

# Ovarian Fibrosis: A Phenomenon of Concern

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## Abstract

**Objective:** Ovarian fibrosis is characterized by excessive proliferation of ovarian fibroblasts and deposition of extracellular matrix (ECM) and it is one of the principal reasons for ovarian dysfunction. This review aimed to investigate the pathogenetic mechanism of ovarian fibrosis and to clarify the relationship between ovarian diseases and fibrosis.

**Data Sources:** We searched PubMed for English language articles published up to November 2016. The search terms included ovarian fibrosis OR fibrosis, ovarian chocolate cyst OR ovarian endometrioma, polycystic ovarian syndrome (PCOS), premature ovarian failure, ECM, matrix metalloproteinases (MMPs), tissue inhibitors of matrix metalloproteinases (TIMPs), transforming growth factor-beta 1 (TGF- $\beta$ 1), connective tissue growth factor (CTGF), peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), vascular endothelial growth factor (VEGF), endothelin-1 (ET-1), and combinations of these terms.

**Study Selection:** Articles were obtained and reviewed to analyze the pathogenic mechanism of ovarian fibrosis and related ovarian diseases.

**Results:** Many cytokines, such as MMPs, TIMPs, TGF- $\beta$ 1, CTGF, PPAR- $\gamma$ , VEGF, and ET-1, are involved in ovarian fibrogenesis. Ovarian fibrogenesis is associated with various ovarian diseases, including ovarian chocolate cyst, PCOS, and premature ovarian failure. One finding of particular interest is that fibrogenesis in peripheral tissues around an ovarian chocolate cyst commonly causes ovarian function diminution, and therefore, this medical problem should arouse widespread concern in clinicians worldwide.

**Conclusions:** Patients with ovarian fibrosis are susceptible to infertility and tend to have decreased responses to assisted fertility treatment. Thus, protection of ovarian function should be a priority for women who wish to reproduce when making therapeutic decisions about ovarian fibrosis-related diseases.

**Key words:** Cytokine; Fibrosis; Ovarian Chocolate Cyst; Polycystic Ovarian Syndrome; Premature Ovarian Failure

## INTRODUCTION

Diffuse ovarian fibrosis is a principal reason for diminished ovarian function, which seriously threatens female reproductive health and quality of life. Fibrosis is a common stage that occurs in the progression of diverse chronic diseases into advanced stages.

Fibrosis is commonly seen in various organs, including heart, liver, lung, and kidney. It is characterized by excessive proliferation of fibroblasts and deposition of extracellular matrix (ECM). Without effective treatment, it can develop into severe scarring that can aggravate organic disorders and lead to functional decline and even organ failure.

Ovarian fibrosis is primarily triggered by ovarian injury caused by different factors, such as surgery, inflammation, and immune abnormalities. During tissue repair, several cytokines interact, facilitate ECM deposition, and consequently fibrogenesis occurs. Although ovarian fibrosis

has not received much attention from researchers worldwide, it plays an important role in the pathophysiological processes of the ovary.

## PATHOGENETIC MECHANISM OF OVARIAN FIBROSIS

The primary pathological features of ovarian fibrosis are a thick capsule, increased mesenchymal connective tissue, and decreased or absent follicles. Many studies have documented that numerous cytokines, including matrix metalloproteinases (MMPs), tissue inhibitors

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of matrix metalloproteinases (TIMPs), transforming growth factor-beta 1 (TGF- $\beta$ 1), connective tissue growth factor (CTGF), peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), vascular endothelial growth factor (VEGF), endothelin-1 (ET-1), and others, are involved in fibrogenesis. A complicated network system is involved in the development and progression of tissue fibrosis. During fibrogenesis, these mutually interacting factors disrupt the balance between ECM synthesis and degradation and stimulate the overproliferation of ovarian mesenchymal fibroblasts and the excessive deposition of ECM.

### Coordinated expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases

The ovary periodically experiences changes of growth, maturation, and degeneration during different stages of life and the menstrual cycle. During these processes, periodic ECM reconstruction is required, such as during follicular development and maturation, ovulation, atresia, luteinization, and luteolysis during the ovarian cycle. The maintenance of ECM homeostasis largely depends on the coordinated expression of MMPs and TIMPs to regulate the synthesis and degradation of ECM.<sup>[1]</sup> MMPs, also sometimes called metalloproteases, belong to the family of proteolytic enzymes that rely on metal ions, such as Ca<sup>2+</sup>, Zn<sup>2+</sup>, and Mg<sup>2+</sup>. TIMPs are specific inhibitors of MMPs and serve as antagonists of MMPs during ECM reconstitution and metabolism. They are both synthesized and secreted by the same cells. MMPs facilitate ECM degradation, improve microenvironment of the ECM, and regulate bioactive molecules. TIMPs dominantly suppress the activation of MMPs and decrease their activities.

### Abnormally high level of transforming growth factor-beta 1

TGF- $\beta$ 1 is a cytokine involved in the formation and development of fibrosis. TGF- $\beta$ 1 is a profibrotic cytokine that is closely associated with the synthesis of ECM components and it can trigger a variety of organ fibrosis. TGF- $\beta$ 1 is a critical cytokine that is involved in multidirectional regulation. It can promote the growth of fibroblasts and osteoblasts and consequently accelerate the development of fibrosis. In addition, it facilitates the expression of ECM, i.e., collagens and laminin, as well as inhibits the degradation of ECM. Furthermore, its expression is important in embryonic development and cytothesis where it helps stimulate the formation of cellular structure, cell proliferation, and differentiation. Previous studies<sup>[2]</sup> have shown that TGF- $\beta$ 1 can suppress the expression and activation of MMPs and upregulate the expression of protease inhibitors such as TIMPs and plasminogen activator inhibitor (PAI). These cytokines are secreted via autocrine and paracrine pathways to further enhance the synthesis of ECM components, such as fibronectin and collagens Type I, II, and III, and repress ECM degradation. Abnormal increases in TGF- $\beta$ 1 levels in the ovary can cause follicular dysplasia and ovulation failure.

### Overexpression of connective tissue growth factor

CTGF has gradually emerged as a novel indicator for identifying tissue fibrosis. CTGF is a cysteine-rich cytokine

with diverse biological functions. CTGF belongs to the CCN family, which comprised polypeptide factors with high homology in their DNA sequences. The CCN family was originally composed of three members, that is, CTGF, cysteine-rich 61 (CYR61), and nephroblastoma overexpressed gene.

Under physiological conditions, CTGF is secreted in the ovary by follicular granulosa cells and fibroblasts via autocrine or paracrine pathway. Harlow *et al.* demonstrated that CTGF messenger RNA (mRNA) was expressed in preantral follicles and early antral follicles of rats as well as follicular granulosa cells and follicular theca of hens.<sup>[3,4]</sup>

CTGF is a downstream response element of TGF- $\beta$ 1 and exhibits similar biological functions to TGF- $\beta$ 1. CTGF promotes cell proliferation, enhances the expression of cell adhesion molecules, and facilitates collagen synthesis and ECM secretion by specific cells. All these biological and pathological functions highlight the pivotal role of CTGF in the development of multiple diseases associated with tissue reconstitution, such as ulcer healing, wound healing, fibrosis, and tumorigenesis.<sup>[5-8]</sup> However, the precise role of CTGF in ovarian tissues remains unclear, but it could be reasonable to postulate that CTGF might be served as a stimulator for the function of luteinizing hormone (LH)-theca cells and the synthesis of ECM in the ovary.

Overexpression of CTGF can positively promote fibrogenesis while inhibition of CTGF can facilitate the recovery of tissue repair function and restore normal structure and function.<sup>[9]</sup> Interactions between TGF- $\beta$ 1 and CTGF regulate the development and progression of tissue fibrosis.<sup>[10]</sup> TGF- $\beta$ 1 triggers the chemotaxis of monocytes, neutrophils, and lymphocytes and enhances the ability of these cells to secrete CTGF. TGF- $\beta$ 1-induced CTGF further acts on mesenchymal fibroblasts via a paracrine mechanism, which in turn stimulates the secretion of TGF- $\beta$ 1. CTGF is a vital cytokine downstream of the TGF- $\beta$ /small mother against decapentaplegic protein (SMAD) signaling pathway. When fibrogenesis occurs, overexpressed TGF- $\beta$ 1 upregulates the expression of its downstream response element, CTGF, and stimulates the production of ECM. Both these factors interact with each other, further promoting increases in collagen production and accelerating the development of fibrosis.<sup>[11]</sup> Blockage of TGF- $\beta$ 1 can inhibit the TGF- $\beta$ 1 signaling pathway and halt the development of fibrosis. Thus, targeting TGF- $\beta$ 1 is anticipated to be an effective treatment against fibrosis.

### Role of peroxisome proliferator-activated receptor-gamma in fibrosis

PPAR- $\gamma$  is a ligand-activated transcriptional factor that belongs to the nuclear hormone receptor superfamily. PPAR- $\gamma$  plays significant roles in regulating glucose and lipid metabolism, the immune system, cell differentiation and apoptosis, and inflammatory responses. Researchers have demonstrated that PPAR- $\gamma$  agonists can suppress the transduction of the TGF- $\beta$ 1 signaling pathway and inhibit the progression of fibrosis.<sup>[12]</sup>

Rosiglitazone is a PPAR- $\gamma$  agonist frequently used in clinical practice. It has various pharmacological activities, including immunoregulation, glycemic control, anti-inflammation, and antifibrosis.<sup>[13]</sup> PPAR- $\gamma$  expression in ovarian granular cells is positively correlated with the concentration of rosiglitazone.<sup>[12]</sup> The results of another study suggest that rosiglitazone can decrease the expression of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in human ovarian granular cells.<sup>[14]</sup> TNF- $\alpha$  can stimulate the proliferation of theca cells and lead to thickening of the tunica albuginea of the ovary and subsequent fibrosis. However, clinical use of rosiglitazone as an antifibrotic drug requires further study.

### Relationship between vascular endothelial growth factor or endothelin-1 and fibrosis

VEGF is a multifunctional cytokine that is expressed in both ovary and endometrium. When it binds to its receptor, proliferation and growth of vascular endothelial cells is promoted and neovascularization increases. Wang *et al.*<sup>[15]</sup> discovered that administration of VEGF and basic fibroblast growth factor, particularly in combination, triggers angiogenesis and inhibits both apoptosis and fibrosis. Luo *et al.*<sup>[16]</sup> found that VEGF significantly upregulates the expression of collagen and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and directly regulates several profibrotic and immune cytokine genes in hepatic stellate cells.

ET-1 is a potent vasoconstrictor that may lead to increased capillary permeability in many different tissues. It is also a strong inflammatory mediator that plays an important role in many inflammatory reactions. ET-1<sup>[17]</sup> can potentiate TGF- $\beta$ 1-induced endothelial-to-mesenchymal transitions and TGF- $\beta$ 1-stimulated expression of mesenchymal cell specific and profibrotic genes and proteins. ET-1 also can induce expression of the TGF- $\beta$  receptor 1 and 2 genes, suggesting an important role for ET-1 in the establishment and progression of tissue fibrosis. It has been reported<sup>[18]</sup> that the ovarian volume and antral follicle count are positively correlated with the concentration of VEGF and negatively correlated with the concentration of ET-1 in follicular fluid. However, there are few studies on ovarian fibrosis and VEGF or ET-1, but they are important cytokines in ovarian fibrosis and further research is necessary into their specific mechanisms and possible role in treatment.

## RELATIONSHIP BETWEEN OVARIAN DISEASES AND FIBROSIS

### Ovarian chocolate cyst and ovarian fibrosis

Ovarian chocolate cysts, also called ovarian endometriosis cysts or chocolate cysts for short, commonly occur in one ovary and more rarely in bilateral ovaries. The most distinctive pathological feature of a chocolate cyst is smooth muscle metaplasia (SMM) and fibrosis. Previously, the pathologist Hughesdon<sup>[19]</sup> reported that 86% of chocolate cysts were characterized by an incomplete cortical capsule and an unclear margin in the cortex caused by SMM, which makes it difficult to perform cystectomy. Schubert

*et al.*<sup>[20]</sup> also found that fibrosis was one of the most common and severe consequences of chocolate cysts while this phenomenon was not observed for ovarian serous and cortical cysts. Donnez *et al.*<sup>[21]</sup> reported in their study of 814 cases of ovarian chocolate cysts that the cyst wall comprised flat endometrial interstitial epithelium and surrounded by fibrotic tissues containing hemosiderin-rich macrophages. Zhang *et al.*<sup>[22]</sup> discovered that the number of follicles around an ovarian chocolate cyst significantly decreased because of SMM and fibrosis. In addition, the pathological degree of SMM and fibrosis and the degree of ovarian dysfunction were positively correlated with time since chocolate cyst onset.<sup>[23]</sup>

In a prior study, Kitajima *et al.*<sup>[24]</sup> compared and analyzed the follicular densities and histological characteristics of the cortex in cystic ovaries and the corresponding healthy contralateral ovary. Macroscopically, the paired ovaries showed no distinct differences in their structure. However, the follicular density in the cyst side was significantly lower than the healthy side and the fibrotic degree was higher microscopically. Among these cysts, 55% cases showed obvious fibrosis and deficiency of follicles in the cortex. It should be noted that the cases in this study had small cysts (<4 cm) in their ovaries, which suggests that ovarian fibrosis and follicle loss occur early after the formation of a chocolate cyst.

Several other researchers<sup>[22,23]</sup> also reported that the follicular density in the ovarian cortex near chocolate cysts was lower than healthy tissues, sometimes as much as two-fold lower in the diseased tissues. This phenomenon was usually related to the pathological changes in the ovarian tissues, including fibrogenesis and vascular loss, but not to tissue stretching caused by increased cyst volume. Coincidentally, similar changes, such as vascular loss, partial fibrosis, and obvious ovarian dysfunction, can also be observed in the ovarian cortex of patients undergoing chemotherapy.

Ovarian chocolate cysts cause fibrogenesis by the following mechanisms:

### Release of inflammatory factors

The pressure inside chocolate cysts gradually increases along with the growth of the cyst and when the pressure significantly increases cracks can form in the capsule wall and result in leakage of liquid containing abundant inflammatory factors. The concentration of proteases and inflammatory factors in chocolate cyst liquid is tens to hundreds of times higher than that in cysts of other types. The interleukin (IL) family cytokines are major inflammatory cytokines that mediate the chemotaxis of leukocytes and cause inflammatory responses. IL-6 and IL-8 levels are higher in patients with chocolate cysts than in patients with other types of cysts.<sup>[25,26]</sup> Increased IL-6 and IL-8 stimulate inflammatory responses and fibrogenesis around ovarian tissues while simultaneously causing ovulation failure.

In addition, cyst rupture generally causes abnormal increases in body temperature, C-reactive protein, and leukocytes,

which is thought to be an acute local inflammatory response triggered by the direct contact of the cyst fluid with intraperitoneal organs. Smith *et al.*<sup>[27]</sup> found that intraperitoneal injection of cyst fluid into rabbits caused severe desmoplasia, which suggests that certain biological molecules contained in the cyst fluid cause inflammation in normal tissues.

#### **Abnormal expression of plasminogen activator system**

The plasminogen activator system is an important controller of ECM degradation that consists of a set of proteolytic enzymes. The key mechanism for activating the plasminogen activator system is to transform inactivated plasminogen into a broad-spectrum serine protease with fibrinolytic activity via extracellular conversion, when remodeling of ECM and basilar membrane is necessary.<sup>[28,29]</sup> The state of this system, activated or inactivated, is correlated with tissue invasion and fibrosis.

PAI is a main modulator of the activity of plasminogen. PAI can decrease fibrinolysis, induce ECM accumulation, and facilitate fibrosis related to inflammatory cells, macrophages, and fibroblasts. Boss *et al.*<sup>[30]</sup> compared and analyzed the activities of the plasminogen activator system in fluid from chocolate cysts and other types of cysts. They found that the concentration of PAI-1 was significantly higher in chocolate cyst fluid than other benign cysts, and the concentration of PAI-2 in chocolate cysts was 50 times higher than in other types of benign cysts.

When cysts rupture, they release excessive products associated with hemagglutination that can cause more serious injury. Previous studies<sup>[31]</sup> have confirmed that chocolate cyst rupture elevates the level of D-dimer, which is a specific cross-linked fibrin derivative catalyzed by fibrous protein and a molecular marker for hypercoagulability *in vivo* and secondary fibrinolytic hyperfunction. Thus, there may be abundant D-dimer in chocolate cyst fluid, which induces fibrinolytic hyperfunction and further accelerates the progression of fibrosis.

#### **Stimulative effect of reactive oxygen species on tissue fibrosis**

In the extracellular interstitium, reactive oxygen species (ROS) exert destructive effects on healthy tissues even when appropriate ROS are required for partial sterilization of the extracellular microenvironment. Ovarian chocolate cysts can induce internal structure disorders in ovaries, trigger inflammation, and produce ROS. In addition, the cyst fluid contains a high concentration of iron that can bind to transferrin or other proteins that have an affinity for cells and the surfaces of endocytic compounds, diffuse into surrounding tissues, and generate more ROS.<sup>[28]</sup>

In patients with chocolate cysts receiving *in vitro* fertilization, the ROS concentration in over one-third of follicles is above 107 cps/400  $\mu$ l, an upper critical value for high-quality embryos. ROS can increase cell membrane penetrability and damage the ovarian tissues around chocolate cysts.<sup>[32-34]</sup> Of greater importance is that ROS can promote tissue fibrosis

synergistically with profibrotic factor PAI and TGF- $\beta$  family members. Fibroblasts, main players in the progression of fibrosis, synthesize collagens and fibronectin.<sup>[35]</sup> TGF- $\beta$ 1 enhances the production of ROS and increases the expression of its downstream target protein, SMAD. PAI-1 controls the activities of plasmin and plasmin-dependent MMPs to regulate the extrinsic and intrinsic reconstitution of collagens.<sup>[29]</sup>

Excessive ROS that cannot be cleared by the intracellular antioxidant system can trigger oxidative stress. 8-hydroxydeoxyguanosine (8-OHdG) is a sensitive marker for DNA damage induced by oxidative stress. In their study on the antioxidant system in the peripheral tissues of chocolate cysts, Matsuzaki and Schubert<sup>[36]</sup> found that the degree of oxidative stress was significantly higher in chocolate cysts than any other ovarian cysts, evidenced by immunostaining intensity of 8-OHdG in ovarian tissues resected during laparoscopic cystectomy. On average, the content of 8-OHdG in chocolate cysts was 10 times more than that in other ovarian cysts, suggesting that there are elevated levels of oxidative stress in normal peripheral tissues around chocolate cysts as compared with other cysts. Oxidative stress in the follicle microenvironment in ovaries is harmful to ova growth, embryonic development, and gestation.<sup>[37-40]</sup> Elevated ROS lead to dysfunction of the normal ovarian cortex around chocolate cysts, implying that ROS might play a role in promoting the development of fibrosis.

Surgical treatment of cysts usually results in resection of normal ovarian tissues and affects the organ's function.<sup>[41,42]</sup> However, a chocolate cyst itself also influences the surrounding tissues. Thus, it is still unclear whether acute or chronic ovarian injury is caused by chocolate cysts and whether surgical treatment can restrict or postpone the damage to the ovaries caused by chronic cysts. Clarifying these issues will address the questions of which type of surgery and timing of surgery is most appropriate for patients with chocolate cysts.

#### **Polycystic ovarian syndrome and ovarian fibrosis**

Polycystic ovarian syndrome (PCOS) is the most common dysgenesis and endocrine metabolic disorder of women of reproductive age. The clinical and pathologic features are chronic anovulation, polycystic ovary, and excessive androgens. As a consequence, insulin resistance and obesity often occur. Recently, many studies have focused on the potent regulative effects of fibrotic factors, such as MMPs and TIMPs, on the balance of ECM in patients with PCOS, since these factors play an important role in PCOS's follicular development disorder through facilitating production of ovarian stromal elements and follicular atresia.<sup>[43]</sup>

Gomes *et al.*<sup>[44]</sup> confirmed that although no significant statistical differences were observed between patients with PCOS and healthy volunteers in levels of serum MMP-2, MMP-8, MMP-9, and TIMP-1, there was a decrease in the serum level of TIMP-2 in PCOS patients. Both the ratio of

MMP-9 to TIMP-1 and the ratio of MMP-2 to TIMP-2 were significantly increased in PCOS patients. Subsequently, similar results were reported by Lewandowski *et al.*<sup>[45]</sup> Furthermore, it was also found that testosterone level was positively correlated to the ratio of MMP-9 and TIMP-1 and negatively associated with the level of TIMP-2. Moreover, testosterone has been recognized as an independent predictor of the ratio of MMP-9 and TIMP-1 and the level of TIMP-2. It can be hypothesized that excessive secretion of androgens might disrupt the balance of MMPs and TIMPs in the ovary under physiological conditions, resulting in the progression of fibrosis in patients with PCOS.

TGF- $\beta$ 1 might be closely related to the development of PCOS based on the abnormal expression of TGF- $\beta$ 1 mRNA in the ovary and an increased level of TGF- $\beta$ 1 in the follicular fluid.<sup>[46]</sup> In ovarian tissue, theca cells, a major source of androgens in normal ovaries, play a pivotal role in maintaining the integrity of the follicle and its function. Abnormal expression of TGF- $\beta$ 1 promotes the overgrowth of theca-interstitial cells, which results in increased production of androgens in patients with PCOS. Endogenous and exogenous stimuli induce the elevation of endogenous TGF- $\beta$ 1, which causes excessive accumulation of ECM and promotes ovarian interstitial fibrosis by disrupting the balance between MMPs and TIMPs.<sup>[47]</sup>

An animal model of PCOS has been established by dehydroepiandrosterone administration<sup>[48]</sup> wherein the amount of CTGF protein in both ovarian tissue and serum is significantly higher than that of control animals. Zhang *et al.*<sup>[49]</sup> also found that the expression of CTGF at both the mRNA and protein levels was significantly increased in ovarian and uterine tissues in PCOS rats compared with controls. Taken together, these findings convincingly demonstrate that CTGF is involved in the occurrence and development of ovarian fibrosis in PCOS.

The current therapeutic strategies for ovarian fibrosis in patients with PCOS have been increasingly studied in animal models. Miao *et al.*<sup>[48]</sup> confirmed that rosiglitazone administration could alleviate ovarian fibrosis by reducing the levels of TGF- $\beta$ 1 and CTGF in serum and ovarian tissue in rats with PCOS. In addition, an *in vitro* study conducted by Bulut *et al.*<sup>[50]</sup> revealed that Jun N-terminal kinase (JNK), a stress-activated protein kinase, mediated the inductive effect of TGF- $\beta$ 1 on the expression of fibronectin and CTGF in fibroblasts, implying that the JNK signaling pathway was involved in ovarian stromal inflammation and fibrogenesis. JNK inhibitors not only inhibit the JNK pathway but also reduce the production of oxidative stress metabolites.<sup>[51]</sup> Further *in vivo* studies in rats demonstrated that SP600125, a JNK inhibitor, thinned the theca cell layer, reduced interstitial collagen deposition, and attenuated inflammation. As a result, ovarian fibrosis was apparently diminished on some level.

Currently, the studies on ovarian fibrosis of PCOS are confined to animal models, clinical specimens of tissue

fluid, and cultured cells. Therefore, it is of great significance to further elucidate the role of these fibrotic factors in the development of ovarian fibrosis of PCOS and provide a theoretical basis for clinical treatments by discovering the role of these fibrotic factors in an *in vivo* system in terms of expression, biological function, and regulative mechanisms.

### Premature ovarian failure and ovarian fibrosis

Premature ovarian failure (POF) has an incidence of approximately 1%. It refers to the loss of function of the ovaries due to depletion of ovarian follicles or ovarian failure caused by iatrogenic injury in women before the age of 40 years.<sup>[52]</sup> There are no known ideal therapeutic strategies to provide proper treatment for POF so far, and ovarian failure is the outcome of ovarian fibrosis.

POF can be classified into two categories according to the histologic features of the ovary. One type is follicle depletion, which is characterized by fibrous tissue or ovarian stroma filled in the ovarian cortex, a thickened ovarian capsule, and extremely rare or completely absent follicles. The other category has a normal number of undeveloped primordial follicles in the follicle cortex but a low sensitivity to gonadotropin (Gn).

The exact etiology of POF is still unclear. Generally speaking, POF can be caused by genetic disorders; abnormal levels of Gn and its receptor; enzyme defects; autoimmune diseases; diabetes mellitus; idiopathic factors; ovarian destructive factors such as radiotherapy, chemotherapy, surgery, and infection; abnormal inhibin; too few eggs or an exhausted egg reserve; folliculogenesis disorders; and others with an unclear etiology. For example, in diabetes mellitus rat models,<sup>[53]</sup> the nuclear factor- $\kappa$ B immunoprotein levels are significantly elevated, which causes follicle degeneration and stromal fibrosis. Erbas *et al.*<sup>[54]</sup> used sunitinib to treat diabetic rats and found lower rates of POF.

Chemotherapy and radiotherapy<sup>[55]</sup> lead to ovarian damage and can cause POF. Cyclophosphamide<sup>[56]</sup> induces the hyperactivation of the phosphatidylinositol-3-kinase/protein kinase B/the mammalian target of rapamycin signaling pathway in ovaries, which leads to primordial follicle loss and increased follicle apoptosis. Although firm evidence is lacking, dioxin<sup>[54]</sup> exposure may also influence the ovarian reserve, and cigarette smoke<sup>[57]</sup> induces dysfunction of mitochondrial repair mechanisms, leading to autophagy-mediated follicle death. However, there are other ovarian failure and fibrosis etiological factors requiring further research. Notably, researchers have demonstrated that TGF- $\beta$  is increased in patients with POF, and it might be involved in fibrogenesis by accelerating the speed of follicular atresia.<sup>[58]</sup> So far, no effective agent that can treat or delay POF has been found yet.

### CONCLUSIONS

Ovarian fibrosis seriously affects ovarian function, and there are many cytokines, including MMPs, TIMPs, TGF- $\beta$ 1,

CTGF, PPAR $\gamma$ , VEGF, and ET-1 involved in its etiology. Many ovarian diseases may induce fibrosis, and these patients are at increased risk of infertility and low quality of life. Therefore, it is important to prevent the formation of ovarian fibrosis to protect the normal function of the ovaries.

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### Conflicts of interest

There are no conflicts of interest.

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