radical Biology and Medicine, 2019. **137**: p. 74-86.

62. Huang, W., et al., *Aflatoxin B1 promotes autophagy associated with oxidative stress-related PI3K/AKT/mTOR signaling pathway in mice testis.* Environmental Pollution, 2019. **255**: p. 113317.

63. Huang, P., 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, 2016. **14**(6): p. 5116-5124.

64. Kim, S.T., K. Omurtag, and K.H. Moley, *Decreased spermatogenesis, fertility, and altered Slc2A expression in Akt1−/− and Akt2−/− testes and sperm.* Reproductive Sciences, 2012. **19**(1): p. 31-42.

65. Liu, D.-L., et al., *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, 2022. **23**(19): p. 11163.

66. Ranawat, P., C.M. Pathak, and K.L. Khanduja, *A new perspective on the quercetin paradox in male reproductive dysfunction.* Phytotherapy Research, 2013. **27**(6): p. 802-810.