Review

DOI: 10.5582/bst.2025.01193

Therapeutic strategies in traditional Chinese medicine for premature ovarian failure: Modulation of oxidative stress and autophagy–apoptosis *via* the AMPK/mTOR pathway

Wenli Cao[1,§], Siling Liu[2,§], Leifang Zhang[2], Xiayan Fu[1], Haiyang Li[1], Feijun Ye[1], Jialu Bei[1], Chunzhi Ren[1], Yanping Ni[1], Jun Zhu[3,*], Ling Wang[4,5,*]

[1] Zhoushan Women and Children Hospital, Zhoushan, Zhejiang, China;
[2] Zhejiang Ocean University, Zhoushan, Zhejiang, China;
[3] The First People's Hospital of Wenling (Taizhou University Affiliated Wenling Hospital), School of Medicine, Taizhou University, Taizhou, Zhejiang, China;
[4] Department of Obstetrics and Reproductive Immunology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China;
[5] Department of Obstetrics, The First Affiliated Hospital, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China.

SUMMARY: Premature ovarian failure (POF), also referred to as premature ovarian insufficiency (POI), is a multifactorial reproductive endocrine disorder characterized by amenorrhea, infertility, hypoestrogenism, and elevated gonadotropin levels before the age of 40. Emerging evidence links its pathogenesis to oxidative stress and dysregulation of the autophagy–apoptosis balance in ovarian cells. Excessive accumulation of reactive oxygen species (ROS) impairs mitochondrial function in oocytes, while aberrant autophagy and granulosa cell apoptosis accelerate the depletion of primordial follicles. The AMP-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) pathway serves as a critical nexus between energy metabolism, oxidative stress, and cell survival. Traditional Chinese medicine (TCM), with its multi-component and multi-target characteristics, has demonstrated unique advantages in modulating the AMPK/mTOR pathway to restore ovarian function. This review synthesizes recent findings on single herbs, classical formulas, and non-pharmacological therapies (acupuncture and moxibustion). Mechanistic studies have revealed that these interventions can activate AMPK, inhibit mTOR overactivation, enhance Nrf2-mediated antioxidant defenses, reduce ROS production, and rebalance autophagy and apoptosis *via* pathways such as PI3K/Akt and SIRT1/p53. By aligning stage-specific regulation of AMPK/mTOR signaling with the TCM principle of syndrome differentiation, this integrative approach provides theoretical guidance for precise, personalized treatment to optimize multi-target strategies for POF management.

Keywords: premature ovarian failure, AMPK/mTOR, oxidative stress, autophagy, apoptosis, traditional Chinese medicine, acupuncture, moxibustion, Nrf2, PI3K/Akt

1. Introduction

Premature ovarian failure (POF), also known as premature ovarian insufficiency (POI), is a multifactorial and heterogeneous disease. It is characterized by amenorrhea, infertility, lower estrogen levels, and elevated gonadotropin levels in women as a result of ovarian failure before the age of 40. These conditions significantly impair female reproductive function and quality of life (*1,2*). Clinical diagnostic criteria are amenorrhea (lasting ≥ 4 months), reduced estradiol (E_2) levels, abnormally high follicle-stimulating hormone (FSH) levels (more than 4 weeks between consecutive tests, FSH > 40 IU/L), and a significant reduction in fertility (*3,4*). Worldwide, the rate of POF is between 0.9% and 1.2% (*5*). Only 5–10% of these women can conceive naturally and give birth (*6*). The causes of POF are complex, and the exact mechanisms are unclear. The generally accepted causes currently include genetic factors, autoimmune factors, medical factors such as surgery, radiotherapy, and chemotherapy, and environmental factors (*7,8*). As the pace of life has accelerated, psychological pressure on women has increased, and many adverse factors such as tumor radiotherapy have increased over the past few years, the incidence of POF has increased yearly and it tends to affect younger women (*9*). POF not only affects a woman's fertility but can also lead to osteoporosis, cardiovascular disease, and other long-term complications (*10*), seriously affecting the patient's

quality of life. Currently, hormone replacement therapy (HRT) is the main treatment for POF in clinical practice (*11*). Still, there are problems of unsatisfactory efficacy, many limitations in the form of contraindications, and an increased risk of other gynecological diseases with long-term use (*12,13*). Therefore, the pathogenesis of POF needs to be investigated and new therapeutic strategies need to be developed. Stem cells have self-repairing and regenerative capabilities, can stimulate follicles and increase hormone levels, and are effective in treating infertility and ovarian failure (*14,15*). In a study, Fu *et al.* found that adipose-derived stem cells promoted the repair of chemotherapy-induced POF by inhibiting granulosa cell apoptosis and aging (*16*). Nevertheless, the clinical use of stem cells still faces significant challenges, including high culturing costs, a limited supply, and immune rejection and ethical concerns in patients (*1*).

In recent years, the role of oxidative stress and dysregulation of cellular autophagy and apoptosis in the pathogenesis of POF has been increasingly emphasized with more in-depth molecular and cellular biology studies. Under physiological conditions, the generation and scavenging of reactive oxygen species (ROS) is dynamically balanced in the ovaries, and ROS act as signaling molecules to drive cellular regulatory pathways (*17,18*). However, stimulation by various internal and external factors leads to oxidative stress, which damages oocytes and granulosa cells and accelerates follicular atresia. Dysregulation of autophagy, an important cellular protective mechanism, can affect follicle development and survival. Apoptosis is the major form of follicular atresia. Excessive apoptosis leads to premature loss of primordial follicles, resulting in ovarian failure. Abnormal apoptosis and autophagy of granulosa cells are considered to be the key pathological mechanisms of POF (*19*).

Traditional Chinese medicine (TCM) has long been used in China for the treatment of a wide variety of illnesses (*20*). In TCM, there is no clear and systematic discussion of POF, but from the point of view of its disease characteristics, it is similar to the ancient description of a "pre-menstrual closure" and "premature menstrual cessation" type of disease, so it can be categorized as "amenorrhea," "infertility," or the like (*21*). Due to its multi-component and multi-target properties, TCM has shown unique advantages in improving ovarian function (*22*). Studies have shown that the effectiveness of TCM combined with HRT in the treatment of POF is more effective than HRT alone (*23*). Research has found that Chinese herbs can improve ovarian function by modulating the AMP-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) signaling pathway, reducing oxidative stress damage and restoring the balance between autophagy and apoptosis. AMPK, a key regulator of cellular energy metabolism, can be activated by a variety of stress conditions and then promote cellular autophagy by inhibiting mTOR signaling. This pathway plays an important role in linking oxidative stress to cellular autophagy and apoptosis (*8*).

In this study, we summarized the mechanisms of oxidative stress and cellular autophagy and apoptosis in POF and explored the therapeutic strategies based on the AMPK/mTOR pathway to provide new ideas for the clinical treatment of POF in TCM.

2. Oxidative stress and POF

Oxidative stress, characterized by excessive production of ROS or impaired antioxidant regulation, occurs in biological systems under normal and pathological conditions (*24*). ROS are the general term for oxygen derivatives with high oxidative capacities, including superoxide anion, hydrogen peroxide, and hydroxyl radicals (*25*). ROS accumulation promotes the generation of advanced oxidation protein products (AOPPs), which are key biomarkers indicative of oxidative stress. Abnormally elevated ROS attack biological macromolecules and organelles, resulting in oxidative damage to DNA, proteins, and lipids. Oxidative stress has a chronic impact on many diseases, such as diabetes, cardiovascular disease, and polycystic ovary syndrome (*26,27*). The mechanisms by which oxidative stress impairs ovarian function are complex and diverse. Excessive ROS attack oocytes and granulosa cells, leading to granulosa cell damage (*28*), weakening of nutritional support for oocytes from granulosa cells, and accelerated follicular atresia (*29*). In addition, oxidative stress inhibits steroid hormone synthases (*e.g.,* CYP19A1, which is responsible for estrogen synthesis), leading to reduced estrogen levels (*30*). Moreover, ROS promote the release of inflammatory factors (TNF-α, IL-6, *etc.*) (*31*), leading to deterioration of the ovarian microenvironment, exacerbation of ovarian fibrosis, and reduction of the available follicular reserve, as shown in Figure 1. Oxidative stress activates a variety of signaling pathways, such as the NF-κB and MAPK pathways, and promotes the release of inflammatory factors that exacerbate the deterioration of the ovarian microenvironment. This ultimately leads to a decline in ovarian function (*32-34*).

A variety of factors can induce oxidative stress in the ovaries. Environmental factors such as the plasticizer diisononyl phthalate can induce oxidative stress in ovarian granulosa cells, which in turn triggers autophagy and apoptosis. Bisphenol A (BPA) is a widespread endocrine-disrupting chemical with estrogen-like effects that has been found to promote autophagy in ovarian granulosa cells by inducing the AMPK/mTOR/ULK1 signaling pathway (*35*). Medical factors, such as metabolites of the chemotherapy drug cyclophosphamide, can induce the production of large amounts of ROS in the ovaries, leading to a dramatic reduction in follicular reserve (*36,37*). In addition, the

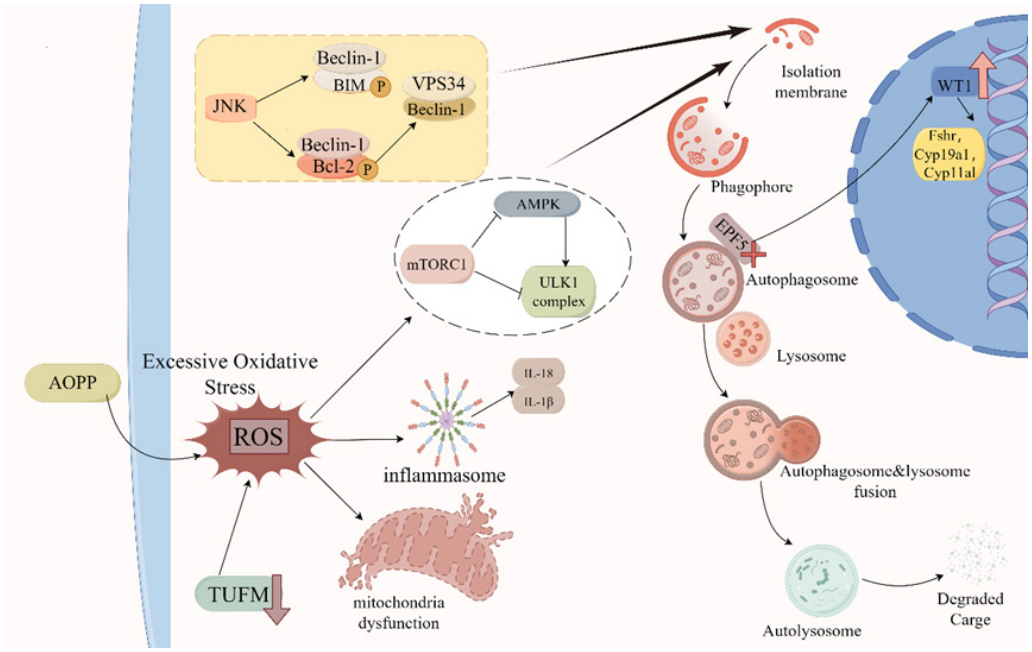

Figure 1. Signaling pathways involved in the pathogenesis of premature ovarian failure.

antioxidant capacity of the ovaries naturally declines with age, which is one of the major causes of ovarian hypoplasia in older women (*38*).

TCM may improve POF by scavenging ROS or by enhancing the antioxidant defense system. A number of active ingredients in TCM and Chinese herbal compounds have been shown to have significant antioxidant effects. For example, Angelica sinensis polysaccharide (AP) is the main chemical component of Angelica sinensis, with a content of up to 15%. AP tonifies the blood, regulates immunity, combats viruses, and is antioxidative. Li *et al.* found that (*39*) AP significantly increased the ovarian index, increased the activity of superoxide dismutase (SOD), and decreased the activity of malondialdehyde (MDA), thus inhibiting the level of oxidative stress in immune POF mice. It also reduced the levels of IL-1β and IL-6 and suppressed the level of inflammation. The Astragal polysaccharide in Astragalus can upregulate the expression of SOD, thereby scavenging ROS and slowing the development of POF. Lyceum barbarum polysaccharide (LBP) can inhibit the production of MDA and protect mitochondrial function (*40*). Salidroside can increase the proliferation of the granulosa-like tumor cell line (KGN) induced by dihydrotestosterone (DHT), activate the phosphorylation of AMPK, induce the translocation of nuclear factor E2 (Nrf2)-related factors, and target antioxidant proteins to inhibit oxidative stress damage (*41*). Danggui Shaoyao San can inhibit oxidative stress and reduce apoptosis by regulating the SIRT1/p53 signaling pathway, thereby protecting against cyclophosphamide-induced POF (*42*), and Zishen Tongmai Decoction inhibits granulosa cell apoptosis through the PI3K/Akt/mTOR pathway (*43*). These studies provide a scientific basis for TCM to combat POF by reducing oxidative stress.

3. Cellular autophagy-apoptosis and POF

Cellular autophagy and apoptosis are two important cellular processes that regulate ovarian function, and their balance is essential for maintaining normal follicular development and ovarian reserve (*19,44*). Autophagy is a complex process involving the degradation of abnormal proteins and organelles by autophagosomes (*45*). It maintains metabolism and homeostasis by selectively and non-selectively sequestering macromolecules and organelles, recognizing autophagy-selective substrates through specific receptors (*46*). Not only does autophagy play a key role in normal cellular metabolism, but it is also closely related to the onset and progression of many diseases (*47*). In the ovaries, autophagy is involved in the regulation of several physiological processes, including follicular development, atresia, and luteal degeneration, where it acts as a balance to maintain cellular homeostasis (*48*). Studies have shown that abnormal levels of autophagy are closely associated with the development of POF, and its specific role is as a "double-edged sword." Basic levels of autophagy can help remove damaged mitochondria and proteins and maintain granulocyte activity, but over-activated autophagy can lead to programmed cell death. Apoptosis, the main form of programmed cell death, is closely associated with POI and is thought to be the primary mechanism of cell death in oocytes lost during maturation from primordial follicle to antral follicle or secondary to chemotherapy (*49*). Normally, apoptosis is involved in regulating follicular atresia and maintaining the homeostasis of the primordial follicle pool. However, excessive activation of apoptosis

is an important feature of POF and can lead to premature depletion of the primordial follicle pool (*50,51*).

There is a complex relationship between autophagy and apoptosis. During mild stress, autophagy is activated as a protective mechanism to promote cell survival. However, when an injury is severe, autophagy can switch to a pro-death mechanism that acts in concert with apoptosis. This transition is regulated by multiple signaling pathways, as shown in Figure 1. Under nutrient-rich conditions, the mechanistic target of rapamycin complex 1 (mTORC1) pathway, which is activated by rapamycin, inhibits the Unc-51-like autophagy-activating kinase 1 (ULK1) complex. When nutrient levels are low, AMP-activated protein kinase (AMPK) becomes activated, which leads to the inhibition of mTORC1 and the subsequent activation of the ULK1 complex (*52,53*). Under endoplasmic reticulum stress, JNK activation phosphorylates Bcl-2, causing the release of Beclin-1. Further disruption of Bcl-2/Beclin-1 interaction is caused by JNK-phosphorylated BIM, resulting in the activation of the PI3K complex, consisting of VPS34 and Beclin-1, by ULK1 (*54*). This generates PI3P and initiates autophagic nucleation (*55*). Dysregulation of the autophagy-apoptosis balance is a key component in the pathogenesis of POF. Studies have shown that the autophagy-related proteins LC3-II and Beclin-1 are abnormally expressed in the ovarian tissue of POF patients, along with increased activity of the apoptosis marker caspase-3 (*42,56*). This dysregulation of autophagic apoptosis accelerates the depletion of the follicle pool.

Experimental studies have confirmed that a variety of herbal components and compounds can regulate the autophagy/apoptosis balance through different pathways. Baicalein inhibits excessive apoptosis through the PI3K/Akt pathway; curcumin can simultaneously activate protective autophagy through the AMPK/mTOR pathway while inhibiting apoptosis-associated protein expression (*57*). Resveratrol-βcd was able to restore the proportion and function of macrophages in the ovarian environment, delay cell autophagy and apoptosis, inhibit the progression of POF, and maintain normal ovarian function (*58*). Bushen Ningxin Soup can significantly reverse the VCD-induced reduction of primary follicles in ovarian tissue, increase FSH and luteinizing hormone (LH) concentrations, decrease serum E_2 and anti-Mullerian hormone (AMH) concentrations, decrease oocyte number and oocyte mitochondrial dysfunction, and alleviate POF (*59*). These findings provide a cell biological basis for TCM intervention in POF.

4. Therapeutic strategies based on the AMPK/mTOR pathway in TCM

Adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved serine/threonine kinase consisting of the catalytic subunit α (α1/α2), the regulatory subunit β (β1/β2) and γ (γ1/γ2/γ3) (*8*). AMPK acts as an intracellular energy receptor present in all eukaryotic cells and maintains cellular energy homeostasis mainly by regulating metabolic and immune functions. When intracellular energy levels decrease, AMPK is activated, which in turn inhibits energy-consuming anabolic processes (*e.g.*, mTOR-dependent anabolic pathways) and activates energy-producing catabolic processes (*e.g.*, autophagy) (*60*).

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase belonging to the phosphatidylinositol kinase-associated kinase (PIK) family and is a key regulator of cellular growth and metabolism that functions primarily by binding to cofactors to form two complexes, mTORC1 and mTORC2 (*61,62*). mTORC1 has been widely studied; it is sensitive to rapamycin and is mainly involved in ribosome and protein synthesis (*63*), cell autophagy (*64*), and lipid and glucose metabolism. The activity of mTORC1 is regulated by a variety of factors, including the availability of nutrients, growth factors, and energy status. When nutrients are sufficient, mTORC1 is activated to promote cell growth and anabolism, whereas when energy is insufficient, mTORC1 activity is inhibited and the cell enters a catabolic state. There are currently limited studies on mTORC2 in regard to cytoskeletal regulation, cell migration, and apoptosis.

Studies have shown that AMPK and mTORC1 are both key regulators of autophagy in response to various stressful conditions (*8*). mTORC1 activity is inhibited by AMPK through phosphorylation of tuberous sclerosis complex 2 (TSC2) (*8,65*), which inhibits cell growth and anabolism and promotes autophagy. Conversely, mTORC1 can inhibit the activity of AMPK by phosphorylating the α-subunit of AMPK, thereby maintaining the anabolic state of cells. This reciprocal regulatory mechanism allows cells to flexibly adjust their metabolic activity according to energy and nutrient status to maintain cellular homeostasis.

4.1. The AMPK/mTOR pathway and oxidative stress

The AMPK/mTOR signaling pathway is an important bridge between cellular energy metabolism and oxidative stress. As a cellular energy receptor, AMPK is activated during oxidative stress and mitigates oxidative damage through multiple pathways. On the one hand, AMPK can directly phosphorylate and activate the antioxidant transcription factor Nrf2 to promote the expression of antioxidant enzymes such as HO-1 (Heme Oxygenase-1) and NQO1 (NAD(P)H Quinone Dehydrogenase 1) (*66*). On the other hand, AMPK can reduce the source of ROS production by inhibiting mTOR, as overactivation of mTOR increases mitochondrial electron transport chain activity and ROS generation (*67*).

Research has found that in animals with POF, reduced AMPK activity associated with mTOR overactivation

led to increased oxidative damage in ovarian tissue. The use of AMPK agonists such as AICAR can significantly improve this situation. TCM renal tonics such as Icariin have been shown to enhance ovarian antioxidant capacity through activation of the AMPK/Nrf2 pathway (*68*).

Notably, activation of AMPK has dose- and time-dependent effects. Moderate activation of AMPK enhances cellular antioxidant defenses, but excessive or prolonged activation can lead to energy depletion (*69*). This explains why certain TCMs have antioxidative effects at low doses, while high doses may have the opposite effect. Therefore, the concept of 'balancing yin and yang' emphasized in the treatment of POF by TCM is highly compatible with the bidirectional regulation of the AMPK/mTOR pathway.

4.2. The AMPK/mTOR pathway and cellular autophagy-apoptosis

The AMPK/mTOR pathway is a central signaling pathway that regulates autophagy in cells, with AMPK initiating autophagosome formation by directly phosphorylating ULK1 (*70*) and simultaneously deregulating autophagy by inhibiting mTOR. Under conditions of energy stress, this regulation ensures that cells are able to maintain survival by recycling damaged components through autophagy. In apoptosis, AMPK reduces the expression of pro-apoptotic proteins by inhibiting mTOR while activating anti-apoptotic pathways such as CREB (*71*).

In POF, dysregulation of the AMPK/mTOR pathway leads to an imbalance between autophagy and apoptosis. Ovarian tissue showed blocked autophagic flux and excessive activation of apoptosis. TCMs such as Zuo Gui Wan can dual-regulate autophagy and apoptosis through the mTOR pathway: promoting protective autophagy to remove damaged mitochondria and inhibiting the caspase cascade reaction to reduce cell death (*72*). Tanghinin IIA, in contrast, restored autophagic flux and attenuated granulocyte apoptosis in an AMPK-dependent manner (*73*).

Of particular interest are possible differences in the regulatory role of the AMPK/mTOR pathway in different follicle types. Moderate autophagy in primordial follicles is important for maintaining dormancy, whereas growing follicles require more energetic support. The holistic regulatory features of TCM may be better suited to this complex situation, *e.g.*, kidney-tonifying drugs may regulate AMPK activity globally, while blood-activating drugs may be more targeted to the local microenvironment of specific follicle groups.

4.3. AMPK/mTOR pathway-based TCM for treatment of POF

Based on the multi-target regulatory properties of the AMPK/mTOR pathway, TCM has demonstrated unique therapeutic advantages in the treatment of POF. Its mechanism of action may regulate the AMPK/mTOR pathway through multiple links and pathways (Figure 2).

Kidney-tonifying herbs such as Radix rehumanize, Ginsenoside Rg1, Corni Fructus (*74*), and Epimedium glycoside are rich in polysaccharides and flavonoids, which can activate AMPK to boost antioxidant defenses. Experiments have shown that polysaccharides from Radix rehumanize can dose-dependently increase p-AMPK expression and improve ovarian reserve. Blood-activating drugs such as Salvia miltiorrhiza, Rhizome Ligusticum Chuanxiong, and Saffron contain phenolic acid components that inhibit mTOR overactivation and regulate autophagic flux. Liver-sparing drugs such as Chaihu and Xiangfu may indirectly affect AMPK activity by improving ovarian microcirculation.

In terms of compound studies, Yougui Pills may

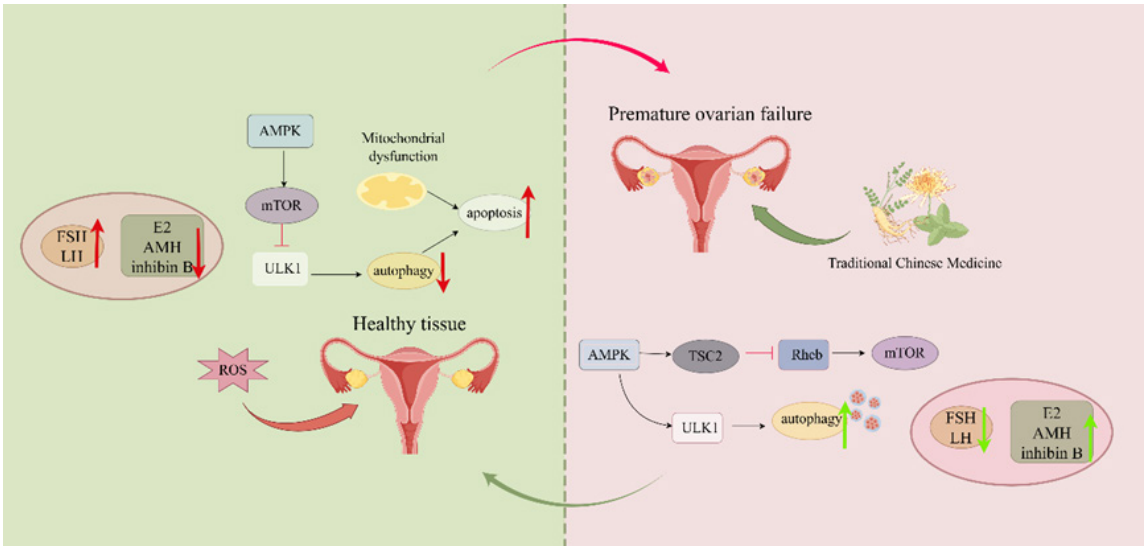

Figure 2. Schematic diagram of traditional Chinese medicine based on the AMPK/mTOR signaling pathway for the treatment of premature ovarian failure.

protect cyclophosphamide-injured ovaries by promoting autophagy and inhibiting apoptosis *via* the AMPK/mTOR pathway. The two immortal gums, turtle and deer, displayed the ability to regulate the AMPK/Nrf2/HO-1 pathway and reduce oxidative stress injury. These combinations reflect the 'multi-component, multi-target' nature of TCM, which is better suited to regulating complex network pathways such as AMPK/mTOR.

In addition, TCM can also treat POF through acupuncture and moxibustion (*20*). Acupuncture and moxibustion treatment for POF is based on the principle of "tonifying the kidney and filling the essence, regulating Chong Ren," and the main acupoints such as Guan Yuan, the Uterus, and Sanyinjiao, with evidence-based acupuncture points that are used to regulate the function of the hypothalamus-pituitary-ovary axis (HPOA) through acupoint stimulation, while directly affecting the local microenvironment of the ovaries. Acupuncture stimulation activates the phosphorylation of the AMPKα subunit, promotes the expression of Nrf2-mediated antioxidant enzymes (*e.g.*, SOD, CAT), and reduces the level of MDA in ovarian tissue. At the same time, it promotes autophagosome formation and scavenges protein aggregates that have accumulated due to oxidative damage by inhibiting mTORC1 signaling and upregulating Beclin-1 and LC3-II protein expression. Randomized controlled clinical trials have shown that acupuncture combined with HRT can significantly increase serum AMH levels in patients with POF and that its efficacy is superior to that of HRT alone, without hormone-related adverse effects.

Moxibustion is a traditional Chinese therapy using a burned moxa stick made from dried mugwort. The combustion of the mugwort permits transmission of heat to the body that causes various pathologic changes (*75*). Available research suggests that moxibustion therapy has significant therapeutic value in the areas of arthritis (including osteoarthritis of the knee) and pain management (*76,77*). This pleiotropic effect includes improvement of immune function and inhibition of oxidative stress and apoptosis (*78*). Moxibustion has also displayed unique benefits in the treatment of POF. Research has shown that moxibustion can help to regulate the menstrual cycle and increase blood flow to the ovaries (*79*). It can also balance hormone levels (such as E_2, aromatase, and testosterone) and enhance ovarian function (*80*). Its therapeutic principle is based on the TCM theory of 'warming the kidney and filling the essence, regulating the Chong ren,' which improves ovarian function by stimulating specific acupoints with warmth and heat. Commonly used acupuncture points include Guanyuan, Qihai, and Sanyinjiao. Together with back acupuncture points such as Ren Yu and Vital Gate, it constitutes a therapeutic program of anterior and posterior matching points. It increases ATP levels in ovarian tissue, activates the AMPK-sensing energy state, and then inhibits the over-activation of mTOR. Studies (*78,79,81*) have shown that moxibustion can effectively increase levels of E_2 and AMH while decreasing levels of the hormones FSH and LH. Moxibustion can also activate the Nrf2/HO-1 and PI3K/Akt signaling pathways in the ovaries, thereby reducing inflammatory injury and improving ovarian reserve function. Activation of the AMPK/mTOR pathway by moxa displays "biphasic regulation." In the early phase (1-2 weeks of treatment), it mainly inhibits mTOR and promotes autophagy to remove the damaged components; in the late phase (3-4 weeks), it maintains cellular homeostasis through the balance of AMPK/mTOR, which is highly compatible with the therapeutic principle of TCM, which is "first pass and then replenish."

5. Conclusion and future perspectives

POF is a complex gynecological endocrine disease with a pathogenesis that is closely related to the imbalance of oxidative stress and autophagy and apoptosis (*37,82*), and the AMPK/mTOR signaling pathway, as a core regulatory network linking energy metabolism, oxidative stress, and autophagy and apoptosis (*83*), plays a key role in the development of POF. This review systematically described the mechanisms of oxidative stress and autophagy-apoptosis in POF. This work has synthesized current evidence on TCM interventions targeting the AMPK/mTOR pathway and, for the first time, highlighted how the stage-specific regulatory roles of AMPK/mTOR signaling align with the TCM principle of syndrome differentiation and treatment.

Numerous studies have shown that TCM can effectively regulate the AMPK/mTOR pathway, reduce oxidative stress damage, and restore the balance of cellular autophagy and apoptosis through its multi-component and multi-target properties, thus improving ovarian function. These findings provide an important theoretical basis and practical guidance for the combined treatment of POF with Chinese and Western medicine (Table 1).

However, the current study had several limitations. First, most of the cited studies are still at the animal experiment or cellular level, with insufficient evidence for clinical translation. Second, studies on the mechanisms by which TCM regulates the AMPK/mTOR pathway lack depth, and there is a lack of studies on spatio-temporal specific regulation in particular. In addition, network pharmacological studies on the interactions between the complex components of TCM complexes and the AMPK/mTOR pathway need to go into further depth.

That said, combining Chinese and Western medicine in the treatment of POF is worth exploring. Examples include combining TCM with assisted reproductive technology to improve oocyte quality and endometrial tolerance by regulating the AMPK/mTOR pathway or combining TCM with HRT to reduce adverse effects and

Table 1. Summary of traditional Chinese medicine interventions for POF *via* oxidative stress and autophagy-apoptosis pathways

Name	Formula	Mechanism of action	Signaling pathway	Type of research	Experimental model	Key findings	*Ref.*
Red Ginseng, RG	Saponins, sugars, amino acid derivatives	Antioxidant, promotes granular cell proliferation	Nrf2/HO-1,PI3K-Akt	Animal experiments, cell experiments	Cyclophosphamide/ D-galactose (D-Gal)-induced POF model	Reduces the expression of aging-related proteins and has estrogen-like effects	(*84, 85*)
Dodder	Flavonoid, quercetin	Inhibits cell apoptosis	/	Animal experiments	Tripterygium glycoside /cyclophosphamide-induced POF	Promotes the restoration of hypothalamic-pituitary gonadotropic function, enhances the responsiveness of the pituitary gland and ovaries to hormones, and promotes follicular development	(*86, 87*)
Curcuma longa Linn	Curcumin	Reduce oxidative stress and inhibits apoptosis	AMPK/mTOR,Nrf2/ HO-1,PI3K-Akt	Animal experiments, cell experiments	Human ovarian granulosa cell line + mice with POF model	Alleviate ovarian dysfunction and apoptosis caused by oxidative stress	(*57, 88*)
Astragalus	Astragaloside IV	Inhibits oxidative stress and improves ovarian reserve function	Nrf2/ARE	Animal experiments	Cyclophosphamide induced POF in rats	Reduces apoptosis of granulosa cells in rats with POF and increases the number of primordial follicles, primary follicles, and antral follicles	(*89*)
Danggui Shaoyao Powder	*Paeoniae Alba Radix* (Bai Shao), *Angelicae Sinensis Radix* (Dang Gui), *Atractylodes Macrocephala Koidz* (Bai Zhu), *Poria* (Fu Ling), *Chuanxiong Rhizoma* (Chuan Xiong), *Alismatis Rhizoma* (Ze Xie)with a 1:3:4:4:8:8 ratio	Inhibits follicular atresia	AMPK/mTOR, PI3K/Akt/FOXO3a	Animal experiments	Cyclophosphamide/ D-galactose (D-Gal)-induced POF model	Raises estrogen levels, decreases the Bax/Bcl2 ratio	(*42, 90, 91*)
Dingkun Pill	*Ginseng Rubra Radix*(Hong Shen), *Cervi Pantotrichum Cornu* (Lu Rong), *Croci Stigma*(Xi Hong Hua), *Spatholobi Caulis*(Ji Xue Teng Gao), *Paeoniae Alba Radix* (Bai Shao), *Rehmanniae Praeparata Radix* (Shu Di Huang), *Angelicae Sinensis Radix* (Dang Gui), *Scutellariae Radix* (Huang Qin), *etc.*	Reduce oxidative stress and inhibit apoptosis	PI3K/Akt/mTOR	Animal experiments, Randomized controlled trial	Tripterygium glycoside induced POF	Upregulate the expression levels of Bax, Cyt C, and Caspase-3, downregulate the expression level of Bcl-2, and downregulate the ratio of Bcl-2 to Bax	(*92, 93*)

Table 1. Summary of traditional Chinese medicine interventions for POF *via* oxidative stress and autophagy-apoptosis pathways (continued)

Name	Formula	Mechanism of action	Signaling pathway	Type of research	Experimental model	Key findings	*Ref.*
Bu-Shen-Ning-Xin decoction	*Rehmanniae Praeparata Radix* (Shu Di Huang), *Paeoniae Alba Radix* (Bai Shao), *Corii Asini Colla*(E Jiao), *Coptidis Rhizoma* (Huang Lian), *Scutellariae Radix* (Huang Qin), *Poria* (Fu Ling), *Nelumbinis Plumula* (Lian Zi Xin), *Schisandrae Chinensis Fructus* (Wu Wei Zi), *Cornus*(Shan Yu Rou), *Uncariae cum Uncis Ramulus* (Gou Teng) and *Concha Cypraeae Violacae* (Zi Bei Chi)	Inhibition of oxidative stress	PI3K/AKT/mTOR, rno_circRNA_012284/ rno_miR-760–3p/ HBEGF(Heparin-binding epidermal growth factor-like growth factor)	Animal experiments, cell experiments	VCD induced the *in vivo* and *in vitro* POF model.	Reduces mitochondrial oxidative stress and apoptosis in ovarian germ cells (OGCs), improves ovarian microenvironment	(*59, 94*)
Yu Linzhu	*Panax ginseng*(Ren Shen)6g, *Poria* (Fu Lin)6g, *Atractylodes Macrocephala Koidz* (Bai Zhu)6g, Radix *Glycyrrhizae* (Gan Cao)3g, *Angelica Sinensis* (Dang Gui)12g, *Rehmanniae Preparata Radix* (Shu Di Huang)12g, *Ligusticum sinense* (Chuan Xiong)3g, *Paeoniae Alba Radix* (Bai Shao)6g, *Cuscutae Semen* (Tu Si Zi)12g, *Eucommia Ulmoides Oliv* (Du Zhong)6g, *Cervus nippon Temminck* (Lu Jiao Shuang)6g, *Zanthoxyli Pericarpium* (Hua Jiao)6g	improve oocyte mitochondrial function, ovarian oxidative stress, and ovarian microenvironment	hif1α/cx43,mTOR	Animal experiments, Randomized controlled trial	Mouse zona pellucida 3 (Zp3)/VCD induced POF model	Improves ovarian function by alleviating hormone levels, ovarian morphology, follicular development, the proliferation and energy metabolism of OGCs	(*95-98*)
Acupuncture and moxibustion	Guanyuan point, Sanyinjiaopoints	Inhibition of excessive autophagic damage in ovarian granulosa cells and reduction of follicular atresia	PTEN/AKT,PI3K/ Akt	Animal experiments, Randomized controlled trial	Cyclophosphamide-induced POF model	Regulate the expression of autophagy signaling pathways and key proteins, adjust the autophagy level of ovarian granulosa cells	(*99-102*)
Moxibustion	Guanyuan point, Zhongwan point, Shenque point	Improvement of reproductive hormone levels, reduction of inflammatory responses, modulation of immunity	PI 3 K/Akt/mTOR	Randomized controlled trial	Tripterygium glycoside induced POF	Increases estrogen levels and reduces inflammatory cell infiltration, thereby reducing inflammatory damage	(*78, 99*)

improve efficacy. As understanding of the AMPK/mTOR pathway grows and research into modernizing TCM continues, new avenues will open up for the prevention and treatment of POF.

Funding: This work was supported by a grant from Medical and Health Technology Plan Projects of the City of Zhoushan, Zhejiang Province (grant No. 2023YB04 to Wenli Cao), a grant from the Zhejiang Provincial Department (grant No. 2024KY549, Jun Zhu), and a grant from the National Natural Science Foundation of China (grant No. 82374243, Ling Wang).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Li M, Xiao YB, Wei L, Liu Q, Liu PY, Yao JF. Beneficial effects of traditional Chinese medicine in the treatment of premature ovarian failure. Evid Based Complement Alternat Med. 2022; 2022:5413504.
2. Kim HK, Kim TJ. Current status and future prospects of stem cell therapy for infertile patients with premature ovarian insufficiency. Biomolecules. 2024; 14.
3. Shi YQ, Zhu XT, Zhang SN, Ma YF, Han YH, Jiang Y, Zhang YH. Premature ovarian insufficiency: A review on the role of oxidative stress and the application of antioxidants. Front Endocrinol (Lausanne). 2023; 14:1172481.
4. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, Vermeulen N. Eshre guideline: Management of women with premature ovarian insufficiency. Hum Reprod. 2016; 31:926-937.
5. Chen Q, Sun L, Huang J, Huang F, Guo S, Chai Y. Three-dimensional transvaginal ultrasonography in the evaluation of diminished ovarian reserve and premature ovarian failure. Pak J Med Sci. 2023; 39:747-751.
6. Nelson LM. Clinical practice. Primary ovarian insufficiency. N Engl J Med. 2009; 360:606-614.
7. Goswami D, Conway GS. Premature ovarian failure. Hum Reprod Update. 2005; 11:391-410.
8. Chun Y, Kim J. AMPK-mTOR signaling and cellular adaptations in hypoxia. Int J Mol Sci. 2021; 22.
9. Juan L, Ling X, Yang G. Research progress and status quo of pathogenesis and treatment of premature ovarian failure. China Med Pharm. 2021; 11:58-61. (in Chinese)
10. Wu J, Zhuo Y, Liu Y, Chen Y, Ning Y, Yao J. Association between premature ovarian insufficiency and gut microbiota. BMC Pregnancy Childbirth. 2021; 21:418.
11. Panay N, Anderson RA, Nappi RE, Vincent AJ, Vujovic S, Webber L, Wolfman W. Premature ovarian insufficiency: An International Menopause Society White Paper. Climacteric. 2020; 23:426-446.
12. He Y, Chen D, Yang L, Hou Q, Ma H, Xu X. The therapeutic potential of bone marrow mesenchymal stem cells in premature ovarian failure. Stem Cell Res Ther. 2018; 9:263.
13. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288:321-333.
14. Shareghi-Oskoue O, Aghebati-Maleki L, Yousefi M. Transplantation of human umbilical cord mesenchymal stem cells to treat premature ovarian failure. Stem Cell Res Ther. 2021; 12:454.
15. Li H, Zhao W, Wang L, Luo Q, Yin N, Lu X, Hou Y, Cui J, Zhang H. Human placenta-derived mesenchymal stem cells inhibit apoptosis of granulosa cells induced by IRE1α pathway in autoimmune POF mice. Cell Biol Int. 2019; 43:899-909.
16. Ai G, Meng M, Guo J, Li C, Zhu J, Liu L, Liu B, Yang W, Shao X, Cheng Z, Wang L. Adipose-derived stem cells promote the repair of chemotherapy-induced premature ovarian failure by inhibiting granulosa cells apoptosis and senescence. Stem Cell Res Ther. 2023; 14:75.
17. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, Dong W. ROS and ROS-mediated cellular signaling. Oxid Med Cell Longev. 2016; 2016:4350965.
18. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev. 2014; 94:909-950.
19. Liu YX, Ke Y, Qiu P, Gao J, Deng GP. LncRNA NEAT1 inhibits apoptosis and autophagy of ovarian granulosa cells through miR-654/STC2-mediated MAPK signaling pathway. Exp Cell Res. 2023; 424:113473.
20. Chen M, He QD, Guo JJ, Wu QB, Zhang Q, Yau YM, Xie YF, Guo ZY, Tong ZY, Yang ZB, Xiao L. Electro-acupuncture regulates metabolic disorders of the liver and kidney in premature ovarian failure mice. Front Endocrinol (Lausanne). 2022; 13:882214.
21. Dai YT, Li J, Zhang Y, Wang R, Shui RZ, Li XM, Wang XB. Research progress of PI3K/AKT/FOXO3a signal pathway regulated by traditional Chinese and Western medicine in the treatment of premature ovarian failure. Asia-Pac Tradit Med. 2024; 20:247-250. (in Chinese)
22. Shui RZ, Li XM, Zhang Y, Wang R, Li J, Dai YT, Wang XB. Rearch progress of traditional Chinese medicine treatment of premature ovarian failure based on PI3K/AKT signaling pathway. J Pract Tradit Chin Intern Med. 2024; 38:85-88. (in Chinese)
23. Kou MJ, Ding XF, Chen JX, Liu Y, Liu YY. Traditional Chinese medicine combined with hormone therapy to treat premature ovarian failure: A meta-analysis of randomized controlled trials. Afr J Tradit Complement Altern Med. 2016; 13:160-169.
24. Song QX, Sun Y, Deng K, Mei JY, Chermansky CJ, Damaser MS. Potential role of oxidative stress in the pathogenesis of diabetic bladder dysfunction. Nat Rev Urol. 2022; 19:581-596.
25. Kakinuma K, Kakinuma T. Significance of oxidative stress and antioxidant capacity tests as biomarkers of premature ovarian insufficiency: A case control study. World J Clin Cases. 2024; 12:479-487.
26. Peker N, Turan G, Ege S, Bademkıran MH, Karaçor T, Erel Ö. The effect of clomiphene citrate on oxidative stress parameters in polycystic ovarian syndrome. J Obstet Gynaecol. 2021; 41:112-117.
27. El-Kenawi A, Ruffell B. Inflammation, ROS, and mutagenesis. Cancer Cell. 2017; 32:727-729.
28. Kaltsas A, Zikopoulos A, Moustakli E, Zachariou A, Tsirka G, Tsiampali C, Palapela N, Sofikitis N, Dimitriadis F. The silent threat to women's fertility: Uncovering

the devastating effects of oxidative stress. Antioxidants (Basel). 2023; 12.
29. Tripathi A, Shrivastav TG, Chaube SK. Aqueous extract of azadirachta indica (neem) leaf induces generation of reactive oxygen species and mitochondria-mediated apoptosis in rat oocytes. J Assist Reprod Genet. 2012; 29:15-23.
30. Yang Y, Tao SY, Zhao PW,Lu D,Yang L,Rao CC. Progress in the regulation of granulosa cell apoptosis in premature ovarian failure. J Med Res. 2018; 47:16-19. (in Chinese)
31. Huang Y, Zhang Q, Cao W, Zhang Q, Wang L, Lai D. TNF-α and IFN-γ prestimulation enhances the therapeutic efficacy of human amniotic epithelial stem cells in chemotherapy-induced ovarian dysfunction. Inflamm Regen. 2023; 43:57.
32. Lundgren CAK, Sjöstrand D, Biner O, Bennett M, Rudling A, Johansson AL, Brzezinski P, Carlsson J, von Ballmoos C, Högbom M. Scavenging of superoxide by a membrane-bound superoxide oxidase. Nat Chem Biol. 2018; 14:788-793.
33. Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxid Med Cell Longev. 2019; 2019:5080843.
34. Wang HH, Hu J, Yao GD. Research progress on mechanism of premature ovarian failure and its prevention and treatment strategies. J Reprod Med. 2023; 32:1909-1914. (in Chinese)
35. Lin M, Hua R, Ma J, Zhou Y, Li P, Xu X, Yu Z, Quan S. Bisphenol a promotes autophagy in ovarian granulosa cells by inducing AMPK/mTOR/ULK1 signalling pathway. Environ Int. 2021; 147:106298.
36. Lopez SG, Luderer U. Effects of cyclophosphamide and buthionine sulfoximine on ovarian glutathione and apoptosis. Free Radic Biol Med. 2004; 36:1366-1377.
37. Yang Y, Tang X, Yao T, Zhang Y, Zhong Y, Wu S, Wang Y, Pan Z. Metformin protects ovarian granulosa cells in chemotherapy-induced premature ovarian failure mice through AMPK/PPAR-γ/SIRT1 pathway. Sci Rep. 2024; 14:1447.
38. Sasaki H, Hamatani T, Kamijo S, Iwai M, Kobanawa M, Ogawa S, Miyado K, Tanaka M. Impact of oxidative stress on age-asociated decline in oocyte developmental competence. Front Endocrinol (Lausanne). 2019; 10:811.
39. Li L, Lu P, Gao X. Angelica polysaccharide regulates endocrine function in mice with immune premature ovarian failure *via* AKT/FOXO3 pathway. Genomics Appl Biol. 2019; 38:3268-3272. (in Chinese)
40. Jiang Y, Wang H, Yu X, Ding Y. Lycium barbarum polysaccharides regulate AMPK/Sirt autophagy pathway to delay D-gal-induced premature ovarian failure. China J Chin Mater Med. 2022; 47:6175-6182. (in Chinese)
41. Chen L, Mo Q, Wu Y, Chen W, Deng K, Xiao Y. Ameliorative effect of salidroside on the cyclophosphamide-induced premature ovarian failure in a rat model. Free Radic Res. 2024; 58:107-116.
42. Chen H, Zhang G, Peng Y, *et al*. Danggui shaoyao san protects cyclophosphamide-induced premature ovarian failure by inhibiting apoptosis and oxidative stress through the regulation of the SIRT1/p53 signaling pathway. J Ethnopharmacol. 2024; 323:117718.
43. Wu J, Wen M, Wang Z, Yu K, Jin X, Liu C, Song Q, Zhang G, Wu B, Li Y. Network pharmacological analysis and experimental verification of Zisheng Tongmai decoction in the treatment of premature ovarian failure. Naunyn Schmiedebergs Arch Pharmacol. 2024.
44. Delcour C, Amazit L, Patino LC, Magnin F, Fagart J, Delemer B, Young J, Laissue P, Binart N, Beau I. ATG7 and ATG9A loss-of-function variants trigger autophagy impairment and ovarian failure. Genet Med. 2019; 21:930-938.
45. Li W, He P, Huang Y, *et al*. Selective autophagy of intracellular organelles: Recent research advances. Theranostics. 2021; 11:222-256.
46. Qian H, Chao X, Williams J, Fulte S, Li T, Yang L, Ding WX. Autophagy in liver diseases: A review. Mol Aspects Med. 2021; 82:100973.
47. Galluzzi L, Baehrecke EH, Ballabio A, *et al*. Molecular definitions of autophagy and related processes. Embo j. 2017; 36:1811-1836.
48. Ding Z, Shao G, Li M. Targeting autophagy in premature ovarian failure: Therapeutic strategies from molecular pathways to clinical applications. Life Sci. 2025; 366-367:123473.
49. Takahashi A, Yousif A, Hong L, Chefetz I. Premature ovarian insufficiency: Pathogenesis and therapeutic potential of mesenchymal stem cell. J Mol Med (Berl). 2021; 99:637-650.
50. Chen HT, Liu H, Mao MJ, Tan Y, Mo XQ, Meng XJ, Cao MT, Zhong CY, Liu Y, Shan H, Jiang GM. Crosstalk between autophagy and epithelial-mesenchymal transition and its application in cancer therapy. Mol Cancer. 2019; 18:101.
51. Vallet N, Boissel N, Elefant E, Chevillon F, Pasquer H, Calvo C, Dhedin N, Poirot C. Can some anticancer treatments preserve the ovarian reserve? Oncologist. 2021; 26:492-503.
52. Ito M, Yurube T, Kakutani K, Maeno K, Takada T, Terashima Y, Kakiuchi Y, Takeoka Y, Miyazaki S, Kuroda R, Nishida K. Selective interference of mTORC1/RAPTOR protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism with Akt and autophagy induction. Osteoarthritis Cartilage. 2017; 25:2134-2146.
53. Eisenberg-Lerner A, Kimchi A. The paradox of autophagy and its implication in cancer etiology and therapy. Apoptosis. 2009; 14:376-391.
54. Haberzettl P, Hill BG. Oxidized lipids activate autophagy in a JNK-dependent manner by stimulating the endoplasmic reticulum stress response. Redox Biol. 2013; 1:56-64.
55. Liu S, Yao S, Yang H, Liu S, Wang Y. Autophagy: Regulator of cell death. Cell Death Dis. 2023; 14:648.
56. Sun P, Nie M. Effect and mechanism of angelic shaoyaosan mediated AMPK/SIRT1 positive feedback loop to promote autophagy and regulate the systemic inflammatory response in acute pancreatitis. Cell Mol Biol (Noisy-le-grand). 2021; 67:101-108.
57. Duan H, Yang S, Yang S, Zeng J, Yan Z, Zhang L, Ma X, Dong W, Zhang Y, Zhao X, Hu J, Xiao L. The mechanism of curcumin to protect mouse ovaries from oxidative damage by regulating AMPK/mTOR mediated autophagy. Phytomedicine. 2024; 128:155468.
58. Hu B, Zheng X, Zhang W. Resveratrol-βcd inhibited premature ovarian insufficiency progression by regulating granulosa cell autophagy. J Ovarian Res. 2024; 17:18.
59. Dou X, Jin X, Chen X, Zhou Q, Chen H, Wen M, Chen W. Bu-Shen-Ning-Xin decoction alleviates premature ovarian insufficiency (POI) by regulating autophagy of granule cells through activating PI3K/AKT/mTOR pathway. Gynecol Endocrinol. 2022; 38:754-764.

60. Li Y, Chen Y. AMPK and autophagy. Adv Exp Med Biol. 2019; 1206:85-108.
61. Nnah IC, Wang B, Saqcena C, Weber GF, Bonder EM, Bagley D, De Cegli R, Napolitano G, Medina DL, Ballabio A, Dobrowolski R. TFEB-driven endocytosis coordinates MTORC1 signaling and autophagy. Autophagy. 2019; 15:151-164.
62. Moro-García MA, Mayo JC, Sainz RM, Alonso-Arias R. Influence of inflammation in the process of T lymphocyte differentiation: Proliferative, metabolic, and oxidative changes. Front Immunol. 2018; 9:339.
63. Kim YC, Guan KL. mTOR: A pharmacologic target for autophagy regulation. J Clin Invest. 2015; 125:25-32.
64. Shimobayashi M, Hall MN. Making new contacts: The mTOR network in metabolism and signalling crosstalk. Nat Rev Mol Cell Biol. 2014; 15:155-162.
65. Marcondes-de-Castro IA, Reis-Barbosa PH, Marinho TS, Aguila MB, Mandarim-de-Lacerda CA. AMPK/mTOR pathway significance in healthy liver and non-alcoholic fatty liver disease and its progression. J Gastroenterol Hepatol. 2023; 38:1868-1876.
66. Tonelli C, Chio IIC, Tuveson DA. Transcriptional regulation by Nrf2. Antioxid Redox Signal. 2018; 29:1727-1745.
67. Hardie DG, Ross FA, Hawley SA. AMPK: A nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012; 13:251-262.
68. Li F, Zhu F, Wang S, Hu H, Zhang D, He Z, Chen J, Li X, Cheng L, Zhong F. Icariin alleviates cisplatin-induced premature ovarian failure by inhibiting ferroptosis through activation of the Nrf2/ARE pathway. Sci Rep. 2024; 14:17318.
69. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell. 2008; 30:214-226.
70. Lin YH, Chen YH, Chang HY, Au HK, Tzeng CR, Huang YH. Chronic Niche inflammation in endometriosis-associated infertility: Current understanding and future therapeutic strategies. Int J Mol Sci. 2018; 19.
71. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol. 2011; 13:1016-1023.
72. Wang Y, Zhang QW, Pan KY, Zhao S. Research progress of zuogui pill in treatment of premature ovarian failure. Chin Arch Tradit Chin. 2025; 43:143-149. (in Chinese)
73. Huang Y, Yu SH, Zhen WX, *et al*. Tanshinone I, a new EZH2 inhibitor restricts normal and malignant hematopoiesis through upregulation of MMP9 and ABCG2. Theranostics. 2021; 11:6891-6904.
74. Sobeh M, Mahmoud MF, Abdelfattah MAO, El-Beshbishy HA, El-Shazly AM, Wink M. Hepatoprotective and hypoglycemic effects of a tannin rich extract from Ximenia americana var. caffra root. Phytomedicine. 2017; 33:36-42.
75. Yao F, Zhang Y, Kuang X, Zhou Q, Huang L, Peng J, Du S. Effectiveness and safety of moxibustion on constipation: A systematic review and meta-analysis. Evid Based Complement Alternat Med. 2020; 2020:8645727.
76. Chen FQ, Ge JF, Leng YF, Li C, Chen B, Sun ZL. Efficacy and safety of moxibustion for chronic low back pain: A systematic review and meta-analysis of randomized controlled trials. Complement Ther Clin Pract. 2020; 39:101130.
77. Chen L, Huang Z, Cheng K, Wu F, Deng H, Lin L, Zhao L, Shen X. The efficacy of jade moxibustion in knee osteoarthritis. Medicine (Baltimore). 2020; 99:e19845.
78. Lu G, Wang Q, Xie ZJ, Liang SJ, Li HX, Shi L, Li Q, Shen J, Cheng J, Shen MH. Moxibustion ameliorates ovarian reserve in rats by mediating Nrf2/HO-1/NLRP3 anti-inflammatory pathway. Evid Based Complement Alternat Med. 2021; 2021:8817858.
79. Shen J, Ai B, Shen M. Effectiveness of mild moxibustion for sub-health conditions in pre- and post-menopausal women: A randomized controlled clinical trial. Med Sci Monit. 2018; 24:2907-2911.
80. Zhao QY, Sun Y, Zhou J, Gao YL, Ma GZ, Hu ZH, Wang Y, Shi Y. Effectiveness of herb-partitioned moxibustion combined with electroacupuncture on polycystic ovary syndrome in patients with symptom pattern of kidney deficiency and phlegm-dampne. J Tradit Chin Med. 2021; 41:985-993.
81. Li HX, Shi L, Liang SJ, Fang CC, Xu QQ, Ge L, Wang Q, Chen J, Shen J, Shen ML. Moxibustion alleviates decreased ovarian reserve in rats by restoring the PI3K/AKT signaling pathway. J Integr Med. 2022; 20:163-172. (in Chinese)
82. Feng Y, Zhang W, Xu X, Wang W, Xu Y, Wang M, Zhang J, Xu H, Fu F. Protective effect of Luffa cylindrica fermentation liquid on cyclophosphamide-induced premature ovarian failure in female mice by attenuating oxidative stress, inflammation and apoptosis. J Ovarian Res. 2024; 17:24.
83. Wang L, Tang J, Wang L, Tan F, Song H, Zhou J, Li F. Oxidative stress in oocyte aging and female reproduction. J Cell Physiol. 2021; 236:7966-7983.
84. He L, Ling L, Wei T, Wang Y, Xiong Z. Ginsenoside Rg1 improves fertility and reduces ovarian pathological damages in premature ovarian failure model of mice. Exp Biol Med (Maywood). 2017; 242:683-691.
85. Tang ZY, Jiang W, Li XM, Huang QS, Zhao HH, Lai H. Protective effects of ginsenoside Rb1 on cyclophosphamide-induced premature ovarian failure in rats. Chin J Histochem Cytochem. 2014; 23:60-63. (in Chinese)
86. Wang YX, Ma N, Zhong XM, Cui R, Miao ZL. Study of TFSC on ovarian function in rat model with premature ovarian failure. Med Recapitulate. 2019; 25:2695-2699. (in Chinese)
87. Huang CS, He SD, Guan YC, Zhou RY, Tan N, Luo SC, Luo ML. Effects of dodder flavonoids and quercetin on the ovarian function in rat model of premature ovarian failure induced by tripterygium glycosides. Chin J Clin Pharmacol. 2020; 36:667-670. (in Chinese)
88. Yan Z, Dai Y, Fu H, Zheng Y, Bao D, Yin Y, Chen Q, Nie X, Hao Q, Hou D, Cui Y. Curcumin exerts a protective effect against premature ovarian failure in mice. J Mol Endocrinol. 2018; 60:261-271.
89. Li YX, Wang Y, Ren H, Zheng Y. Exploring the effect and mechanism of astragaloside IV on ovarian reserve function in premature ovarian failure rats based on oxidative stress. J Nankai Univ (Natural Science). 2025; 58:96-102. (in Chinese)
90. Jiang SS, Wang YJ, Pang QY, You C, Shao YW, Zhang YP. The effect of danggui shaoyao powder on the PI3K/AKT/FOXO3a signaling pathway in mice with decreased ovarian reserve function. J Li-shizhen Tradit Chin Med. 2021; 32:1335-1338. (in Chinese)
91. Wang SJ, Li D, Lu LY, Chen F. Exploring the protective effect of danggui shaoyao powder on ovarian function in

cyclophosphamide induced premature ovarian failure rats based on AMPK/mTOR signaling pathway. Acta Med Univ Sci Technol Huazhong. 2025; 54:80-85. (in Chinese)
92. Ma K, Chen Y, Fan X, Yuan Y, Wang K, Tian C, Li M. Dingkun Pill replenishes diminished ovarian reserve through the PI3K/AKT/mTOR signaling pathway in TWP-induced mice. J Ethnopharmacol. 2020; 262:112993.
93. Ke XH. A clinical study on dingkun pill for treating menorrhagia in cases of kidney deficiency and liver depression syndrome. Pract Clin Integr Tradit Chin West Med. 2022; 54:80-85. (in Chinese)
94. Xu X, Wang J, Jin X, Ma Q, Li H, Zhou Q, Chen W. Bu-Shen-Ning-Xin decoction ameliorates premature ovarian insufficiency by suppressing oxidative stress through rno_circRNA_012284/rno_miR-760-3p/HBEGF pathway. Phytomedicine. 2024; 133:155920.
95. Dong XY, Li SY. Yulinzhu treatment of yang deficiency of spleen and kidney type of premature ovarian failure. Chin Arch Tradit Chin Med. 2016; 34:2364-2366. (in Chinese)
96. Ruan X, Wang P, Wei M, Yang Q, Dong X. Yu linzhu alleviates primary ovarian insufficiency in a rat model by improving proliferation and energy metabolism of granulosa cells through hif1α/cx43 pathway. J Ovarian Res. 2024; 17:89.
97. Dong XY, Feng XX, Zheng XR, Cui S, Zhou WY, Cao WN. Effects of yulin pills on ovarian morphology, follicle development and mTOR signaling pathway of mice with primary ovarian insufficiency. Chin J Inf Tradit Chin Med. 2018; 25:49-53. (in Chinese)
98. Yang Z, Wei ML, Dong XY. Effects of yu linzhu on ovarian function and oocyte mitochondria in natural aging mice. Aging (Albany NY). 2021; 13:23328-23337.
99. Yang Z, Zhu Z, Yang X, Zhang W, Chen P, Jin L, Luo Q, Meng Y, Liu J. The correlation of acupuncture and moxibustion points for the treatment of diminished ovarian reserve and premature ovarian failure: A systematic review based on complex network. Medicine (Baltimore). 2024; 103:e40185.
100. Kalibinuer Y, Pei LX. Research progress on the mechanism of acupuncture in the treatment of premature ovarian insufficiency. Tradit Chin Med Rehabil. 2025; 2:50-55. (in Chinese)
101. Cui WZ, Zhu KX, Li R, Dong JW, Cheng YX, Zhang J. Exploring effects of acupuncture on ovarian function and autophagy-related factor expression in premature ovarian failure rats based on PTEN/AKT signaling pathway. New J Tradit Chin Med. 2025; 57:116-124. (in Chinese)
102. Xia W, Huang J, Zhou H, Zhong Y, Xue H, Wang Zhen DOG, Ma Q, Zhang Y, Zeng Q. Curative effect of assisted reproduction technology by traditional Chinese medicine multi-channel interventional therapy on 95 cases of *in vitro* fertilization and embryo transfer failure. J Tradit Chin Med. 2017; 37:681-687.

Received July 7, 2025; Revised August 21, 2025; Accepted August 25, 2025.

[§]These authors contributed equally to this work.

*Address correspondence to:
Ling Wang, Department of Obstetrics and Reproductive Immunology, Shanghai East Hospital, Tongji University School of Medicine, No. 1800 Yuntai Road, 200120 Shanghai, China.
E-mail: dr.wangling@vip.163.com

Jun Zhu, The First People's Hospital of Wenling (Taizhou University Affiliated Wenling Hospital), School of Medicine, Taizhou University, No. 333 Chuan'an South Road, Zhejiang 317599, China.
E-mail: ZJzhujun888@163.com

Released online in J-STAGE as advance publication August 29, 2025.