ISSN 2706-6819 Vol.7, Issue 2: 18-29, DOI: 10.25236/IJFM.2025.070204

Exploring the Patterns and Mechanisms of Treatment for Male Infertility by the Famous Lingnan Physician Feng Chonglian Based on Data Mining and Network Pharmacology

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Abstract: *Based on clinical information system,network pharmacology and data mining technology were used to explore the drug use rules and potential mechanism of Guangdong famous doctor of traditional Chinese medicine Feng Chonglian in the treatment of male infertility. Cases diagnosed as "male infertility" or "infertility" in the hospital information systerm (HIS) from January 2020 to December 2022 were extracted and imported into Excel database for analysis by data mining and network pharmacology. The study found that 2,531 male infertility patients received treatment with Chinese herbal slices,using 299 kinds of Chinese herbs, among which 36 were high-frequency herbs.Cluster analysis identified five effective clusters; association analysis yielded 13 association rules,ultimately determining "dioscoreae rhizoma,moutan cortex,poria cocos,alismatis rhizoma,ligustri lucidi fructus" as the core prescription for treating male infertility.This prescription contains 62 active ingredients,involves 181 gene targets, 1,385 disease targets,and 99 intersection targets between the core prescription and the disease.Through PPI network analysis,GO analysis,and KEGG pathway enrichment analysis,it was concluded that quercetin,kaempferol and luteolin might be the key active ingredients for the treatment of male infertility. Guangdong famous doctor of traditional Chinese medicine Feng Chonglian in the treatment of male infertility to tonifying kidney and essence, invigorating spleen and liver,promoting blood circulation as the main ideas,according to different symptoms of patients to carry out syndrome differentiation treatment,the use of Liuwei Dihuang pill as the basic prescription clinical addition and reduction effect is accurate,rational, there is evidence to follow.*

Keywords: *Infertility, Network pharmacology, data mining, Medication rule, Liuwei Dihuang pill*

1. Introduction

Male infertility refers to the condition where a pregnant couple has not used any contraceptive measures and the female partner has not become pregnant for more than one year due to male factors. The World Health Organization has identified infertility as the third leading disease of the 21st century, second only to tumors and cardiovascular diseases.Approximately 8% -12% of couples of childbearing age worldwide suffer from infertility,with male factors accounting for about half of the cases[1].

There are no names for "oligospermia" and "asthenozoospermia" in ancient Chinese medicine literature, and infertility is also known as "infertility" or "lack of offspring" in Chinese medicine literature. Oligozoospermia is related to the traditional Chinese medicine concepts of "oligospermia", "clarity of essence",and "thinness of essence",while asthenozoospermia is related to the traditional

ISSN 2706-6819 Vol.7, Issue 2: 18-29, DOI: 10.25236/IJFM.2025.070204

Chinese medicine concepts of "coldness of essence" and "coldness of essence"[2]. In recent years, traditional Chinese medicine has achieved good clinical efficacy by starting with methods such as tonifying the kidney and nourishing essence,strengthening the spleen and soothing the liver,and promoting blood circulation and unblocking collaterals.Basic research on the effective ingredients and mechanisms of traditional Chinese medicine is also increasing[3],but clinical research is not deep enough and the understanding of its mechanisms is not yet complete.

Professor Feng Chonglian is currently the director of the Department of Traditional Chinese Medicine at the Third Affiliated Hospital of Guangzhou Medical University.He has been awarded titles such as Guangdong Province Famous Traditional Chinese Medicine,the third batch of National Excellent Clinical Talents in Traditional Chinese Medicine,and the second batch of Guangdong Province Famous Traditional Chinese Medicine Master Project Guidance Teacher.I have studied under Chinese medical masters Professor Deng Tietao and Professor Sun Guangrong for more than 30 years,with extensive knowledge and rigorous treatment.I am skilled in traditional Chinese medicine diagnosis and treatment of male,gynecological,spleen and stomach,liver diseases,etc.Therefore,based on data mining and network pharmacology,this study uses statistical methods to summarize the experience of the Guangdong Famous Traditional Chinese Medicine Studio team, providing a good reference for the inheritance of traditional Chinese medicine.

2. Information and Methods

2.1. Prescription Collection

The data used in this study were all sourced from the Hospital Information System (HIS) of the Third Affiliated Hospital of Guangzhou Medical University from January 2020 to December 2022. The included cases were all male infertility or sterility diagnosed based on the main disease and syndrome, and treated with traditional Chinese medicine decoction pieces. Two individuals alternately audited a total of 2531 valid prescriptions for traditional Chinese medicine decoction pieces.

2.2. Case Collection

2.2.1. Inclusion Criteria

(1)Simultaneously meeting the diagnostic criteria for male infertility in the "Guidelines for Multidisciplinary Diagnosis and Treatment of Male Infertility with Integrated Traditional Chinese and Western Medicine" (2023 edition)[4] and oligoasthenozoospermia in the WHO "Laboratory Test Manual for Human Semen and Sperm Cervical Mucus Interaction" 5th edition[5]; (2)Complete medical records; (3)First visit prescription of traditional Chinese medicine;(4)The names, dosages, and usage of traditional Chinese medicine prescriptions are accurately recorded.

2.2.2. Exclusion Criteria

(1)Infertility caused by female reasons;(2)Accompanied by other diseases that affect semen quality; (3) Severe sexual dysfunction;(4)Suffering from mental illness;(5)Taking medication or coming into contact with objects that affect sperm production function; (6)The information is incomplete.

2.3. Data Standardisation

Using the HIS system to collect patient related information, import it into Excel to establish a database, and standardize the names of traditional Chinese medicine decoction pieces according to the "Pharmacopoeia of the People's Republic of China" (2020 edition)[4], "Clinical Medication Guidelines of the Pharmacopoeia of the People's Republic of China" (2015 edition)[5],and the "Twelfth Five Year Plan" textbook "Traditional Chinese Medicine"[6].For example,"salt morindae officinalis" is collectively referred to as "morindae officinalis radix",while "pinelliae rhizoma praeparatum","pinelliae rhizoma praeparatum cum zingibere et alumine","pinelliae rhizoma praeparatum cum alumine" are collectively referred to as "pinelliae rhizoma",etc; sort and classify the four nature,five flavors, meridian tropism and efficacy of drugs.

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2.4. Data Entry

The data entry work is carried out under a system where two individuals alternate audit responsibilities. The 2531 valid cases were entered into the Microsoft Office Excel standardised male infertility case database,alternately reviewed and corrected by two individuals to ensure data source accuracy reliable.

2.5. Data Analysis

Using Microsoft Office Excel 2010 and OriginPro 2021 software,descriptive statistics were conducted on the frequency,nature,flavor,meridian tropism of drugs,and the resulting high-frequency drug clustering analysis was performed;Using the Apriori algorithm of IBM SPSS Modeler 18.0 software and other tools,effective association rule analysis is achieved,and a network diagram of high-frequency drug associations is established to summarize and obtain core prescriptions.

2.6. Network Pharmacology Analysis

By applying TCMSP[7] to identify the active ingredients of core prescription Chinese medicine, screening was conducted under the conditions of oral bioavailability (OB)≥30% and drug likeness (DL)≥0.18 to obtain the main active ingredients and related target genes.Using the keyword "male infertility", search the GeneCards database to identify the target genes for male infertility,and standardize the target genes for male infertility using the Uniprot database.By using Venny2.1 software to draw Venny diagrams and taking the intersection,potential targets for the core prescription treatment of male infertility can be obtained.The screened drug disease common target proteins were analyzed using the STRING platform for PPI network analysis.Cytoscape 3.7.2[8] and MetScape[9] were used to explore the functions and metabolic pathways of target genes related to core prescription therapy for male infertility.GO function (Gene ontology) and KEGG metabolic pathway enrichment analysis were performed.Finally,the "disease component target pathway" network diagram was established using Cytoscape 3.7.2 software.

3. Results

3.1. General Information of the Case

This study ultimately included 2531 male infertility patients who received treatment with traditional Chinese medicine decoction pieces,including 320 male patients (12.64%) aged≤30 years old;1749 male patients (69.10%) aged between 30 and 40 years old;419 male patients (16.55%) aged between 40 and 50 years old; There were 43 male patients (1.70%) aged over 50 years old, with an average age of 36 years.

3.2. Clinical Prescription Statistics

3.2.1. Drug Frequency Analysis

This study involved 299 types of traditional Chinese medicine decoction pieces,with 36 high-frequency drugs (frequency≥10%) included in the analysis,and the remaining 187 drugs used with a frequency≤2%,as shown in Table 1.According to frequency of use and ranking,the top 10 drugs are:poria cocos,moutan cortex,salviae miltiorrhizae radix et rhizoma,alismatis rhizoma,epimedii fplium, dioscoreae rhizoma,morindae officinalis radix,licorice,ligustri lucidi fructus and rehmannia radix.

Table 1: Frequency analysis of medication administration (frequency ≥ 10%)

No.	Medicine	Frequency	Percent (%)	No.	Medicine	Frequency	Percent (%)
1	poria cocos	1719	67.92	19	polygonati rhizoma	490	19.36
2	moutan cortex	1139	45.00	20	cinnamomi ramulus	484	19.12
3	salviae miltiorrhizae radix et rhizoma	1032	40.77	21	cynomorii herba	484	19.12
4	alismatis rhizoma	1019	40.26	22	curculiginis rhizoma	469	18.53

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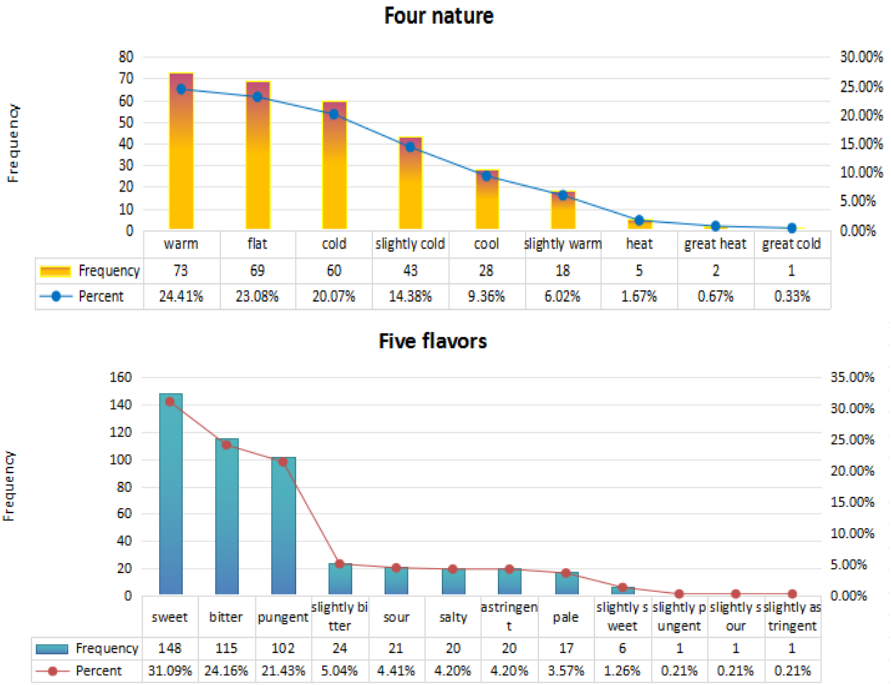
5	epimedii fplium	983	38.84	23	dioscoreae hypoglaucae rhizoma	446	17.62
6	dioscoreae rhizoma	922	36.43	24	astragali complanati semen	439	17.34
7	morindae officinalis radix	870	34.37	25	notoginseng radix et rhizoma	432	17.07
8	licorice	826	32.64	26	hordei fructus germinatus	426	16.83
9	ligustri lucidi fructus	705	27.85	27	astragali radix	424	16.75
10	rehmannia radix	697	27.54	28	phellodendri chinensis cortex	423	16.71
11	cuscutae semen	660	26.08	29	curcumae radix	396	15.65
12	rhodiolae crenulatae radix et rhizoma	646	25.52	30	bupleuri radix	342	13.51
13	lonicerae japonicae caulis	626	24.73	31	anemarrhenae rhizoma	337	13.31
14	achyranthis bidentatae radix	616	24.34	32	plantaginis semen	323	12.76
15	atractylodia macrocephalae rhizoma	615	24.30	33	gardeniae fructus	277	10.94
16	liquidambaris fructus	615	24.30	34	amomi fructus	261	10.31
17	curcumae rhizoma	600	23.71	35	rosae laevigatae fructus	259	10.23
18	eucommiae cortex	588	23.23	36	junci medulla	255	10.08

3.2.2. Analysis of the Four Qi, Five Flavors, and Meridian Returning Characteristics of Drugs

Using Microsoft Office Excel 2010 software,a statistical analysis was conducted on the characteristics of the four nature,five flavors,and meridian tropism of 299 drugs.The results showed that the main Chinese medicinal properties for treating male infertility in traditional Chinese medicine were warm (24.41%),flat (23.08%) and cold (20.07%);The medicinal flavors are mainly sweet(31.09%), bitter (24.16%) and pungent (21.43%);The main meridian is the liver meridian (21.58%),followed by the lung meridian (15.94%),stomach meridian (13.68%),spleen meridian (12.55%) and kidney meridian (12.55%). The frequency of drug use in the pericardium meridian and the Sanjiao meridian is ≤ 1%,as shown in Figure 1.



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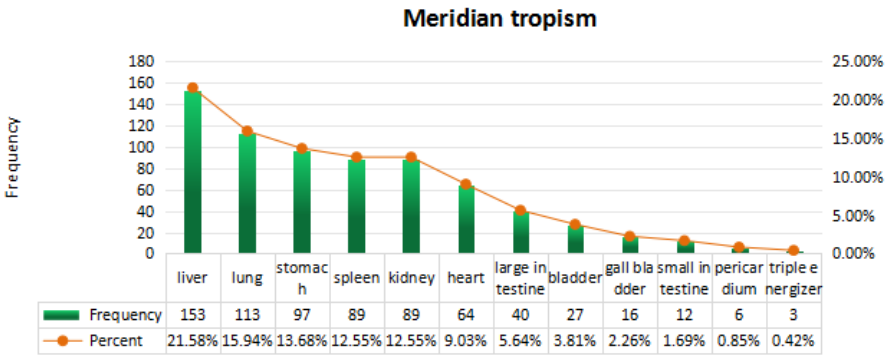

Figure1: Chart of traditional Chinese medicine flavor normalization

3.2.3. Statistical Analysis of Drug Efficacy Classification

The statistical results of drug efficacy classification show that the top 5 drugs used in the frequency of traditional Chinese medicine treatment for male infertility were,in order:tonifying yang drugs (16.03%), damp-draining diuretic drugs (15.17%),tonifying qi drugs(12.05%),blood-activating and stasis-resolving drugs (10.39%),and heat-clearing and blood-cooling drugs (8.23%).

The most commonly used types of traditional Chinese medicine were heat-clearing and detoxifying drugs (31 types),followed by blood-activating and stasis-resolving drugs (21 types), tonifying yang drugs(19 types),damp-draining diuretic drugs (19 types),and wind-damp-dispelling drugs (19 types).There were not found cathartic drugs,laxative drugs,blood circulation pain drugs and externally applied drugs.

According to the classification and statistics of the efficacy,requency, and frequency of 36 high-frequency drugs,the results showed that the use of tonifying yang drugs in the treatment of male infertility in traditional Chinese medicine was the highest,followed by damp-draining diuretic drugs, tonifying qi drugs,blood-activating and stasis-resolving drugs,and heat-clearing and blood-cooling drugs.Among the high-frequency drugs,there were no wind-damp-dispelling drugs,phle-gmresolving drugs and qi regulating drugs.See Table 2.

Table 2: Classification and statistical analysis of high-frequency drug efficacy

Function classification	Frequency	Percent(%)
tonifying yang drugs	4493	13.87
damp-draining diuretic drugs	4377	13.51
tonifying qi drugs	3433	10.60
blood-activating and stasis-resolving drugs	2644	8.16
heat-clearing and blood-cooling drugs	2173	6.71
tonifying yin drugs	1195	3.69
heat-clearing and detoxifying drugs	903	2.79
xin wen relieving drugs	484	1.49
hemostasis drugs	432	1.33
resolving food stagnancy drugs	426	1.31
heat-clearing and dampness-drying drugs	423	1.31
cool acrid exterior-resolving drugs	342	1.06
damp -resolving drugs	261	0.81
astringent drugs	259	0.80

3.3. Cluster Analysis And Association Rule Analysis

3.3.1. Cluster Analysis Of High Frequency Drugs

Use the Hierarchical clustering algorithm in OriginPro 2021 software to perform cluster analysis on 36 high-frequency drugs,set the number of clusters to 5,and draw relevant circular graphs as shown in Figure 2. According to the clustering analysis results,a total of 5 effective clustering groups were identified. They are Class1:poria cocos,rhodiolae crenulatae radix et rhizoma,moutan cortex,alismatis rhizoma, dioscoreae rhizoma,ligustri lucidi fructus,rehmannia radix,curcumae radix,junci medulla, curc-uliginis rhizoma,epimedii fplium,cynomorii herba,cinnamomi ramulus,morindae officinalis radix; Class2:bupleuri radix,gardeniae fructus,phellodendri chinensis cortex,anemarrhenae rhizoma,salviae

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miltiorrhizae radix et rhizoma, curcumae rhizoma; Class3: amomi fructus;Class4:atractylodia macrocephalae rhizoma,astragali radix,dioscoreae hypoglaucae rhizoma,licorice,liquidambaris fructus, lonicerae japonicae caulis,achyranthis bidentatae radix,polygonati rhizoma,hordei fructus germinatus, notoginseng radix et rhizoma,plantaginis semen;Class5:cuscutae semen,astragali complanati semen, rosae laevigatae fructus,rosae laevigatae fructus.

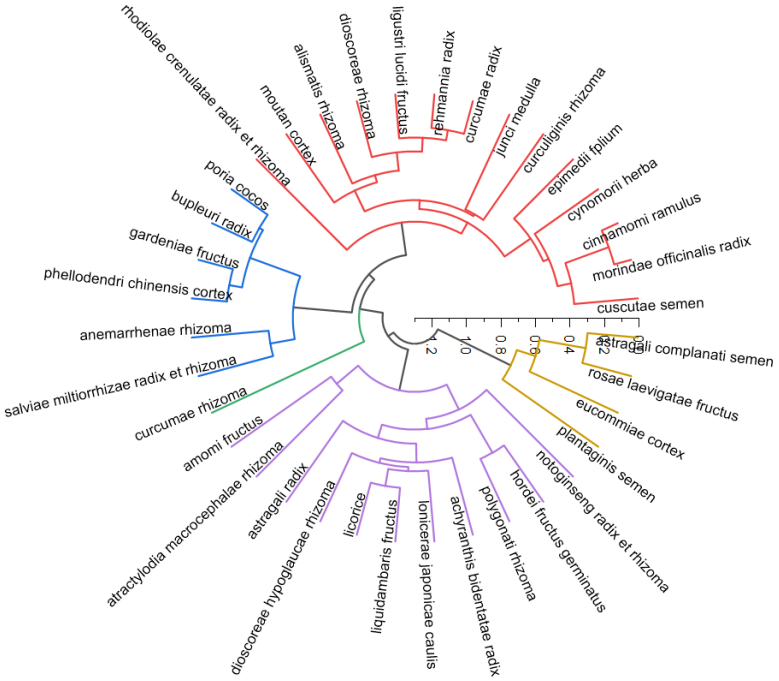

Figure2:Cluster analysis diagram of high frequency drugs

3.3.2. Analysis of Association Rules for High Frequency Drugs

Association rules can not only explore high-frequency drug pairs and commonly used drug combinations in traditional Chinese medicine, discover compatibility rules, but also provide a basis for clinical rational drug use and new drug development. Use the Apriori algorithm of IBM SPSS Modeler 18.0 software to analyze the association rules of 36 high-frequency drugs. Setting the maximum number of leading terms to 2, the minimum confidence level to 90.00%, and the minimum support level to 27.80%, a total of 13 association rules were obtained. Among them, the combination with the highest confidence level was dioscoreae rhizoma,moutan cortex and poria cocos (99.72%), sorted by support percentage. The results are shown in Table 3.

Table 3: High-frequency drug association rule analysis

No.	Consequent	Antecedent	Instances	Degree of support(%)	Confidence level(%)
1	poria cocos	moutan cortex	1139	45.00	96.14
2	poria cocos	alismatis rhizoma	1019	40.26	95.49
3	poria cocos	alismatis rhizoma-moutan cortex	810	32.00	98.40
4	alismatis rhizoma	dioscoreae rhizoma-poria cocos	794	31.37	93.20
5	moutan cortex	dioscoreae rhizoma-alismatis rhizoma	747	29.51	92.24
6	poria cocos	dioscoreae rhizoma-alismatis rhizoma	747	29.51	99.06
7	ligustri lucidi fructus	dioscoreae rhizoma-moutan cortex	707	27.93	91.80
8	alismatis rhizoma	dioscoreae rhizoma-moutan cortex	707	27.93	97.45
9	poria cocos	dioscoreae rhizoma-moutan cortex	707	27.93	99.72
10	dioscoreae rhizoma	ligustri lucidi fructus	705	27.86	93.62
11	alismatis rhizoma	ligustri lucidi fructus	705	27.86	94.04
12	moutan cortex	ligustri lucidi fructus	705	27.86	94.04
13	poria cocos	ligustri lucidi fructus	705	27.86	96.17

Establish a network diagram of associations between 36 high-frequency drugs using web nodes in IBM SPSS Modeler 18.0 software,as shown in Figure 3.The thicker the line, the stronger the

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connection. Therefore,the core prescription for treating male infertility with traditional Chinese medicine is "dioscoreae rhizoma,moutan cortex,poria cocos,alismatis rhizoma,ligustri lucidi fructus", which is a modified formula of Liuwei Dihuang Pill.The treatment is mainly aimed at nourishing yin and tonifying the kidneys," strengthening water to control sunshine".

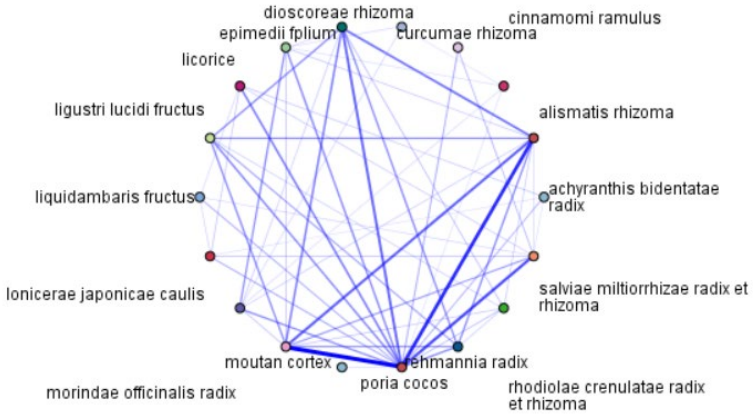

Figure3: High frequency drug association network

3.4. Network Pharmacological Analysis

3.4.1. Core Prescription Main Active Ingredients And Disease Target Screening

By querying the TCMSP database, a total of 62 main active ingredients and 181 gene targets were obtained (excluding duplicate components and targets between drugs).Among them, dioscoreae rhizoma contains 16 active ingredients and 103 gene targets;moutan cortex contains 11 active ingredients and 190 gene targets;poria cocos contains 15 active ingredients and 21 gene targets; alismatis rhizoma contains 10 active ingredients and 9 gene targets;ligustri lucidi fructus contains 13 active ingredients and 279 gene targets.A total of 1385 targets for male infertility were obtained through the GeneCards database (5931 targets were obtained,and 1385 disease targets were obtained by taking the median of two measurements).Using the Venny2.1 auxiliary platform, a total of 99 "core prescription disease" intersection targets were obtained by calculating 181 gene targets in the core prescription and 1385 action targets in male infertility.The specific analysis results are shown in Table 4 and Figure 4.

Table 4: 99 "core prescription-disease" intersection targets

No.	drug	No.	drug	No.	drug	No.	drug
1	PGR	26	VCAM1	51	IL6	76	CHEK2
2	NCOA2	27	CYP1B1	52	TP53	77	PPARA
3	PTGS1	28	GSTP1	53	NFKBIA	78	CRP
4	SCN5A	29	AHR	54	POR	79	CXCL10
5	PTGS2	30	INSR	55	CASP8	80	SPP1
6	NOS2	31	GSTM1	56	RAF1	81	CTSD
7	AR	32	AKR1C3	57	HIF1A	82	IGFBP3
8	ACHE	33	SLPI	58	ERBB2	83	IGF2
9	RELA	34	ESR1	59	CAV1	84	CD40LG
10	AKT1	35	ADRB2	60	MYC	85	PON1
11	BCL2	36	MMP3	61	F3	86	CAT
12	BAX	37	EGFR	62	GJA1	87	SLC6A4
13	TNF	38	VEGFA	63	IL1B	88	APOB
14	JUN	39	CCND1	64	CCL2	89	CCNA2
15	CASP3	40	BCL2L1	65	CXCL8	90	MDM2
16	XDH	41	FOS	66	BIRC5	91	PCNA
17	MMP1	42	CDKN1A	67	NOS3	92	TYR
18	STAT1	43	CASP9	68	HSPB1	93	IL4
19	PPARG	44	PLAU	69	IL2	94	MET
20	HMOX1	45	MMP2	70	PLAT	95	SLC6A3
21	CYP3A4	46	MMP9	71	SERPINE1	96	ESR2
22	CYP1A2	47	MAPK1	72	IFNG	97	ADRB1
23	CYP1A1	48	IL10	73	IL1A	98	NR3C1
24	ICAM1	49	EGF	74	MPO	99	MTOR
25	SELE	50	RB1	75	NFE2L2		

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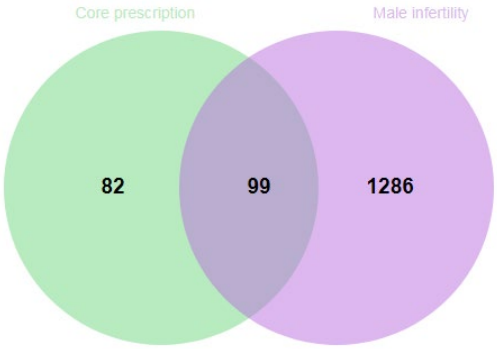

Figure 4: Disease-Core prescription target Wayne diagrams

3.4.2. Protein Interaction Network Construction

Importing 99 common targets of "Core prescription - Male infertility" through the STRING platform and constructing a PPI network diagram, as shown in Figure 5.The results showed that a total of 99 nodes were obtained,representing target proteins with 871 edges between them.The average node degree value was 17.6,and the average local clustering coefficient was 0.585.If there are more edges and darker colors between two nodes in the graph,it indicates that the correlation between the two nodes is greater[8].By analyzing the data in descending order of degree values using Cytoscape 3.7.2,the average value was calculated to be 18.532,with a total of 47 key targets greater than the average.The top 10 target proteins were IL6,TP53,TNF,EGFR,IL1β,AKT1,JUN, MMP9,CASP3 and PTGS2.

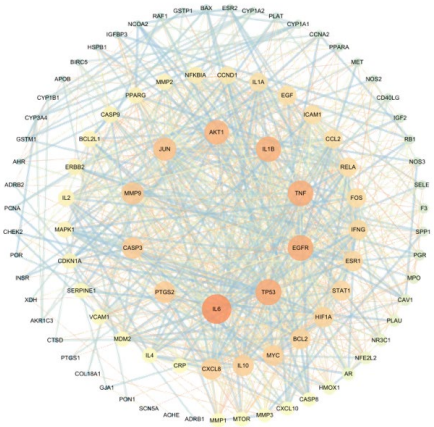

Figure 5: PPI network map of potential targets of core prescriptions

3.4.3. GO Enrichment And KEGG Pathway Analysis

Through Metascape database analysis of 99 intersecting genes,GO analysis identified multiple important biological processes,including 1498 biological processes (BP),such as responses to hormones, inorganic substances,and cellular responses to lipids;there are 60 cellular components (CC) involved,such as membrane rafts,transcriptional regulatory complexes,plasma membrane rafts, etc;There are 146 molecular functions (MF) involved,such as transcription factor binding,cytokine receptor binding,RNA polymerase II specificity,and DNA binding transcription factor binding.Arrange according to the P-Value value,and draw a bar chart with BP,CC,and MF taking the top 10 each.The results are shown in Figure 6.KEGG analysis identified 177 signaling pathways,which were sorted based on P-Value values.The top 20 main pathways were retained and an enriched bubble plot was plotted.The results are shown in Figure 7.Among them,key target genes are mainly enriched in cancer,lipid and atherosclerosis,chemical carcinogenesis-receptor activation,AGE-RAGE in diabetic complications,fluid shear stress and atherosclerosis,prostate cancer,IL-17,TNF,PI3K-Akt,HIF-1 and other signaling pathways.

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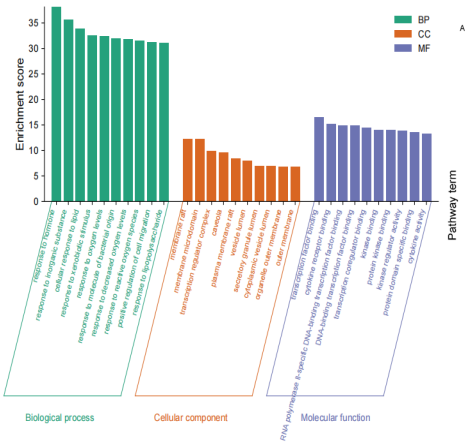

Figure 6: BP, CC, MF triple column chart

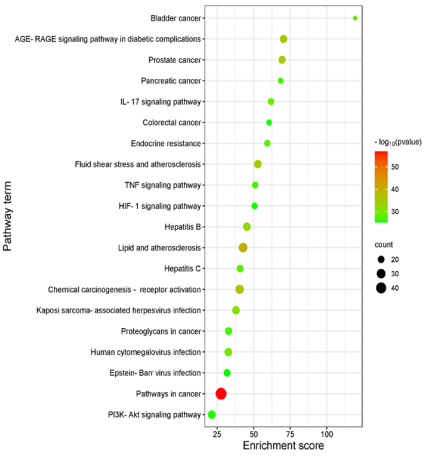

Figure 7: KEGG top 20 path bubble map

3.4.4. "Disease-Component-Target-Pathway" Complex Network Analysis

Import 5 drugs,62 main active ingredients,181 gene targets,and related information on male infertility from the core prescription into Cytoscape 3.7.2 to construct a complex network,as shown in Figure 8. In the network diagram, the red center shape represents male infertility,the irregular green shape around the periphery represents the main pathways,the pink diamond shaped targets represent the active ingredients of core prescription drugs, and the blue hexagons represent target genes.The results showed that there were 239 nodes and 1083 edges,and each target played an interaction with other targets, reflecting the multi-component,multi-target,and multi-path effects of traditional Chinese medicine compound.The top 5 gene targets ranked by degree are AKT1,RELA,PTGS2,MAPK1 and NCOA2; the top three active ingredients in terms of degree ranking are quercetin,kaempferol and luteolin, indicating that they may play a key role in the core prescription treatment of male infertility.

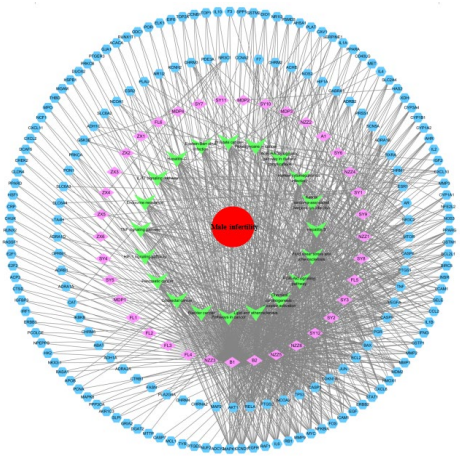

Figure 8: "Disease - component - target - pathway" network diagram

4. Discussion

Through data mining and analysis,this study found that the frequency of tonifying yang drugs is the highest in the efficacy classification,followed by damp-draining diuretic drugs, tonifying qi drugs,and blood-activating and stasis-resolving drugs.It can be inferred that modern male infertility patients are mainly caused by kidney deficiency.According to traditional Chinese medicine theory, the kidneys store essence and regulate the meridians,which is known as the foundation of sealing and storage and plays an important role in opening and closing the essence.Therefore,the treatment of infertility mostly relies on the regulation of the kidneys,and is also related to factors such as liver depression and blood stasis, spleen deficiency and weak qi,and damp heat blockade. Through the analysis of the high-frequency medicinal properties and flavors,it can be found that the meridian is mainly based on the liver meridian,followed by the lung meridian,stomach meridian,spleen meridian,and kidney meridian. In clinical medication,sweet,warm and flat drugs are mainly used to tonify the liver and

ISSN 2706-6819 Vol.7, Issue 2: 18-29, DOI: 10.25236/IJFM.2025.070204

kidneys, replenish qi and spleen, and reduce the use of cold, bitter, and pungent drugs to dry dampness, eliminate heat, and remove blood stasis, treating both symptoms and root causes.

The analysis of association rules resulted in the core prescription of "dioscoreae rhizoma,moutan cortex, poria cocos-alismatis rhizoma,ligustri lucidi fructus".Dioscoreae rhizoma, moutan cortex,poria cocos and alismatis rhizoma are important components of the Liuwei Dihuang Pill.Dioscoreae rhizoma has a sweet and mild taste,and can be used to nourish the spleen and tonify deficiency.In the book "Jingyue Quanshu" it is said that dioscoreae rhizoma can" nourish the spleen and tonify deficiency, astringent essence and strengthen the kidneys" and can supplement innate deficiencies[10].Moutan cortex, poria cocos,and alismatis rhizoma can nourish kidney yin,promote spleen dampness,and eliminate kidney turbidity.Previous studies have shown that ligustri lucidi fructus has a pharmacological effect on protecting the liver and kidneys.After being brewed with alcohol,its nourishing effect on the liver and kidneys is enhanced.Ligustri lucidi fructus improves the balance of the endocrine system, reduces the release of inflammatory factors and oxidative stress damage to liver cells,and plays a protective role against liver and kidney yin deficiency caused by emotional disorders in rats[11]. The core prescription includes two tonics and three laxatives,tonifying kidney yin,promoting damp heat,strengthening spleen and protecting liver,which is the core combination for treating male infertility of kidney yin deficiency type.

The core components identified through network pharmacology analysis are quercetin, kaempferol,and luteolin.According to literature review,it was found that quercetin and luteolin can improve mitochondrial function in asthenozoospermia samples by stimulating respiratory activity[12]. Quercetin has strong antioxidant properties and effectively prevents and controls problems in the male reproductive system by improving sperm quality and alleviating testicular damage[13]. There are research reports that kaempferol has the effects of inhibiting beta cell apoptosis, reducing inflammatory response and oxidative stress,and can effectively alleviate kidney tissue damage and inflammatory response in a high glucose environment,improving kidney function[14]. Research has shown that luteolin has an inhibitory effect on the disease target protein MMP9, promoting the destruction and remodeling of the extracellular matrix,thereby promoting the invasion and metastasis of cancer cells[15].

The core targets and pathways were identified through PPI network and KEGG pathway analysis. Research has shown that prostaglandins play a crucial role in the synthesis of PTGS2 in the body,and can also affect a series of processes such as ovulation,fertilization,implantation and childbirth[16].RelA is an important transcription factor in the NF-κB family,by affecting the expression of downstream target genes, it influences cell proliferation, inflammatory responses, immunity and differentiation, and plays a significant role in the occurrence and development of tumors[17].The single nucleotide polymorphism of TP53 may be one of the genetic risk factors for idiopathic infertility patients[18]. Related studies have shown that the levels of IL-6 and TNF in the seminal plasma of infertile patients are elevated[19]. This prescription can alleviate the inflammatory response of testicular tissue by regulating the secretion of IL-6 and IL1β.TNF is an immune regulatory factor mainly produced by activated macrophages,NK cells,and T lymphocytes. Research has shown that TNF has a certain impact on fertility decline caused by high-fat diets [20,21].By affecting TNF-α targets on the TNF signaling pathway,it is possible to enhance sperm motility.Research has found that the PI3K/Akt signaling pathway plays an important role in the development of the male reproductive system by regulating the proliferation of testicular spermatogonial stem cells and the microenvironment of epididymal sperm maturation,improving sperm motility and semen quality[22].Hypoxia inducible factor-1 is only stably expressed in the body under hypoxic conditions,and is rapidly degraded when oxygen is sufficient.It is involved in both the PI3K Akt and HIF-1 signaling pathways[23].

5. Summary

In summary, this study systematically analyzed the medication patterns and mechanisms of action of the Feng Chonglian Famous Traditional Chinese Medicine Studio team in treating male infertility through data mining and network pharmacology techniques,as well as the network pharmacology analysis of the core prescription for treating kidney yin deficiency type male infertility.This reflects the treatment principle of nourishing yin over supplementing yang, clarifies that traditional Chinese medicine prescriptions are a multi-component,multi-target,and multi pathway method that can exert the overall treatment mechanism,and provides a new idea and feasibility for clinical treatment of male infertility.

ISSN 2706-6819 Vol.7, Issue 2: 18-29, DOI: 10.25236/IJFM.2025.070204

Acknowledgements

The research is supported by: Fund projects:2022 Guangdong Province Traditional Chinese Medicine Research Project Yue Zhongyi Han [2021] No.425 (20221247);2023 Guangdong Province Traditional Chinese Medicine Research Project Yue Zhongyi Han [2022] No.197 (20232108) (20231241); 2022 Guangzhou Traditional Chinese Medicine and Integrated Traditional Chinese and Western Medicine Technology Project Suiwei Han [2022] No.80 (20222A011020) (20222A011018); 2024 Guangzhou Traditional Chinese Medicine and Integrated Traditional Chinese and Western Medicine Technology Project Suiwei Han [2023] No.2250(20242A010020); "1+N" system construction (high-quality development) project of acupuncture and moxibustion Hospital of Guangzhou City; 2024 Municipal School (Institute) Enterprise Joint Funding Project (SL2023A03J00340).

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