ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

Application and Mechanism of Action of Zuogui Pill in the Treatment of Premature Ovarian Failure

Li Yi[1,a], Xiaoping Cui[1,b,*], Gege Li[1,c], Mingzhu Huang[1,d], Juanjuan Zhang[1,e], Xinchun Xiao[2,f], Xia Liu[2,g]

[1]No. 1, Middle Section of Century Avenue, Chenyangzhai, Qindu District, Xianyang City, Shaanxi Province, Shaanxi University of Chinese Medicine, Xianyang City, China, 712046
[2]Deputy No. 2, Weiyang West Road, Qindu District, Xianyang City, Shaanxi Province, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang City, China, 712046
[a]1144757089@qq.com, [b]13892079999@126.com, [c]1787408809@qq.com, [d]2952594236@qq.com, [e]2218386650@qq.com, [f]1029475976@qq.com, [g]1596166326@qq.com.

Abstract: *The incidence of premature ovarian failure is increasing year by year, which brings serious harm to women's physical and mental health. Western medicine is mostly based on hormone replacement therapy, which may increase the risk factor for vaginal bleeding and endometrial cancer with long-term use. According to Traditional Chinese Medicine, the treatment of premature ovarian failure is based on kidney tonification and treatment according to the evidence. Zuogui Pill can regulate immune factors and reproductive endocrine function, inhibit rapid apoptosis of granulosa cells, promote angiogenesis, increase local blood supply, thereby improving ovarian function, regulating menstrual status, and alleviating perimenopausal syndrome. However, a standardized and comprehensive treatment system needs to be explored in depth.*

Keywords: *Premature Ovarian Failure, Zuogui Pill, Chinese Medicine Treatment, Ovarian function*

Premature ovarian failure [1] is a gynecological clinical syndrome in which the ovaries of women fail prematurely. The main clinical manifestations are menstrual cessation, infertility, atrophy and degeneration of sexual organs before the age of 40, with varying degrees of serum hypergonadotropic and hypoestrogenic syndrome, such as mood disorders, facial flushing, hot flashes and night sweats, and osteoporosis. The incidence of premature ovarian failure in women is 1% to 3% [2-3], and the trend is increasing year by year. Currently, Western medicine mostly uses hormone replacement therapy, ovarian transplantation, immunotherapy, stem cell and gene therapy, etc. [4], which do provide relief for the condition. However, it can cause adverse effects such as vaginal bleeding and an increased risk factor for endometrial cancer [5]. Through herbal medicine and acupuncture and other treatments, Traditional Chinese Medicine regulates hormone levels, awakens follicles, improves endocrine and ovarian functions, thus relieving patients' clinical symptoms and achieving the purpose of treating premature ovarian failure. According to Traditional Chinese Medicine, the pathogenesis of premature ovarian failure is based on the disorder of the internal organs, and phlegm, dampness and blood stasis are the symptoms of the pathology. Mostly caused by deficiency of endowment, loss of nourishment, emotional disorders and poor diet, mainly manifested as deficiency and actual evidence. Deficiency is a deficiency of the spleen and kidneys, the sea of blood is depleted without a source, no blood can go down and cause amenorrhea. The excess causes phlegm and dampness to block the meridians, poor flow of qi and blood, stagnation of qi and blood stasis, and irregular menstruation. With the continuous development of Chinese medicine, the clinical efficacy of using Chinese medicine to treat premature ovarian failure has become increasingly significant. The application and mechanism of action of Zuo Gui Pill in the treatment of premature ovarian failure are reviewed.

1. Chinese medicine's understanding of premature ovarian failure

1.1 History Trace

Premature ovarian failure does not have a clear Chinese medical name. According to its clinical characteristics, it belongs to the categories of “amenorrhea”, “infertility” and “symptoms before and after menopause”. Traditional Chinese Medicine's understanding of its historical evolution is divided into 4 main stages [6]. The first stage is the period from the Spring and Autumn and Warring States to

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

the Sui Dynasty, represented by The Yellow Emperor's Internal Canon of Medicine. There are "can know the seven losses and eight benefits, then the two adjustable, do not know to use this, is the early decline of the section", "When you are forty years old and half of your yin energy, your life is also in decline" and "Tian Gui is exhausted and the channels are blocked, so the body is aging and childless". First to propose the disease of "premature aging" and to describe its etiology and pathogenesis. The second stage is the Tang, Song and Jin Yuan period. Woman complete and effective prescription cloud: "Sadness, thought and sadness, heart injury and blood rebellion exhaustion, the gods first scattered, the moon water first closed". This period is the most extensive period of understanding of the etiology of amenorrhea by all schools of thought. The third stage is the Ming Dynasty period. The compendium of Jiyin said: "The menstruation does not work, either because of abortion and multiple births injuring the blood, or because of long-suffering tidal fever pinning the blood, or because of long-suffering night sweats consuming the blood, or because the spleen and stomach are not in harmony with the diet and thus do not produce blood, or because of dysentery losing blood... Maybe due to the sadness of the seven emotions, the heart Qi stagnation, so the blood is closed and does not work... ". This period is the stage of medical doctors to summarize and improve this disease. The fourth stage is the Qing Dynasty period. In Fu Qingzhu Women's Science, it is said that "menstruation stops early before old age" and "menstruation stops early before reaching 49 years of age". This period is the stage of continued development and the characteristics of the disease onset are documented.

1.2 Etiology

The etiology of premature ovarian failure is complex and no clear pathogenesis has been given by Western medicine. According to Traditional Chinese Medicine, premature ovarian failure is mostly caused by deficient endowment, loss of nourishment, excessive emotions, poor diet and phlegm, dampness and blood stasis. "Menstrual water comes out of the kidneys" [7], the nature-nurture is not nourished, the kidney essence deficiency, essence deficiency, blood deficiency, no blood can be down and amenorrhea and even infertility; emotional and mental disorders lead to loss of drainage of the liver, stagnation of qi and blood stasis, and poor menstrual flow; preferring fatty, sweet and thick flavors, the spleen fails to transport and transform, gathering dampness into phlegm, blocking the uterus and making menstruation difficult or even infertile. Some studies in the literature add that "deficiency and cold accumulation" can also lead to the development of the disease [8]. The pathogenesis is mainly due to blood deficiency, then the lack of essence and blood can hardly nourish the uterus. Cold Qi accumulation, collection and lead to stagnation, then the blood vessels are not blocked, the flow of menstruation is not smooth. Jiang Ying [9] suggested that premature ovarian failure is related to six climatic pathogens, surgery, drugs and other injuries. Lu Jing et al. [10] summarized the results of previous studies on premature ovarian failure, suggesting that a poorly regulated lifestyle, such as diet and living, can also lead to the development of the disease. According to Dong Xiaoying et al. [11], the long-standing deficiency of the spleen and stomach or the wasting of the body is due to the lack of essence and blood, which makes it difficult for the Tiangui to reach the uterus and leads to the interruption of menstruation. Wang Xia et al. [12] suggested that multiple abortions may increase the chance of mechanical damage to the uterus and cervix, infection, and increased effects on neuroendocrine function. Damage to the uterus, deficiency of essence and blood, difficulty in regulating menstruation, resulting in the onset of the disease. Based on the recognition of previous research results, Qiao Huaili et al. [13] suggested that the six climatic pathogens could also contribute to the development of this disease. When the climatic pathogens infiltrate the cellular vessels, and the Chong Ren is not regulated, it is difficult for the blood to fill up on time, and the menstruation cannot come as scheduled.

1.3 Types of symptoms

The classification of premature ovarian failure is based on the subjective experience of various medical practitioners and there is no uniform standard yet. The types of evidence of each scientist are summarized below. Luo Songping [14] identified the pathogenesis from the perspective of yin and yang, and concluded that the pathogenesis of premature ovarian failure is a deficiency of yin essence and a deficiency of yang, suggesting that the classification of symptoms can be based on a deficiency of kidney essence and a deficiency of spleen and yang. Zhang Yuzhen [15] believes that the basic cause of premature ovarian failure is kidney deficiency, and subdivides kidney deficiency into 3 types: spleen and kidney yang deficiency, kidney deficiency and liver depression, and kidney deficiency and blood stasis. Tao Lixin et al. [16] classified 162 patients with premature ovarian failure into 3 categories based on TCM symptoms and signs, and the distribution of their symptoms was 21 with spleen and kidney

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

deficiency, 52 with kidney deficiency and liver deficiency, and 89 with liver deficiency and spleen deficiency in that order. He Jing et al. [17] investigated the distribution pattern of TCM evidence of POF, and the study showed that liver-depression and kidney-deficiency evidence predominated, supplementing the remaining subtypes as liver-kidney-yin-deficiency evidence, spleen-kidney-yang-deficiency evidence, heat-damaging evidence, and liver-depression and spleen-deficiency evidence. Based on previous studies, Tang Minghui [18] added the POF syndrome typology and proposed the spleen-stomach weakness type and the heart-kidney non-interaction type. Based on the analysis of the TCM inheritance-assisted platform, Chen Yingying [19] added the evidence-based typology of phlegm-damp obstruction type POF. Liu Chunni [20] believes that premature ovarian failure is mostly a mixture of deficiency and reality, or only deficiency evidence, with less pure reality evidence. In addition to the above, the distribution of the evidence types are Qi deficiency and blood stasis, kidney deficiency and liver depression with blood stasis, kidney deficiency and liver depression with Qi deficiency, kidney deficiency and Qi deficiency and kidney deficiency and Qi deficiency with blood stasis. Based on the basic theory of Chinese medicine, Li Dieying [21] used evidence-based medicine and literature survey to analyze 94 patients with premature ovarian failure and concluded that the disease was classified into four types of evidence: kidney deficiency, qi and blood deficiency, liver depression and qi stagnation, and yin deficiency. Huang Hongli et al. [22] analyzed and summarized the literature related to the distribution of ovarian premature failure, and added seven types of evidence as kidney deficiency and blood depletion, qi deficiency and blood stasis with phlegm-damp obstruction, blood deficiency and fire, kidney deficiency and liver depression (with stasis), liver and kidney yin deficiency (with blood dryness), qi and blood deficiency (with stasis), and heat and fluid injury.

2. Zuogui Pill and the Treatment of Premature Ovarian Failure

Chinese medicine mainly through examination, diagnosis and treatment, the four diagnoses together to identify the evidence. The treatment is very effective. However, the failure to have a unified and mature system of clinical prescriptions and medicines in Chinese medicine. Therefore, it is especially important to build a unified standardized expert consensus based on the scientific support of basic theories of TCM and to develop an authoritative system of diagnosis and treatment evaluation standards. Zuogui Pill is recommended for patients with kidney yin deficiency type of premature ovarian failure. This formula is a combination of Radix Rehmanniae Praeparata, Turtle Board Gum, Antler Gum, Cornus Officinalis, Yam, Fructus Lycii, Semen Cuscutae and Radix Achyranthes Bidentatae, which is effective in nourishing Yin, tonifying the kidney and filling the marrow. Radix Rehmanniae Praeparata is the ruling herb, nourishing kidney yin and filling the essence; turtle board gum and deer horn gum are flesh and blood sentient products, which can nourish the essence, nourish the yin and kidney and help the yang, with the effect of "seeking yin in yang"; cornus officinalis nourishes the liver and nourishes the kidneys; yam nourishes the spleen and benefits the kidneys; Chinese wolfberry [23] can nourish the kidney and benefit the essence, induce ovulation, improve fertility and delay aging; cuscuta seeds are a flat tonic for yin and yang, enhance immunity, promote follicle development, regulate endocrine, antioxidant and anti-aging, etc.; cuscuta seeds - Lycium barbarum contains a variety of cellular biological processes and signaling pathways that can effectively treat premature ovarian failure [24]; Radix Achyranthes Bidentatae [25] can nourish the liver and kidneys and strengthen the muscles and bones. Pharmacological studies have shown that the total saponin of hyssop can significantly excite the smooth muscle of the uterus. The combination of all the herbs together can achieve the function of pure tonification without drainage and the function of steadily replenishing true yin, which is exactly the pathogenesis of premature ovarian failure. Because the main pathogenesis of premature ovarian failure is precisely kidney deficiency [26]. If the kidney essence is deficient, the Tiangui does not arrive, the Ren Chong Qi and blood do not fill, the uterine veins are not nourished, the kidney - Tiangui - Chong Ren - uterine axis is dysfunctional, and menstruation does not occur, resulting in the occurrence of this disease. A researcher [27] investigated Zuogui Pill for the treatment of premature ovarian failure and concluded that it can effectively relieve dizziness and tinnitus, baking heat and sweating, five heartburn and lumbar weakness, and effectively improve endometrial thickness and serum hormone levels with low adverse effects. It has been shown that Zuogui Pill [28] is effective in improving the symptoms of perimenopausal syndrome, and it has been suggested that it can increase the level of anti-mullerian hormone and the number of sinus follicles, effectively improve the endometrial tolerance, and increase the fertility and conception rate.

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

3. Mechanism of action

3.1 Modulating immune factors

It has been documented [29] that another important cause of premature ovarian failure is autoimmune disorders. Zhu Ling et al. [30] built a model of immune ovarian premature failure and treated with different doses of Zuogui Pill, which was found to reduce immune inflammatory damage and protect ovarian function in mice by balancing the Fas/FasL system, regulating immune response and cytotoxic lymphocyte function, enhancing B cell apoptosis and preventing antibody aggregation. Hu Qinglian et al. [31] found that Zuogui Pill significantly reduced FSH content and significantly increased E2 content to effectively improve ovarian function through follicular apoptosis regulation mechanism. It can significantly reduce NK cells, suggesting that it can effectively inhibit NK cell activity and prevent apoptosis from occurring. It can significantly reduce AZAb value and apoptosis rate, suggesting that it can effectively inhibit AZAb and prevent the occurrence of inflammatory damage. It also inhibited Fas-mediated follicular cell apoptosis and significantly increased Fas-L protein, Bcl-2 protein in the high dose group. The above results show that it relies more on the regulation of immunity, gonadal endocrine, follicular apoptosis rate and ovarian tissue microstructure to effectively improve ovarian function and thus treat premature ovarian failure.

3.2 Modulating reproductive endocrine function

Studies have shown [32] that Zogui Pill can significantly reduce follicle stimulating hormone and luteinizing hormone, increase estradiol and uterine and ovarian organ indices and thus promote reproductive organ development. Zhu Ling et al. [33] investigated that Zuo Gui Pill can inhibit the production of anti-ovarian antibody (AoAb) and reduce its inhibition of growing follicles, thus increasing the number of positive oocyte long differentiation factor-9 (GDF-9) mRNA, increasing the number of growing and mature follicles, increasing E_2 content, promoting feedback regulation, decreasing FSH content, and regulating neuroendocrine function to play a role in the treatment of premature ovarian failure. Yang Songwei et al. [34] found that Zuo Gui Wan could effectively alleviate the state of kinetic cycle in mice with premature ovarian failure caused by cyclophosphamide (CTX), increase E_2 level, decrease FSH level, ensure the quality of genitalia, improve ovarian tissue lesions, thus increasing follicle number, delaying aging and restoring ovarian function. Cheng Binbin et al. [35] confirmed that it was effective in increasing E_2 levels, ovarian and thymic mass in female rats with yin deficiency, but no significant effect was seen on FSH, thus confirming that Zuo Gui Pill was effective in improving reproductive endocrine function.

3.3 Inhibiting of ovarian granulosa cell apoptosis

It was found [36] that an important factor triggering Premature Ovarian Failure is follicular atresia due to excessive apoptosis of ovarian granulosa cells. Zhu Ling et al. [37-38] used mouse zona pellucida 3 as antigen and multi-point injection of immunized BALB/C female mice to model the changes of ovarian ultrastructure in immunized Premature Ovarian Failure mice by electron microscopy with unequal doses of Zogui pill. It was concluded that the nuclei of granulocytes at low doses were solidly shrunken, and apoptotic cells were present at medium and high doses, but with neatly arranged and normal morphological structure. The expression of follicular and interstitial Bcl-2 protein increased in all three dose groups, and the expression of Bax protein decreased in all three dose groups, indicating that Zuo Gui Pill could significantly improve its ultrastructure and ovarian function by inhibiting rapid apoptosis of granulosa cells, and showed quantitative effect. Researchers concluded [39-40] that 6.0 Gy triggers follicular DNA damage, activates p53, and has a key role in mediating follicular atresia triggered by granulosa cell apoptosis. Zhao Fenqin et al. [41] concluded that Zuo Gui Pill may have a promoting effect on granulosa cell value-added and follicle development by inhibiting ovarian tissue apoptosis and P53 protein expression, increasing ovarian E2, and alleviating radiation-damaged ovaries by negative feedback regulation to reduce FSH and FSH/LH levels.

3.4 Promote the growth and proliferation of ovarian vascular endothelial cells

Su Yan et al. [42] selected cyclophosphamide chemotherapy as a model and found that Zuo Gui Wan also caused significant expression of vascular endothelial growth factor and significantly promoted the growth and proliferation of ovarian vascular endothelial cells, thus changing the ovarian tissue microstructure and promoting the development of reproductive organs. Most scholars agree [43] that the

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

development of reproductive organs induced by Zogui Pill may be associated with increased estrogen levels, angiogenesis and increased local blood supply. Wu Di et al. [44] used rat tail gum as a substrate to construct a three-dimensional model to investigate the effects of Zuo Gui Pill containing serum on vascular endothelial cell proliferation and angiogenesis in an in vitro three-dimensional model. The results confirmed that vascular endothelial cells could form tube-like structures under the influence of Zogui pill-containing serum, and that angiogenesis was achieved mainly through the proliferation of vascular endothelial cells, the increase of tubule area, length, tube diameter, tube-forming index, budding and aggregation, and the increase of serum E2 and vascular endothelial growth factor levels. Using 40 recently aged rats as study subjects, Tang Liming et al. [45] suggested that Zuo Gui Pill increased the levels of placental growth factor, tyrosine kinase receptor, hepatocyte growth factor, mesenchymal epithelial transforming factor protein and mRNA, which in turn induced the production of ovarian blood vessels and improved ovarian function, and the high dose was superior to the low dose.

4. Summary

In the treatment of premature ovarian failure, TCM fully embodies the concept of holistic and evidence-based treatment, and analyzes its causes in detail. The treatment is based on the four diagnostic methods, and the prescriptions are selected according to the evidence and appropriately added or subtracted to relieve the patient's symptoms, thus achieving the purpose of treating premature ovarian failure. Zogui Pill can fundamentally regulate immune factors and reproductive endocrine function, inhibit rapid apoptosis of granulosa cells, promote angiogenesis, increase local blood supply and thus improve ovarian function, effectively regulate menstrual status and alleviate perimenopausal syndrome. With the continuous development of Chinese medicine, Chinese medicine is increasingly accepted and recognized for its precise dialectic, excellent curative effect and minimal toxic side effects. However, there is still a lack of consensus on dialectic and standardized diagnosis and treatment system in TCM, and the subjective and empirical nature of TCM evidence classification, the immaturity of the clinical prescription system and the lack of clear criteria for evaluating efficacy are still the directions that TCM practitioners urgently need to continue to investigate. Therefore, TCM practitioners should increase their research efforts in related areas in order to provide more scientific basis for standardized treatment in TCM.

Acknowledgement

This work was supported in part by the National Natural Science Foundation of China (No. 81373681), Natural Science basic Research Program of Shaanxi Province (No. 2017JM8091).

References

[1] Li Zuang, Cui Xiaoping, Ma Rui, et al. Research progress in the classification of premature ovarian failure symptoms[J]. Journal of Changchun University of Traditional Chinese Medicine, 2021, 37(04): 920-923.
[2] Zhang Bing, Liu Jinxing. Clinical research and treatment progress of premature ovarian failure in Chinese and Western medicine[J]. Journal of Modern Traditional Chinese and Western Medicine, 2020, 29(32): 3643-3648.
[3] Yang Lixia. Ideas and methods of Chinese medicine evidence and treatment of premature ovarian failure [J]. Sichuan Traditional Chinese Medicine, 2005(08): 11-12.
[4] Duan Feiyan, Li Qin, Liu Lu, et al. Advances in the diagnosis and treatment of premature ovarian failure[J]. China Medical Science, 2016, 6(07): 38-42+66.
[5] Wang Yuanshuo, Zhang Limei. Advances in Chinese medicine research on premature ovarian failure[J]. Journal of Qiannan National Medical College, 2019, 32(02): 100-103.
[6] Zhou Mei. Overview of ancient Chinese medical research on premature ovarian failure [J]. Special Health, 2019(23): 109.
[7] Wang Yaru, Su Jian, Di XiaoQian, et al. Research advances in premature ovarian failure[J]. Medical Review, 2019, 25(19): 3895-3899.
[8] Qu Liyuan, Zhao Wenjuan, Xie Jiejie, et al. Exploring the Chinese medical etiology and pathogenesis of premature ovarian failure[J]. Chinese Ethnic Folk Medicine, 2016, 25(15): 70-71.

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

[9] Jiang Ying. Epidemiological survey of premature ovarian failure and progress of Chinese medicine treatment[J]. Asia-Pacific Traditional Medicine, 2011, 7(04): 163-165.
[10] Lu Jing, Zou Shuxuan. An analysis of the etiology and pathogenesis of premature ovarian failure from the perspective of Chinese medicine[J]. Asia-Pacific Traditional Medicine, 2018, 14(05): 128-129.
[11] Dong Xiaoying, Li Donghua, Liu Shunyu, et al. The pathogenesis and treatment of premature ovarian failure under the perspective of Ezinc Ginseng and Western medicine[J]. Journal of Traditional Chinese Medicine, 2014, 20(03): 1-3.
[12] Wang Xia, Shi Yanqiu. Chinese medicine etiology and treatment of premature ovarian failure[J]. Jilin Chinese Medicine, 2009, 29(11): 939-940.
[13] Qiao Huaili, Liu Jingjun. Advances in Chinese medicine etiology and treatment of premature ovarian failure[J]. Gansu Traditional Chinese Medicine, 2009, 22(09): 80-81.
[14] Zhu Ling, Luo Songping. Luo Songping's treatment of premature ovarian failure from the theory of yin and yang[J]. Chinese Journal of Basic Chinese Medicine, 2020, 26(06): 841-843.
[15] Wan Yunhui. Prof. Zhang Yuzhen's experience in the treatment of premature ovarian failure[J]. Shi-Zhen Guomao, 2014, 25(07): 1721-1722.
[16] Tao Lixin, Teng Xiuxiang, Zhao Fengchao, et al. The application of latent category model in the classification of premature ovarian failure in Chinese medicine[J]. Beijing Traditional Chinese Medicine, 2012, 31(08): 566-568.
[17] He Jing, Wang Ying. Study on the distribution pattern of TCM evidence and factors influencing premature ovarian failure[J]. Journal of Guiyang College of Traditional Chinese Medicine, 2018, 40(04): 49-52.
[18] Tang Minghui. A trial of Chinese medicine treatment for premature ovarian failure[J]. Journal of Practical Chinese Medicine, 2013, 29(07): 598.
[19] Chen Yingying. Analysis of the distribution of TCM evidence and medication pattern in premature ovarian failure based on the TCM heritage assistance platform[D]. Gansu University of Traditional Chinese Medicine, 2019.
[20] Liu Chunni. Study on the distribution of Chinese medical evidence in premature ovarian failure [D]. Chengdu University of Traditional Chinese Medicine, 2011.
[21] Li Dieying. Study on the pattern of Chinese medical evidence and its correlation with influencing factors in premature ovarian failure [D]. Guangzhou University of Traditional Chinese Medicine, 2010.
[22] Huang Hongli, Dong Li. A brief description of the distribution characteristics of the evidence patterns of premature ovarian failure[J]. Guangming TCM, 2015, 30(05): 1146-1148.
[23] Wang Jing. Analysis of the pharmacological effects and clinical application value of Lycium barbarum[J]. Asia-Pacific Traditional Medicine, 2014, 10(07): 50-51.
[24] Li Jinying, Zhang Zhaoping, Ye Jinfei, et al. Study on the mechanism of action of Cuscuta sinensis-Lycium barbarum medicine in the treatment of premature ovarian failure based on network pharmacology[J]. Chinese Pharmacy, 2020, 31(18): 2202-2209.
[25] Zhang Fengfeng, Cui Xiaoping, Zhao Panting, et al. Experience of Dr. Cui Xiaoping in the treatment of premature ovarian failure[J]. Western Chinese Medicine, 2022, 35(02): 48-51.
[26] Che Yongzhi, Ma Hongbo. Advances in Traditional Chinese Medicine Research on Premature Ovarian Failure[J]. Asia-Pacific Traditional Medicine, 2018, 14(11): 87-89.
[27] Jiao Cun, Lu Xianjie. Meta-analysis of Zuo Gui Wan plus and minus formula combined with hormones in the treatment of premature ovarian failure[J]. Chinese Journal of Physicians, 2019, 21(07): 1002-1006.
[28] Zhao Panting, Cui Xiaoping. Overview of research on the treatment of gynecological diseases with Zuo Gui Wan[J]. China Medicine Herald, 2020, 17(32): 40-43.
[29] Chen Guansu, Shi Yanqiu. Overview of research on the etiology and mechanism of premature ovarian failure and its treatment in Chinese medicine[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2014, 16(12): 205-208.
[30] Zhu Ling, Luo Songping, Xu Limian, et al. Effect of Zuojiuwan on the expression of Fas and Fas-L in immune ovarian premature failure[J]. Journal of Jiangxi College of Traditional Chinese Medicine, 2008, 20(1): 52-55.
[31] Hu Qinglian, Sun Wenjun. Study on the therapeutic effect of Zuojiwan on immune premature ovarian failure [J]. Clinical Research in Traditional Chinese Medicine, 2018, 10(19): 1-4.
[32] Duan Heng, Zhou Ying. Effect of Zuo Gui Wan on the development of the uterus in immature rats [J]. World Science and Technology - Modernization of Traditional Chinese Medicine, 2014, 16(2): 364-367.

ISSN 2706-6819 Vol.4, Issue 8: 46-52, DOI: 10.25236/IJFM.2022.040808

[33] Zhu Ling, Luo Songping, Xu Limian, et al. Protective effect of Zuo Gui Wan on autoimmune ovarian injury in mice[J]. Chinese Journal of Integrative Medicine, 2005(10): 920-924.
[34] Yang Songwei, Sun Xiaofeng, He Youshun, et al. Effects of Zuo Gui Wan on ovarian function in mice with premature ovarian failure caused by chemotherapy[J]. Chinese Patent Medicine, 2016, 38(04): 717-722.
[35] Cheng Binbin, Zhu Ling, Li Changzheng, et al. Effects of Zuo Gui Wan on reproductive endocrine secretion in female mice with Yin deficiency model[J]. Journal of Modern Traditional Chinese and Western Medicine, 2003(13): 1362-1364.
[36] Massin N, Méduri G, Bachelot A, Misrahi M, Kuttenn F, Touraine P. Evaluation of different markers of the ovarian reserve in patients presenting with premature ovarian failure. Mol Cell Endocrinol. 2008 Jan 30;282(1-2):95-100.
[37] Zhu Ling, Luo Songping, Xu Limian. Effect of Zuo Gui Wan on the ultrastructure of ovaries in mice with immune ovarian premature failure[J]. Shi-Zhen Guomao, 2016, 27(01): 42-44.
[38] Zhu Ling, Luo Songping, Xu Limian, et al. Effects of Zuo Gui Wan on ovarian Bcl-2 and Bax protein expression in mice with immune ovarian premature failure[J]. New Drugs in Chinese Medicine and Clinical Pharmacology, 2012, 23(04): 381-386.
[39] Yan Jie, Qiao Jie. Protection and preservation of reproductive capacity[J]. Chinese Journal of Practical Gynecology and Obstetrics, 2010, 26(10): 772-776.
[40] Tilly KI, Banerjee S, Banerjee PP, Tilly JL. Expression of the p53 and Wilms' tumor suppressor genes in the rat ovary: gonadotropin repression in vivo and immunohistochemical localization of nuclear p53 protein to apoptotic granulosa cells of atretic follicles. Endocrinology. 1995 Apr; 136(4):1394-402.
[41] Zhao Fenqin, Zhao Yan, Liu Jieying, et al. Effects of Zuo Gui Wan on apoptosis and p53 protein expression in ovarian tissues of rats with 6.0 Gy γ-ray injury[J]. Global Chinese Medicine, 2022, 15(03): 378-383.
[42] Su Yan, Xue Ruixia, Li Sha, et al. Effect of sequential yin-yang therapy on ovarian VEGF expression in rats with chemotherapy-induced POF [J]. Journal of Shaanxi College of Traditional Chinese Medicine, 2014, 37(5): 83-87.
[43] Wu Xiaoyan, Cui Xiaoping, Fan Meiling, et al. Clinical and experimental research progress of Zuo Gui Wan in the treatment of premature ovarian failure[J]. Journal of Chinese Medicine, 2020, 35(03): 568-572.
[44] Wu Di, Duan Heng. Effect of Zuo Gui Wan drug-containing serum on in vitro three-dimensional model of angiogenesis[J]. Chinese Pharmacology and Clinical Practice, 2016, 32(05): 1-4.
[45] Tang Liming, Duan Heng, Xiao Caixian, et al. Effects of Zuo Gui Wan on the relationship between ovarian angiogenesis and placental growth factor, hepatocyte growth factor and their receptors in rats [J/OL]. Chinese Journal of Integrated Traditional Chinese and Western Medicine: 1-7.