Molecular mechanism of reproductive toxicity induced by *Tripterygium Wilfordii* based on network pharmacology

Qing Ding, MBa,b,c, Yuanhao Wu, MDa,b,c,*, Wei Liu, MDa,b,c

**Abstract**

To explore the possible molecular mechanism of reproductive toxicity of *Tripterygium wilfordii* from the perspective of network pharmacology and bioinformatics.

The compounds of *T wilfordii* were obtained by querying the relevant Chinese medicine database, the effective compounds were screened and the corresponding targets were obtained, and then compared with the reproductive toxicities related to disease targets obtained from the disease gene database to infer the potential toxic targets of reproductive toxicity of *T wilfordii*. Then, the key targets of reproductive toxicity of *T wilfordii* were screened using Search Tool for the Retrieval of Interacting Genes/Protein and Cytoscape. The gene ontology function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, as well as module analysis, were performed on the key targets using Database for Annotation, Visualization, and Integrated Discovery and Cytoscape, respectively. Finally, the network between effective compounds-toxic targets was conducted to see how the compounds interacted.

A total of 48 effective compounds and 482 potential toxic targets related to the reproductive toxicity of *T wilfordii* were screened. The enrichment analysis results showed that the key targets were mainly enriched in biological processes such as response to drug, ionotropic glutamate receptor signaling pathway, and KEGG pathways such as neuroactive-ligand-receptor interaction, cAMP signaling pathway. In the protein-protein interaction network of potential toxic targets, there were 78 key targets such as TP53, INS, IL6, AGT, ADCY3, and so on. Enrichment analysis of the top module with 19 genes from module analysis indicated that *T wilfordii* might cause reproductive toxicity by gene ontology terms and KEGG pathways such as regulation of vasocostriction, G-protein coupled receptor signaling pathway, inflammatory response, cAMP signaling pathway, and so on. In the network between effective compounds of *T wilfordii* and key targets, there were 5 compounds with high degree including Tingenone, Wilfordic Acid, Abruslactone A, Nobilin, and Wilforlide B.

The complex molecular mechanism of reproductive toxicity of *T wilfordii* can be preliminarily elucidated with the help of the network pharmacology method, and the analysis results can provide some reference for the further mechanism research of reproductive toxicity of *T wilfordii*.

**Abbreviations:** BATMAN-TCM = Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine, DAVID = Database for Annotation, Visualization and Integrated Discovery, GO = gene ontology, BPs = biological processes, KEGG = Kyoto Encyclopedia of Genes and Genomes, STRING = Search Tool for the Retrieval of Interacting Genes/Proteins, PPI = protein-protein interaction, MCODE = molecular complex detection, TP53 = tumor protein 53, INS = insulin, IL6 = interleukin-6, AGT = Angiotensinogen, ADCY3 = adenylyl cyclase 3.

**Keywords:** molecular mechanism, network pharmacology, reproductive toxicity, *Tripterygium wilfordii*

---

*Editor: Subhashchandra Naik.*

This study was supported by Scientific Research Project of Tianjin Municipal Commission of Education (2019ZD12); High-Level Talent Selection and Training Project of Tianjin Health and Family Planning Industry (20205wuyuanhao); and National Natural Science Foundation of China (81673927).

The authors have no conflicts of interest to disclose.

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

Department of Rheumatology and Immunology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China; National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China; Tianjin Key Laboratory of Translational Research of TCM Prescription and Syndrome, Tianjin, China.

*Correspondence: Yuanhao Wu, Department of Rheumatology and Immunology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China (e-mail: doctor.wuyh@gmail.com).

Copyright © 2021 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Ding Q, Wu Y, Liu W. Molecular mechanism of reproductive toxicity induced by *Tripterygium Wilfordii* based on network pharmacology. Medicine 2021;100:26197.

Received: 22 December 2020 / Accepted: 12 May 2021

http://dx.doi.org/10.1097/MD.00000000000026197
1. Introduction
As a traditional Chinese herbal medicine, *Tripterygium wilfordii* has a long history in the application of traditional Chinese medicine. Modern pharmacological researches have shown that the components of *T. wilfordii* are complex, mainly including alkaloids, terpenoids, sugars, and so on and have the functions of regulating immunity, anti-inflammatory, anti-tumor, anti-fertility, anti-angiogenesis, and so on. Due to its outstanding immunomodulatory and anti-inflammatory effects, *T. wilfordii* is widely used in autoimmune diseases. For example, *T. wilfordii* polyglycosides tablets made from extracts of *T. wilfordii* are commonly used in the treatment of rheumatoid arthritis. On the other hand, it harms, including hepatotoxicity, nephrotoxicity, anti-angiogenesis, and so on. Due to its outstanding immunomodulatory and anti-inflammatory effects, *T. wilfordii* is widely used in autoimmune diseases. For example, *T. wilfordii* polyglycosides tablets made from extracts of *T. wilfordii* are commonly used in the treatment of rheumatoid arthritis. However, on the one hand, *T. wilfordii* has a good effect; on the other hand, it harms, including hepatotoxicity, nephrotoxicity, reproductive toxicity, and cardiovascular system toxicity, which not only causes harm to patients’ health but also greatly limits the clinical application of *T. wilfordii*. Recent studies have suggested that the reproductive toxicity of *T. wilfordii* is related to the atrophy of reproductive organs, function decline, and apoptosis of reproductive cells, whose molecular mechanism is still unclear due to the complex composition. The purpose of this study is to predict the biological processes and related pathways involved in the reproductive toxicity of *T. wilfordii* by means of network pharmacology, and to explore the molecular mechanism of reproductive toxicity of *T. wilfordii*, so as to provide certain theoretical reference for further molecular experiments and attenuation studies on the reproductive toxicity of *T. wilfordii*.

2. Materials and methods
Since this study is an analysis of data from online databases and the privacy of patients will not be disclosed, so patients’ informed consent and ethical approval are all not required.

2.1. Acquisition of effective compounds and targets of *T. wilfordii*
The chemical compounds contained in *T. wilfordii* and their targets were searched from a Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm/),[5] with the score cutoff value of 20 and the adjusted P value of .05. Then, the dataset A of effective compounds and targets of *T. wilfordii* was collected.

2.2. Acquisition of reproductive toxicity targets of *T. wilfordii*
The terms “reproductive toxicity, gonad toxicity, sexual dysfunction, reproductive damage, and gonad damage” were used to retrieve the target genes of reproductive toxicity from the human gene database GeneCards (version5.0, https://www.genecards.org).[6] Reproductive toxicity target dataset B was obtained by excluding the duplicate results. Subsequently, the dataset C of overlapping genes dataset between A and B was gained using a Venn diagram, which was produced by Venny (version2.1, http://bioinfogp.cnb.csic.es/tools/venny/index.html).[7]

2.3. Analysis of GO and KEGG pathway enrichment
For further analysis of the molecular mechanism of reproductive toxicity of *T. wilfordii*, the key targets would be input into Database for Annotation, Visualization and Integrated Discovery (DAVID; version6.8, https://david.ncifcrf.gov/),[8,9] limiting the species as Homo sapiens to conduct the analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. It was considered to be statistically significant when P value <.05.

2.4. Construction and module analysis of PPI network
To explore the internal relationship between the potential gene targets of reproductive toxicity of *T. wilfordii*, the target genes in dataset C were uploaded to Search Tool for the Retrieval of Interacting Genes/Proteins database (version11, http://www.string-db.org/)[10] to build a protein-protein interaction (PPI) network. Then the analysis of topology characteristics of the PPI network was carried out using Network analyzer plugin in Cytoscape, and key genes were screened and extracted from the network according to the Betweenness Centrality and Closeness Centrality which were larger than the median and the degree which was larger than twice median. Module analysis on the PPI network of key genes was conducted using the molecular complex detection clustering algorithm in Cytoscape. Module analysis is usually used to identify connected gene groups with common functions and analyze the complex relationship of the nodes in the PPI network. The conditions for the module selection contained: node score cutoff = 4, degree cutoff = 4, maximum depth = 100, and k-core = 2. Additionally, the top module was further analyzed for KEGG pathway enrichment using DAVID, with a cutoff of P < .05.

2.5. Construction and analysis of effective compounds-toxic targets network
The effective compounds and reproductive toxicity targets were uploaded to Cytoscape to build their reaction network. The network between the key genes and the effective compounds was then extracted. The greater the degree value of nodes in the network, the greater their role in the network. A large degree of 1 compound indicates that it can react with many targets; similarly, a large degree of 1 target indicates that it can combine with many compounds.

3. Results

3.1. Effective compounds and targets of *T. wilfordii*
Based on BATMAN database, a total of 48 effective compounds of *T. wilfordii* that met the screening conditions were obtained, and a total of 529 gene targets were predicted.

3.2. Reproductive toxicity targets of *T. wilfordii*
There were totally 13,866 gene targets related to reproductive toxicity retrieved from GeneCards database after detracting and resorting. As shown in Figure 1, there were 482 common gene targets between the active compounds of *T. wilfordii* and reproductive toxicity.
3.3. Enrichment analysis of GO and KEGG

Putting 482 common target genes into the DAVID database, 1154 GO terms and 95 KEGG pathways were enriched (Table 1). The top 5 biological processes in key targets of reproductive toxicity of *Tripterygium wilfordii* were a response to drug, ionotropic glutamate receptor signaling pathway, positive regulation of transcription, DNA-templated, positive regulation of gene expression, and positive regulation of transcription from RNA polymerase II promoter. The 5 most enriched KEGG pathways were neuroactive ligand-receptor interaction, nicotine addiction, cAMP signaling pathway, calcium signaling pathway, and amphetamine addiction.

3.4. Construction and module analysis of PPI network

Four hundred eighty two potential reproductive toxicity targets of *T. wilfordii* were uploaded to Search Tool for the Retrieval of Interacting Genes/Proteins database to obtain their interaction relationships. The results were opened by Cytoscape and topology analysis of the network was conducted. According to the Betweenness Centrality and Closeness Centrality which were larger than the median and the degree which was larger than twice the median, 78 key genes were screened finally, whose average degree was 26.17, among which a total of 56 (71.79%) genes had a degree greater than 20. The top 5 of degree among all key genes were TP53, INS, IL6, AGT, and ADCY3 (Table 2).

In molecular complex detection analysis, a total of 5 modules were finally selected (Table 3), including 1 top cluster with 19 nodes and 171 edges (Fig. 2). Enrichment analysis of genes in the top module demonstrated that it may be associated with regulation of vasoconstriction, G-protein coupled receptor signaling pathway, inflammatory response, alpha2-adrenergic receptor activity, epinephrine binding, drug binding, neuroactive ligand-receptor interaction, cGMP-PKG signaling pathway, and cAMP signaling pathway (Table 4).

3.5. Construction and analysis of the network between toxic compounds and reproductive toxicity targets of *T. wilfordii*

The network between effective compounds of *T. wilfordii* and key targets was built by Cytoscape, and then the network between toxic compounds and reproductive toxicity targets of *T. wilfordii* (Figure 3), which contained 111 nodes and 209 edges, involving 33 toxic compounds and 78 reproductive toxicity targets. The top 5 compounds were Tingenone (25 targets), Wilfordic Acid (16 targets), Abruslactone A (16 targets), Nobilin (14 targets), and Wilforlide B (10 targets).

4. Discussion

Not only the decoction pieces of *T. wilfordii* but also the extracts from *T. wilfordii* are widely used in clinical practice. It has been proved that *T. wilfordii* can promote male spermatogenic cell apoptosis, reduce sperm viability, and cause female ovarian function decline. Therefore, it is very important to understand the toxicity mechanism of *T. wilfordii*, and then to develop attenuated strategies for various toxic reactions. This study focused on the reproductive toxicity of *T. wilfordii*, and tried to explore the molecular mechanism based on BATMAN, GeneCards, and other related databases and online tools.

In total, 48 toxic compounds and 482 toxic target genes related to reproductive toxicity of *T. wilfordii* were dugout. Enrichment analysis of these 482 genes preliminary found that the pathogenesis of the reproductive toxicity of *T. wilfordii* might be connected with biological processes such as response to drug, ionotropic glutamate receptor signaling pathway, positive toxicity of *T. wilfordii*.

| Table 1 | GO analysis in BP and KEGG pathway analysis of reproductive toxicity of *Tripterygium wilfordii*, including the top 5 terms selected according to the P value. |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Term** | **Description** | **Count** | **P-value** |
| Top 5 BPs | | | |
| GO:0042493 | Response to drug | 57 | 1.25E-29 |
| GO:0035225 | Ionotropic glutamate receptor signaling pathway | 18 | 1.14E-21 |
| GO:0045893 | Positive regulation of transcription, DNA-templated | 61 | 6.18E-21 |
| GO:0010628 | Positive regulation of gene expression | 44 | 6.53E-21 |
| GO:0045944 | Positive regulation of transcription from RNA polymerase II promoter | 85 | 3.28E-20 |
| Top 5 KEGG pathways | | | |
| hsa04080 | Neuroactive ligand-receptor interaction | 70 | 2.21E-27 |
| hsa05033 | Nicotine addiction | 26 | 6.59E-22 |
| hsa04024 | cAMP signaling pathway | 46 | 2.21E-16 |
| hsa04020 | Calcium signaling pathway | 41 | 2.54E-14 |
| hsa05031 | Amphetamine addiction | 25 | 4.71E-14 |

**BPs** = biological processes, **GO** = gene ontology, **KEGG** = Kyoto Encyclopedia of Genes and Genomes.
regulation of transcription, DNA-templated, positive regulation of gene expression, positive regulation of transcription from RNA polymerase II promoter, and KEGG pathways such as neuroactive ligand-receptor interaction, nicotinic addiction, cAMP signaling pathway, calcium signaling pathway, andamphetamine addiction.

Then, the PPI network of potential toxic targets was further analyzed. Out of them there were 78 key gene targets and Table 2 summarized the top 10 PPI network hub genes including TP53, INS, IL6, AGT, and ADCY3. Most of these genes were associated with reproduction. As a tumor suppressor gene, TP53 can participate in the regulation of the cell cycle, is closely related to cell proliferation and apoptosis, and plays an important role in the occurrence and development of various malignant tumors such as gastric cancer, colon cancer, and ovarian cancer.

In animal experiments, knocking out the TP53 gene in mouse ovarian epithelial cells could accelerate cell proliferation and DNA synthesis, and enhance the ability of cell cloning and ovarian epithelial cells could accelerate cell proliferation and enhance the ability of cell cloning and migration. In addition, clinical studies have found that there is a certain correlation between the TP53 gene and the incidence of male infertility and female infertility. INS is an insulin gene, which plays a certain role in the fertility of mice. ADCY3 gene encodes adenylate cyclase 3 (ADCY3), which is a membrane integrated protein and is one of the key signal molecules downstream of G protein-coupled receptor. It converts the stimulation of extracellular signals into intracellular signals, regulates the synthesis of cyclic adenosine-3'-5'-monophosphate (cAMP), and thus participates in various pathological and physiological processes of the body. However, the current studies on ADCY3 mainly focus on its correlation with diseases such as obesity, fatty liver, and Crohn disease.

The module analysis showed that the PPI network of 78 key target genes contained 5 modules. The enrichment results of the top module with 19 genes indicated that T wilfordii might cause reproductive toxicity by GO terms and KEGG pathways such as regulation of vasoconstriction, G-protein coupled receptor signaling pathway, inflammatory response, alpha2-adrenergic receptor activity, epinephrine binding, drug binding, neuroactive ligand-receptor interaction, cGMP-PKG signaling pathway, and cAMP signaling pathway. These may be the underlying mechanisms of reproductive toxicity of T wilfordii.

In addition to the above, the reproductive system damage of T wilfordii is also related to some other genes. An animal experiment showed that tripterygium glycosides had different degrees of damage to the ovaries of female rats at different times, which may be related to its effect on the expression of circadian rhythm genes CLOCK and BMAL1. The reproductive toxicity of T wilfordii was different in different sex rats. This suggests that administration time, sex, and other factors may also affect the reproductive toxicity of T wilfordii.

Among top compounds with a high degree in the network between toxic compounds and reproductive toxicity targets of T wilfordii, Tingenone is a pentacyclic triterpene, which can induce peripheral antinociception due to opioidergic activation, NO/cGMP, and ATP-sensitive K(+) channels pathway activation and cannabinoid receptors activation in mice. Unfortunately, no studies have been found to explore the direct effect of Tingenone on reproduction. Abruslactone A, also called Wilforlide A, is a triterpenoid from T wilfordii, which has

| Cluster | Score | Nodes | Edges | Node IDs |
|---------|-------|-------|-------|----------|
| 1       | 19    | 19    | 171   | CNR2, CR1, ADRA2C, PF4, BKBR, PTGER3, CXCR1, ADORA1, ADRA2A, ADCY3, DRD4, CHRM2, C5, ADORA3, ADRA2B, AGT, GPER1, ANX1, DRD2 |
| 2       | 6.476 | 22    | 68    | GRIN2A, IFNG, TGF81, GRIA1, CAMK2D, CAMK2A, IL6, ADIPQ, GRIA2, NCOA1, CALM1, TNF, GRIN1, ESR1, PKC9, GRIN2B, PRKCD, IL1B, IL10, IL4, BDNF, NGF |
| 3       | 6.429 | 15    | 45    | F2, DRD1, AVP, CCL2, INS, FFAR1, ADRB2, AGT, UTS2R, CRH, PTGS2, CALCA, PTGER1, AVP, KISS1 |
| 4       | 4.333 | 7     | 13    | MED1, AR, SRC, HDA1C1, FOS, SIRT1, FFAR1 |
| 5       | 4     | 6     | 10    | WNT5A, CTNNB1, MTR, MAPK9, NOTCH1, TP53 |

**Table 3** Five modules from the PPI network satisfied the criteria of MCODE scores >4 and number of nodes >4.
obvious immunosuppressive activity. Abruslactone A can inhibit the activity of adenosine deaminase in HL-60 cells and induce apoptosis.\textsuperscript{[33,34]} The mechanism of Wilforlide A-induced ovarian cell apoptosis may be related to the abnormal expression of ERK/c-fos, and there is a time effect on the expression of apoptosis-related proteins.\textsuperscript{[35]} One study showed that compatibility of \textit{T. wilfordii} and \textit{Astragalus membranaceus} could downregulate the content of Wilforlide A\textsuperscript{[36]}, which suggests that \textit{T. wilfordii} combined with \textit{A membranaceus} may reduce reproductive toxicity. However, so far, there have been no studies on Wilfordic Acid, Nobilin, or Wilforlide B.

### 5. Conclusions

The results of this study are consistent with those of published literature, indicating that it is feasible to predict the molecular mechanism from the perspective of bioinformatics by means of network pharmacology. The data from this study may provide

---

**Table 4**

| Term Name | GO:0019229 Regulation of vasoconstriction | GO:0007186 G-protein coupled receptor signaling pathway | GO:0006954 Inflammatory response | GO:0004938 Alpha2-adrenergic receptor activity | GO:0051379 Epinephrine binding | GO:0008144 Drug binding | GO:0004938 | GO:0051379 | GO:0008144 |
|------------|------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|---------|---------|---------|
| Count      | 5                                        | 11                                              | 8                               | 3                               | 3               | 4               | 3                   | 3                   | 4               |
| P value    | 4.43E-09                                 | 5.44E-09                                        | 7.25E-08                        | 3.22E-06                        | 1.61E-05               | 6.82E-05               | 3.22E-06          | 1.61E-05          | 6.82E-05          |
| Genes      | BDKR2, ADRA2C, ADRA2B, ADRA2A, AGT       | C3orf1, CHRM2, C5, GPER1, PTGER3, BDKR2, ADRA2C, ADRA2B, ADRA2A, AGT, PF4 | C5, ANXA1, CNR2, GPER1, PTGER3, BDKR2, ADORA1, PF4 | ADRA2C, ADRA2B, ADRA2A | ADRA2C, ADRA2B, ADRA2A | CHRM2, CNR1, DRD2, DRD4 | ADORA2C, ADRA2B, ADRA2A, ADRA1, DRD2, ADRA2C, ADRA2B, ADRA2A, DRD4 | ADORA3, BDKR2, ADORA1, ADCY3, ADRA2C, ADRA2B, ADRA2A | ADORA3, BDKR2, ADORA1, ADCY3, ADRA2C, ADRA2B, ADRA2A |

**Figure 3.** Network of effective compounds and reproductive toxicity targets of Tripterygium wilfordii. The green circles represent targets; the purple arrows represent compounds.
greater insight into the molecular mechanisms of reproductive toxicity of \textit{T. wilfordii}. However, because many of the genes identified in this study had not been previously associated with reproductive toxicity of \textit{T. wilfordii}, further studies will be needed to validate the expression of these genes.

**Author contributions**

**Investigation:** Qing Ding, Yuanhao Wu.  
**Methodology:** Yuanhao Wu.  
**Software:** Qing Ding.  
**Validation:** Qing Ding.  
**Visualization:** Qing Ding.  
**Writing – original draft:** Qing Ding.  
**Writing – review & editing:** Wei Liu, Yuanhao Wu.

**References**

[1] Hu DJ, Peng ZY, He DC. Research progress on pharmacological action of \textit{Tripterygium wilfordii} (Chinese). Herald Med 2018;37:586–92.

[2] Zhang Q, Peng GC, Zhu MJ. Research progress on pharmacological action and toxicity of \textit{Tripterygium wilfordii} (Chinese). Chin J Integr Med Cardio-Cerebrovasc Dis 2016;14:1753–4.

[3] Xu Y, Fan YF, Zhao Y, Lin N. Overview of reproductive toxicity studies on \textit{Tripterygium wilfordii} in recent 40 years. China J Chin Mater Med 2019;44:3406–14.

[4] Xiong W, Chen J. Advances in clinical and animal studies on reproductive toxicity of \textit{Tripterygium wilfordii} (Chinese). J Mod Clin Med 2014;40:403–5.

[5] Liu Z, Guo F, Wang Y, et al. BATMAN-TCM: a Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine. Sci Rep 2016;6:21146.

[6] Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics 2016;54:1–30.

[7] Oliveros, J.C., Venny. An interactive tool for comparing lists with Venn’s diagrams. (2007–2015): https://bioinfogp.cnb.csc.es/tools/venny/index.html.

[8] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.

[9] Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–13.

[10] Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47(D1):D607–13.

[11] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.

[12] He XW, Xu L. Research progress of cell cycle related genes CDKN2A, TP53, RB1 and BRCA2 in malignant tumors (Chinese). J Mod Oncol 2018;26:133–7.

[13] Zhang T, Fan JW, Li D, et al. Gene knockout of TP53 in primary mouse ovarian epithelial cells and its biological characteristics (Chinese). Lab Anim Sci 2018;35:64–9.

[14] Jin Q. Correlation between PGAM4 gene and TP53 gene and idiopathic male infertility (Chinese). Wannan Medical College 2013.

[15] Tan Y. Correlation of SNPs of TP53 and MDM2 gene with treatment outcomes of infertility and IVF (Chinese). Kunming University of Science and Technology 2013.

[16] Lin JL. Molecular mechanism of spermatogenic dysfunction in immune orchitis and insulin regulation of function of hypothyalamus-pituitary-gonadal axis (Chinese). Peking Union Medical College 2018.

[17] Ma J, Han RY, Mei XA, et al. Correlation analysis of insulin resistance index with male reproductive hormone level and semen parameters (Chinese). Natl J Androl 2018;24:695–9.

[18] Ma J, Han RY, Mei XA, et al. Effects of obesity and insulin resistance on semen quality in men (Chinese). Chin J Androl 2018;32:19–24.

[19] Sun XL. Effect of insulin resistance on reproductive function in patients with polycystic ovary syndrome (Chinese). Fudan University 2013.

[20] Ke JW, Zhang T, Duan R. Regulation of hypothalamic-pituitary-gonadal axis (HPG) and hypothalamic-pituitary-adrenal axis (HPAA) by cytokines (Chinese). Jiangxi J Medical Lab Sci 2002;06:379–81.

[21] Li QL, Ni J. Regulation of ovarian function by cytokines (Chinese). Prog Physiol Sci 2000;04:361–3.

[22] Lin Y. Effect of angiotensinogen gene deficiency on fertility of mice (Chinese). Foreign Med Sci (Obstet Gynecol Fascicle) 2000;27:303.

[23] Yang YM, Yang YQ, Song G, et al. Research progress of adenylyl cyclase (Chinese). J Clin Pathol Res 2019;39:390–4.

[24] Pan LY, Cai X, Kong YL, et al. Correlation study between ADCY3 gene polymorphism and fatty liver (Chinese). Acta Nutri Sin 2017;39:337–42.

[25] Sadia S, Amelie B, Filippo T, et al. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. Nat Genet 2018;50.

[26] Zhang CB, Zheng H, Chao K, et al. Polymorphism of ADCY3 and NFIL3 genes and their susceptibility to Crohn’s disease in Chinese population (Chinese). Chin J Clin Pharmacol 2019;33:743–5.

[27] Liu Z. Experimental study of Tripterygium glycosides CIA rats administered at different times on the function of the reproductive female (Chinese). North China University of Science and Technology 2017.

[28] Fan YY, Xu Y, Su XH, et al. Effect of Tripterygium glycosides tablets on reproductive toxicity in female rats with II type collagen induced arthritis (Chinese). China J Chin Mater Med 2019;44:3486–93.

[29] Fan YY, Xu Y, Su XH, et al. Effect of Tripterygium glycosides tablets on reproductive toxicity in male rats with II type collagen induced arthritis (Chinese). China J Chin Mater Med 2020;45:755–63.

[30] Veloso CC, Rodrigues VG, Ferreira RC, et al. Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to opioidergic receptors activation in mice. Eur J Pharmacol 2015;755:1–5.

[31] de Carvalho VC, Rodrigues VG, Ferreira RC, et al. Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to NO/cGMP and ATP-sensitive K(+) channels pathway activation in mice. Eur J Pharmacol 2015;755:1–5.

[32] Veloso CC, Ferreira R, Rodrigues VG, et al. Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to cannabinoid receptors activation in mice. Inflammopharmacology 2018;26:227–33.

[33] Ma Z, Zwillmann A, Schröder R, et al. Correlation of SNPs of TP53 and MDM2 gene with treatment outcomes of infertility and IVF (Chinese). Chin J Prim Med Pharm 2015;8:1615–21.

[34] de Carvalho VC, Rodrigues VG, Ferreira RC, et al. Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to cannabinoid receptors activation in mice. Inflammopharmacology 2018;26:227–33.

[35] Ma Z, Zwillmann A, Schröder R, et al. Correlation of SNPs of TP53 and MDM2 gene with treatment outcomes of infertility and IVF (Chinese). Chin J Prim Med Pharm 2015;8:1615–21.