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The Potential Role of Matrix Metalloproteinase in Polycystic Ovary Syndrome: Implications for Extracellular Matrix Remodeling

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder and a leading cause of infertility primarily due to impaired folliculogenesis and anovulation. Central to ovarian function and follicular development is the extracellular matrix (ECM), which undergoes significant remodeling facilitated by matrix metalloproteinases (MMPs). In PCOS, the ovarian cortex becomes thickened and collagen-rich, creating a rigid environment that disrupts normal follicular growth and oocyte maturation. Altered MMP activity further complicates this scenario by impairing ECM degradation, leading to the accumulation of small, quiescent follicles, and elevated androgen levels. This review aims to explore the intricate roles of ECM and MMP alterations in PCOS pathogenesis, highlighting their impact on folliculogenesis and steroidogenesis. Understanding MMP/TIMP dynamics offers insights into potential therapeutic targets to restore normal ovarian function and improve fertility outcomes for women with PCOS.

1 | Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by polycystic ovaries, biochemical or clinical signs of androgen excess, and anovulation [1]. It is a leading cause of infertility, accounting for approximately 85% of cases with ovulatory disorders, according to the World Health Organization (WHO) [2]. A key feature of PCOS is impaired folliculogenesis, which hinders the normal development and maturation of ovarian follicles, leading to anovulation and reduced fertility [1].

The extracellular matrix (ECM) plays a critical role in ovarian function and folliculogenesis by providing structural support and biochemical signals essential for the development of ovarian follicles [3]. As follicles grow, the ECM undergoes significant alterations, influencing cell behavior and tissue organization through dynamic reciprocity, a bidirectional interplay of forces between cells and their microenvironment [4]. ECM components, such as collagen, fibrin, and glycoproteins like hyaluronan and versican, contribute to the mechanical properties of the ovarian environment [5], ensuring the proper progression of folliculogenesis.

A key aspect of ECM's influence on folliculogenesis is its remodeling, which is regulated by enzymes known as matrix metalloproteinases (MMPs) [6]. MMPs degrade ECM components, facilitating the necessary changes in the ovarian microenvironment required for follicular growth, ovulation, and subsequent formation of the corpus luteum [7]. This dynamic remodeling

process is essential for follicle expansion and ovulation, highlighting the importance of ECM flexibility in normal ovarian function.

In contrast, conditions like PCOS demonstrate the impact of altered ECM dynamics. In PCOS, the ovarian cortex is thicker and contains more collagen compared to normal ovaries [8], creating a rigid and biomechanically nonpermissive environment. This increased stiffness disrupts the normal mechanical signaling pathways within the ovary, limiting follicle expansion and oocyte maturation. Consequently, this leads to the accumulation of small, quiescent follicles in the ovarian cortex [7]. This altered mechanical environment is associated with defects in actin polymerization, increased F-actin content, and abnormal biosynthesis of intracellular matrix proteins, which may disrupt signaling pathways such as the Hippo signaling pathway [9, 10]. Moreover, research indicates that these changes in ECM composition and MMP activity may contribute to elevated androgen levels in polycystic ovaries. The stiffer ECM environment in PCOS can lead to higher androgen secretion from follicles, which is a characteristic feature of the syndrome [3].

The role of MMPs in the pathogenesis of PCOS is an emerging area of interest. In polycystic ovaries, there is often a disruption in the balance between MMPs and their inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs) [11]. This imbalance in ECM remodeling contributes to the rigid cortical environment, which may promote androgen excess and anovulation, hallmarks of PCOS [3]. The altered biomechanical state of the ovary in PCOS may establish a detrimental feedback loop, in which increased cortical rigidity further disrupts folliculogenesis and exacerbates syndrome-related symptoms. The aim of this review is to comprehensively explore the role of MMP-mediated ECM remodeling in ovarian physiology, follicle development, and steroidogenesis, with an emphasis on the pathogenesis of PCOS.

2 | MMPs: Classification, Structure, and Functions

MMPs are a crucial group within the metzincin superfamily of zinc-dependent endopeptidases, essential for the remodeling of ECM components [6]. MMPs encompass various subgroups based on their substrate specificity. Collagenases, such as MMP-1, MMP-8, and MMP-13, are responsible for degrading fibrillar collagens I to IV [12]. Gelatinases, including MMP-2 (gelatinase A) and MMP-9 (gelatinase B), target ECM proteins like type IV collagen, laminin, and the core protein of aggrecan. Notably, while MMP-2 breaks down collagen types I to III similarly to collagenases, MMP-9 employs a distinct mechanism for their degradation [13]. Other MMP subgroups, like stromelysins (MMP-3, MMP-10, and MMP-11), matrilysins (MMP-7 and MMP-26), and membrane-type MMPs (MT-MMPs), further illustrate the diversity within the MMP family, each with specific substrates and physiological roles [14]. For instance, MT-MMPs are anchored to the plasma membrane via a transmembrane domain located near their carboxy-terminal, highlighting their specialized functions within the ECM [15].

Besides the effects of MMPs on the ECM, MMPs can also cleave a variety of other molecules, such as TNF-alpha, E-cadherin, syndecan-1, cytokines, growth factors, and chemokines [16]. The metalloproteinase superfamily includes not only MMPs but also ADAMs (a disintegrin and metalloproteinase), which have an integrin receptor-binding disintegrin domain, and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin-like motifs), each with distinct structural and functional characteristics [17]. Despite sharing similar metalloprotease domains, ADAMs and ADAMTSs exhibit notable structural and functional differences. ADAMs are primarily transmembrane proteins involved in critical processes like cell adhesion, migration, and cell–matrix interactions, whereas ADAMTSs are secreted enzymes that play a crucial role in ECM organization and remodeling due to their thrombospondin repeats that facilitate ECM binding [18]. Unlike MMPs and ADAMs, ADAMTSs do not utilize the "cysteine switch" mechanism for pro-enzyme activation. Instead, their activity is regulated by interactions between their pro-peptide and metalloprotease domains or through the binding of TIMPs [19]. The ADAMTS family, which consists of 19 structurally conserved members in vertebrates, is involved in the degradation of large proteoglycans such as collagen, aggrecan, brevican, versican, and neurocan [20]. This enzymatic activity is critical for various physiological processes, including the organization of connective tissue, inflammation, coagulation, angiogenesis, cell migration, and basal lamina remodeling [19].

3 | Regulation of MMPs

The activity of MMPs is intricately regulated through multiple mechanisms at various levels, ensuring precise control over their enzymatic functions. At the gene expression level, MMP activity is modulated by a range of factors, including microRNAs, growth factors, inflammatory cytokines, and hormones, which influence both transcriptional and post-transcriptional processes [21, 22]. MMPs are initially synthesized as inactive proenzymes (proMMPs) that require conversion to their active forms. This activation process is commonly mediated by serine proteinases or other MMPs, ensuring tightly controlled proteolytic activity. Additionally, nonproteolytic mechanisms, such as the presence of reactive oxygen species (ROS) or exposure to denaturants, can promote the activation of proMMPs [15]. Once activated, MMPs are subject to inhibition by TIMPs or by nonspecific proteinase inhibitors like serum-borne α2-macroglobulin [21]. Among the four known TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4), each exhibits distinct tissue-specific expression patterns and varying inhibitory affinities toward different MMPs. For instance, TIMP-2 has a strong affinity for MMP-2, while TIMP-1 and TIMP-3 are more effective at inhibiting MMP-9 [22, 23]. Specifically, TIMP-3 is recognized as the primary inhibitor of ADAMTS activity, highlighting its essential role in controlling ECM dynamics [24]. The final layer of MMP regulation involves the degradation of the protease itself, a process believed to be governed by specific endocytosis followed by lysosomal degradation [21]. Thus, MMP catalytic activity is tightly controlled through coordinated regulation at the levels of gene expression, proenzyme activation, and inhibition by both specific and nonspecific

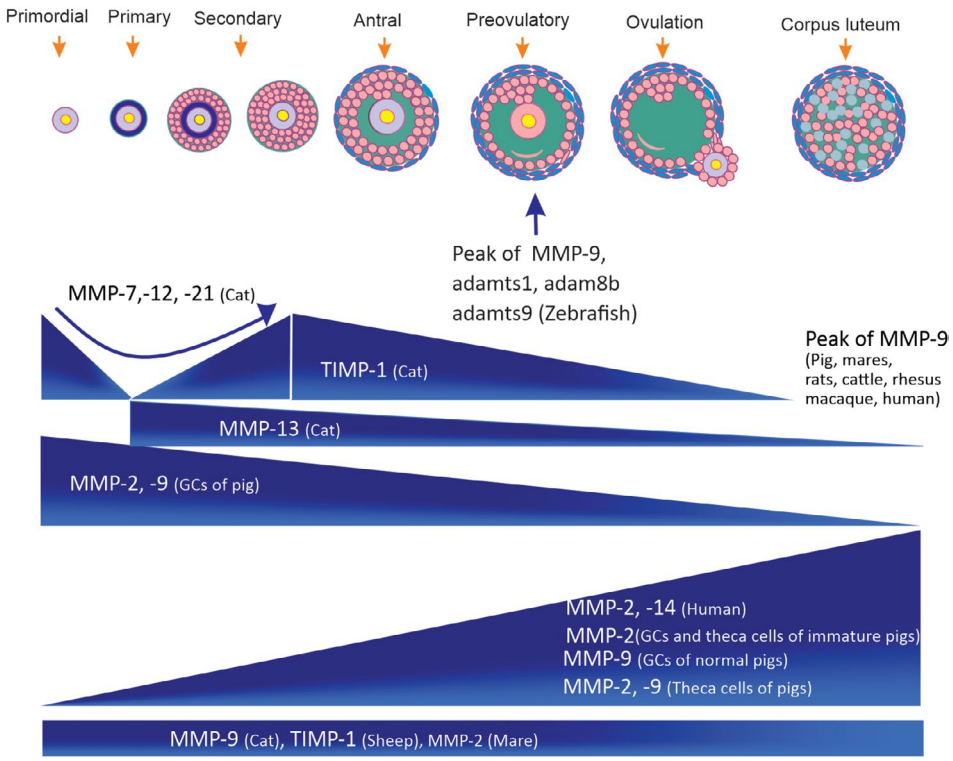

FIGURE 1 | Dynamics of metzincin family members, including MMPs, TIMPs, and ADAMTS, during folliculogenesis across different species.

inhibitors, ensuring their proteolytic functions are precisely modulated.

4 | The Role of MMPs in Ovarian Physiology

MMPs are indispensable in ovarian physiology, particularly in the dynamic remodeling of the ECM during crucial processes such as follicular development, ovulation, and corpus luteum formation. The precise regulation of ECM components by MMPs ensures that the ovarian environment remains conducive to these processes, allowing for the proper growth of follicles. A study on *Bombyx mori* showed that MMP-2 plays a key role in ovarian development by interacting with collagen I and insulin-like peptides, thereby regulating tissue dynamics, creating space for follicle growth, and supporting proper ovarian function [25]. Additionally, both MMP-2 and MT2-MMP contribute to the degradation of collagen within the follicle wall, facilitating the structural changes necessary for follicle rupture during ovulation [26]. The role of MMPs extends beyond ECM remodeling; for instance, the serine protease plasmin not only directly degrades various ECM proteins but also activates pro-MMPs, highlighting a complex network of proteolytic interactions essential for successful ovulation [27]. This network highlights the intricate balance between protease activation and inhibition, ensuring that ECM remodeling occurs precisely when and where it is needed. Tight regulation of MMP activity is crucial, as dysregulation can lead to excessive ECM degradation, impairing follicular development, disrupting oocyte maturation, and hindering fertilization [28]. This delicate balance is maintained by various regulatory mechanisms, as discussed in the previous section.

4.1 | MMPs and TIMPs' Role in Folliculogenesis

Given the presence of MMP substrates in the basal lamina and theca layers of follicles, MMPs are likely to play a critical role in ovarian tissue remodeling and folliculogenesis (Figure 1). Evidence from multiple species [29–34] highlights the role of MMPs in this process, with studies categorized according to the substrate specificity of the MMPs, as detailed below.

4.1.1 | Collagenases

In cat ovaries, early antral follicles showed approximately a 72-fold increase in MMP-1 mRNA expression levels compared to primordial follicles [35]. However, the highest levels of MMP-13 mRNA were detected in cat primary follicles and exhibited a declining trend from the primary follicle stage onward [36].

4.1.2 | Gelatinases

In human studies, the staining intensity of MMP-2 progressively increased during follicle development. Notably, MMP-2 protein expression was observed in stromal cells surrounding tertiary follicles, in contrast to its absence around primordial follicles [37]. However, this finding differs from the observations of Lind et al. [38], who reported positive MMP-2 protein expression in stromal cells around primordial follicles. It is essential to mention that one limitation of the first study relates to the technique used: immunohistochemistry cannot distinguish between active and inactive forms of MMP-2 and can

detect bound MMP-2 [39]. Additionally, MMP-2 concentrations in follicular fluid have been associated with oocyte maturation and fertilization rates in women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), although the stimulation protocols used in these procedures might influence MMP levels [39].

In early antral follicles of cat ovaries, the mRNA expression of MMPs was significantly higher compared to primordial follicles, with MMP-2 showing a 10-fold increase and MMP-9 a ~threefold increase [36]. Among these, MMP-2 was the most predominant in cat follicles, exhibiting an increasing pattern for mRNA expression and immunoreactivity during folliculogenesis. Additionally, the MMP2/TIMP-1 ratio increased as the follicles advanced to the antral stage, signifying a dynamic shift in the MMP–TIMP balance during these critical stages of development [36]. Notably, in a study by Kehoe et al. [30], MMP-9 transcript expression remained unchanged throughout follicle development in cats. However, treatment of cat cortical tissue with MMP-9 in vitro led to a notable reduction in ovarian tissue rigidity [40], underscoring MMP-9's role in altering the extracellular matrix, enhancing tissue remodeling, and improving ovarian function.

In pigs, a study comparing normal and miniature pigs found that the latter, characterized by a lower ovulation rate, reduced litter size, and fewer follicles and corpora lutea, exhibited elevated levels of MMP-2 in both theca interna and granulosa cells throughout follicle development [35]. The protein expression of MMP-9 was also elevated in granulosa cells during follicle development in normal pigs, with lower expression levels in miniature pigs. This difference may explain the compromised fertility observed in miniature pigs, which could be due to a deficiency in MMP-9, necessary for follicular maturation, and an increased expression of apoptosis-inducing MMP-2 [35]. However, contrasting findings from another study reported a rise in MMP-2 and MMP-9 mRNA expression in theca cells during follicle growth, while a decline was observed in granulosa cells as follicular development progressed [31].

In chickens, granulosa cells have emerged as the primary source of MMP-9 secretion during various phases of ovarian development, with growth factors, rather than hormones, playing a significant role in regulating MMP-9 expression [41]. Both mRNA and protein expression levels of MMP-2 were elevated during follicular maturation in the hen ovary [32, 42, 43].

In other animal models, such as the mare, Riley et al. [33] demonstrated that MMP-2 was the most prevalent metalloproteinase in follicular fluid; however, its concentrations remained constant throughout follicular development, suggesting that while MMP-2 is crucial, its role may vary between species and contexts. Interestingly, in sheep, the addition of proMMP-9 during in vitro maturation did not yield beneficial effects on the maturation of oocytes subjected to heat stress [34].

Finally, a study in zebrafish described higher expression levels of MMP-2 and MMP-9 transcripts in immature follicles compared to preovulatory follicles, with a subsequent decline following ovulation, while the mRNA expression of MMP-9 increased in preovulatory follicular cells [44].

MMP-2 and MMP-9 are consistently highlighted as key players, with their expression patterns and activity levels varying between species and follicular stages. In general, MMP-2 is associated with follicle maturation, while MMP-9 plays a significant role in ECM degradation, facilitating follicular rupture and oocyte release. The balance between MMPs and their inhibitors is crucial for maintaining normal folliculogenesis, with disruptions in this balance potentially leading to impaired fertility, as observed in certain animal models.

4.1.3 | Stromelysins

Fujihara et al. [35] observed a gradual rise in MMP-3 mRNA expression throughout folliculogenesis in cat ovaries, with levels increasing approximately 57-fold in early antral follicles compared to primordial follicles. Additionally, in the hen ovary, mRNA and protein expression levels of MMP-3 and MMP-10 showed a notable increase during follicular maturation [32, 42, 43].

4.1.4 | Matrilysins

The transcript expression patterns of MMP-7 declined from primordial to primary follicles, followed by an increase during the transition from primary to secondary follicles in cats [30].

4.1.5 | Other MMPs and TIMPs

During follicle development in humans, the intensity of MMP-14 staining progressively increased. The expression of MMP-12 and MMP-21 transcripts decreased from primordial to primary follicles, followed by an increase thereafter [30].

Furthermore, the elevated levels of TIMP-3 during proestrus in rats further suggest its vital role in the final stages of follicle maturation [45]. Human follicular levels of MMP-9, TIMP-1, and TIMP-2 did not show any significant correlation with the oocyte maturation rate [46]. In rodents, TIMP-1 plays a crucial role in facilitating cellular proliferation and inhibiting apoptosis through the modulation of MMP activity, as well as through MMP-independent mechanisms such as protein–protein interactions [29]. TIMP-1 showed a six-fold increase in secondary follicles but declined thereafter [36]. No significant alteration in TIMP-1 protein expression was observed throughout sheep follicular development [47].

4.1.6 | ADAMTS

The mRNA expression of ADAMTS1 increased in both preovulatory follicular cells and cells after oocyte maturation, whereas ADAM8b and ADAMTS9 were upregulated exclusively in preovulatory follicular cells [48]. A study has reported an association between ADAMTS1 and follicular growth in humans, suggesting its potential as a marker for oocyte competence [49].

4.2 | Follicular Atresia

In addition to their critical roles in follicular development and maturation, MMPs, particularly MMP-2 and MMP-9, are also deeply involved in the process of follicular atresia. Atresia, the degeneration of ovarian follicles that do not reach ovulation, is another context in which MMP activity is significant. Multiple cysts observed in the ovaries of patients with PCOS are believed to result from atresia of early antral follicles that fail to be removed or regress [50], in contrast to the normal follicular atresia, which typically occurs only at the preovulatory stage in healthy ovulatory women. Therefore, it can be speculated that alterations in MMPs may play a role in the inappropriate atresia observed in these patients. It has been demonstrated the altered mRNA expression of MT1-MMP in atretic follicles of dehydroepiandrosterone-induced PCOS rat models, highlighting its potential role in follicular atresia and ovarian dysfunction in PCOS [51]. The dual role of MMPs in both follicle growth and atresia is further supported by studies in guinea pigs, where an increased number of atretic antral follicles subsequent to testosterone propionate treatment has been linked to the upregulation of MMP-2 [52].

MMP-2 contributes to mitochondrial membrane dysfunction and induces apoptosis during follicular atresia, a process that selectively eliminates less viable follicles, allowing only the most competent ones to progress [28]. An augmented MMP-2:TIMP-2 ratio could potentially trigger the degradation of laminin in the follicular basal lamina during atresia [48]. Imai et al. [53] study further underscores the significance of MMP-2 and its inactive precursor, proMMP-2, in the progression of follicular atresia. Similarly, research in ewes has revealed that both MMP-2 and MMP-9 activities are heightened in follicles undergoing degeneration following hypophysectomy, a finding consistent with observations in bovine follicles, where atretic follicles exhibit elevated enzymatic activities and mRNA levels of these gelatinases compared to healthy follicles [42, 54].

These results align with the findings in chicken follicles, as elevated activities of both gelatinases were noted in atretic follicles compared to normal white follicles, the smallest of which were 1–4 mm in size [42].

4.3 | MMPs and TIMPs' Role in Ovulation

Ovulation is the physiological process characterized by the release of a mature oocyte from the surrounding cells within the follicle [17]. An essential occurrence within the process of ovulation is tissue remodeling, encompassing the fragmentation of the ECM, perturbation of the basal lamina and follicular layers, alteration of capillary vessels, the release of the mature oocyte, as well as the restoration of compromised tissue [55]. However, in women with PCOS, oligo- or anovulation, one of the hallmark features of the condition, often results in infertility [56]. This condition is partially attributed to dysregulated ECM remodeling, which plays a crucial role in the ovulatory process. MMPs and their tight regulation are central to ECM remodeling during ovulation. MMPs degrade ECM components and facilitate tissue restructuring, which is essential for follicular rupture and oocyte release. For instance, MMPs like MMP-2, MMP-7, and MMP-9 are known to degrade type IV collagen, a key component of the follicle basal lamina and theca, suggesting their substantial role in the ovulatory process [57]. In PCOS, where ovulation is often compromised, the role of MMPs becomes especially important. For instance, the downregulation of TIMP-1 observed in PCOS patients may be a compensatory mechanism to overcome the thickened ovarian cortex [58]. This section will explore the detailed involvement of MMPs and their inhibitors in the normal ovulatory process, as well as the effects of ovulatory agents on their activity, which will be discussed in a separate section.

4.3.1 | Collagenases

In vitro studies have shown that treating Xenopus oocytes with low concentrations of collagenase can mimic the maturation and rupture of follicles, underscoring the importance of collagen degradation in ovulation [59]. However, the mRNA expression of collagenases, such as MMP-8 and MMP-13, remained unchanged during the periovulatory period in granulosa and theca cells [60].

4.3.2 | Gelatinases

Elevated levels of gelatinases, specifically MMP-2 and MMP-9, were observed in the theca layer of porcine preovulatory follicles, indicating the potential involvement of MMPs in the degradation of ECM proteins prior to ovulation [61]. In macaques, this increase was detected only immediately prior to ovulation in periovulatory granulosa cells. MMP-2 exhibited increased activity during the periovulatory phase across various species, including human [62], rats [63], cat [36], mice [64], monkeys [65], medaka [26], sheep [66], and vase tunicate [67] ovaries. In *Drosophila*, MMP-2 knockdown resulted in anovulation and subfertility, underscoring its importance in successful ovulation [68]. Nevertheless, contrasting findings exist, challenging the central regulatory role of MMP-2 in preovulatory follicular shape modulation [69]. For instance, Lind et al. [38] demonstrated the absence of any significant changes in the protein levels of MMP-2 within human dominant follicles over the course of the ovulatory phase. Moreover, miniature pigs with reduced ovulation rates have been observed to exhibit elevated MMP-2 protein expression in the theca interna and granulosa cells of Graafian and ovulated follicles [35].

MMP-9 is one of the key proteases that demonstrate elevated levels during the periovulatory period in humans [38, 70]. Bilen et al. [48] reported a significant positive correlation between MMP-9 levels and oocyte quality, hypothesizing that MMP-9 may be involved in the degradation of the perivitelline debris, which could explain its beneficial effects on oocyte quality. In chickens, MMP-9 levels rise as follicles mature and continue to rise even after ovulation in postovulatory follicles [41]. In pigs, although MMP-9 protein expression decreases in granulosa cells after ovulation, it increases in the theca lutein and theca externa layers following ovulation [35]. However, other studies failed to detect any discernible alterations in protein expression of this gelatinase in human dominant follicles

during the ovulatory phase [38]. The relatively modest role of MMP-9 in ovulation is further suggested by studies in MMP-9 knockout mice and zebrafish, which did not show significant fertility issues, indicating that MMP-9 may not be essential for ovulation [71].

4.3.3 | MT-MMPs

In addition to their role in triggering other proteases, MT-MMPs also have the capability to stimulate intracellular molecules such as growth factors and cytokines, as well as to degrade matrix proteins like syndecan-1 [72]. The functions of some growth factors and syndecan have been well documented in the mechanisms underlying ovulation and the formation of the corpus luteum [73, 74].

4.3.4 | Other MMPs and TIMPs

Interestingly, in fish, MMP-11 transcripts increased at the time of ovulation compared to the period prior to ovulation [75]. While TIMP-2 protein expression showed a remarkable decrease in the ovulated follicles of normal pigs compared to miniature pigs [35], no discernible alterations were observed in TIMP-2 protein expression within dominant follicles during the ovulation phase in women [38]. In mice, TIMP-3 mRNA expression exhibited an ascending trend during the early ovulatory phase yet remained at lower levels within the intact ovary [76].

4.3.5 | ADAMTS

In mice, deficiency in ADAMTS-1 was associated with impaired ovulation [77], possibly due to unsuccessful cleavage of versican, a key ECM component [78]. In this context, an increase in the expression of ADAMTS-1 has been documented within preovulatory follicles of several species, including mice [79], humans [80], cattle [81], and zebrafish [82].

The conspicuous expression of ADAMTS-9 has been documented in fish and mammalian species during the ovulatory cycle [70, 83]. Given its regulation by progestin and LH, ADAMTS-9 assumes a pivotal role in the process of ovulation. Furthermore, it has been reported that there is an increase in the expression of ADAMTS-8a and ADAMTS-8b transcripts in preovulatory follicles of zebrafish, and a similar upregulation of ADAMTS-4 and ADAMTS-15 in rhesus monkeys [44, 84].

Limited reports exist regarding the impact of metalloprotease knockouts on reproductive functions, including ovulation. However, this scarcity of data does not diminish their potential significance in this context, as compensatory effects by other members of the MMP family may obscure the individual contributions of specific MMPs. Given the broad expression of metalloproteases across various tissues, cautious interpretation of results is warranted, highlighting the importance of employing more targeted knockouts in specific tissues of interest for comprehensive understanding. Elucidating the specific roles of individual MMPs during ovulation could provide critical insights into the molecular mechanisms underlying PCOS and potentially reveal novel therapeutic targets.

4.4 | The Effect of Ovulatory Agents on MMP Activity

4.4.1 | Collagenases

LH stimulation resulted in MMP-1 mRNA upregulation in periovulatory granulosa cells of rhesus monkeys and in the chicken ovary [41, 85]. MMP-1 transcripts are induced after human chorionic gonadotrophin (hCG) administration in humans, as well as in numerous other species. For example, in the rhesus macaque [70], the mRNA expression of MMP-1 in granulosa cells was triggered 12 h post-hCG treatment and exhibited a consistent increase for up to 36 h [86]. However, in whole follicles, the elevated levels of MMP-1 mRNA began to decline at 24 and 36 h after hCG stimulation, before showing a secondary increase at the 36-h post-hCG [70]. Similarly, the gonadotropin surge triggered by gonadotrophin-releasing hormone (GnRH) upregulates MMP-1 expression in bovine preovulatory follicles [87, 88], and comparable stimulatory effects have been documented following hCG administration in the follicular cells of rhesus macaque [70].

4.4.2 | Gelatinases

The stimulatory effects of gonadotropins on MMPs have been documented in rodents [17, 89, 90]. For instance, it has been documented that LH and FSH can enhance MMP-9 enzymatic activity and upregulate its expression at both mRNA and protein levels within porcine granulosa cells [91]. While LH stimulation resulted in MMP-9 mRNA upregulation in periovulatory granulosa cells of rhesus monkeys [85], contrasting inhibitory effects were noted following treatment with LH or progesterone in the corpus luteum [92]. These divergent responses may reflect dynamic regulatory shifts that occur across distinct stages of folliculogenesis [92]. MMP-2 is activated in intact rat ovaries by LH, while MMP-9 showed no significant alteration [79]. An investigation into preovulatory follicles in brook trout revealed that salmon LH (sLH) induced proteolysis, encompassing the upregulation of MMP-2 mRNA expression. This heightened proteolytic activity was consistently observed during sLH-induced ovulation [93]. However, other studies have reported no significant effect of gonadotropins on MMP expression or activity. For instance, LH does not induce changes in the secretion or activity of MMP-2 or MMP-9 in mouse primary granulosa cells [94].

MMP-9 demonstrates elevated levels following hCG treatment in macaques [38, 70]. In rats, MMP-9 mRNA expression significantly increases 24 h after hCG injection, reaching its peak during luteinization [95]. In mouse ovaries, hCG treatment upregulated MMP-9 expression in follicular cells [96]. However, in humans, hCG has been shown to decrease the enzymatic activity of both MMP-2 and MMP-9 [97].

4.4.3 | Stromelysins

The stimulatory effect of LH on MMP-19 expression following ovarian induction has been shown in various species, including mouse [89], rat [98], and fish [93]. The hCG induction yields varying outcomes in different species. For example, MMP-3 transcripts were not detected in murine ovaries, but exhibited an elevation in rat ovaries, while remaining unchanged in human theca and granulosa cells [99]. In addition to interspecies variations, dissimilar results emerged from in vitro and in vivo investigations. While hCG stimulated MMP-3 expression in cultured granulosa cells, MMP-3 transcripts remained unchanged following hCG stimulation in vivo. These differences are likely due to the lack of extensive cellular interactions and endogenous ovarian inhibitory effects in vitro, which are present in the in vivo environment [99].

MMP-10 levels have been observed to be upregulated in both human and rat granulosa cells, as well as in human theca cells and rat ovarian tissue, subsequent to hCG stimulation [99]. An increase in MMP-10 mRNA expression has been observed in the dominant follicles of macaques [70]. Nevertheless, utilizing the northern blot technique, mRNA abundance of this proteinase remained undetectable in intact mouse ovaries after hCG administration [89].

Furthermore, MMP-11 was absent in granulosa cells, and its mRNA expression was reduced in human and rat granulosa and theca cells in response to hCG stimulation [100]. Besides, some studies found no changes in MMP-11 mRNA levels during the periovulatory period following hCG administration in rodent ovaries [89].

Additionally, the transcripts of MMP-19, detected in the thecal-interstitial and granulosa cells of large preovulatory or ovulating mouse, human, and rhesus macaque follicles, exhibited a significant elevation after hCG treatment [86, 98]. It has been proposed that MMP-19 plays a role in facilitating follicular rupture, while TIMP-1 may act to terminate the activity of MMP-19 post-ovulation in mice [89]. The effect of gonadotropin on MMP-19 induction was also reported in granulosa cells of rats and rhesus macaques [70].

4.4.4 | MT-MMPs

In fish, among the proteases involved in the degradation of ECM and follicle rupture, MMP-15 was the only one inducible by LH [101]. In preovulatory follicles of Medaka, LH stimulation resulted in increased MMP-15 expression within granulosa cells [27, 102]. In vitro studies have documented the stimulatory impact of LH on MT2-MMP expression, evident at both mRNA and protein levels [103]. Alterations in the levels of MT-MMPs after hCG stimulation have also been documented. For instance, MMP-14, MMP-16, and MMP-25 transcripts were elevated in rats. Correspondingly, the protein levels of MMP-14 and MMP-16 exhibited a parallel increase in rats and were similarly upregulated in human granulosa cells after hCG administration. Nonetheless, the mRNA levels of MMP-16 and MMP-25 exhibited no significant change in human granulosa and theca cells, with a subsequent decrease after ovulation. In macaque granulosa cells, while only the mRNA of MMP-16, not MMP-14, exhibited an increase upon hCG administration, the active form of MMP-14 protein also displayed an elevation following hCG treatment [81, 82]. These findings suggest the significant role of MT-MMPs in the processes of ovulation [104].

4.4.5 | TIMPs

The expression of MMP regulators, such as TIMPs, is influenced during the process of ovulation. An increase in TIMP-1 transcript levels has been observed following LH stimulation in rats [105], mice [89], and sheep [106]. In sheep, TIMP-1 expression following LH induction was localized to granulosa cells and luteal tissue [106]. Similarly, LH stimulation upregulated TIMP-1 mRNA expression in peri-ovulatory granulosa cells of rhesus monkeys [85]. In addition, induction of preovulatory follicles in brook trout by sLH increased the mRNA expression of TIMP-2 [93].

A significant increase in mRNA levels of TIMP-1 was observed 12 h after hCG administration, as well as within the preovulatory granulosa cells [105]. Similar findings were reported regarding both mRNA and protein expression of TIMP-1 within goat granulosa cells by hCG [107]. LH/hCG treatment induced the expression of TIMP-1 and TIMP-2 in both monkeys and humans [55]. Elevated concentrations of TIMPs might play a role in halting the activity of MMPs, which experience an upsurge during follicular rupture to degrade the ECM structure [17].

In human granulosa cells, there was a notable 2-fold increase in TIMP-3 transcripts during the late ovulatory phase compared to the preovulatory phase [108]. Similar periovulatory augmentation of TIMP-3 following hCG stimulation has been documented in various other species. The upregulation of TIMP-3 was observed within rat follicles 12 h after the administration of hCG [63], and in cultured granulosa cells, dual peaks of TIMP-3 overexpression were discerned at 2 and 16 h post-stimulation [105]. Furthermore, GnRH injection led to a significant increase in TIMP-3 transcripts after 12 h. TIMPs are multifunctional proteins that execute their roles through both MMP-dependent and MMP-independent pathways [45].

4.4.6 | ADAMTS

In human granulosa cells, ADAMTS-1 transcripts were upregulated in response to hCG stimulation [86]. In rodents, ADAMTS-1 mRNA expression increased 6–12 h after hCG administration, while in macaques, this elevation occurred solely post-ovulation, irrespective of progesterone levels, suggesting a role of this proteinase in corpus luteum function [70, 109].

Moreover, in humans and macaques, there is an observed upregulation in the expression of ADAMTS-9 within granulosa cells and preovulatory follicles following hCG treatment, respectively. This heightened expression persisted until ovulation in both [70]. Similarly, following hCG/forskolin treatment, adam17 mRNA expression was upregulated in theca interna and granulosa cells of preovulatory follicles in cows [110].

4.5 | MMPs and TIMPs' Role During the Luteal Phase

In the luteal phase, the remaining follicle cells post-ovulation undergo a reorganization and neovascularization to develop into the corpus luteum, responsible for producing progesterone. This remodeling during the luteal phase is accompanied by extensive alterations in ECM, which can subsequently impact various cellular mechanisms including apoptosis, genetic expression, mitosis, and cellular differentiation [111]. MMPs play a crucial role throughout the luteal phase, including in corpus luteum formation, midluteal maintenance, and luteal regression [15].

It has been reported that polycystic ovaries have a reduced number of corpora lutea due to anovulation or oligo-ovulation. In some PCOS phenotypes that still experience ovulatory cycles, the luteal phase is often shorter than normal, leading to luteal phase defects (LPD) [112]. Dysregulation of MMP activity may further compromise luteal function, thereby exacerbating the reproductive challenges associated with PCOS.

The concentrations of MMPs increase during the luteal phase in various species, including rodents [113], humans [114], and bovines [115]. For example, both the transcription and enzymatic activity of gelatinases, along with collagenolytic activity, increase during this phase after pseudopregnancy induction [116]. While the role of MMPs and their regulators in maintaining normal ovarian function is well documented, there is a lack of comprehensive information regarding their contributions to the mechanism of ECM remodeling in ovarian pathologies, such as PCOS. In this section, we will discuss the role of MMPs in the luteal phase.

4.5.1 | Collagenases

The crucial function of MMP-1 in the involution of postovulatory follicles has been evidenced in hens, rabbits, and pigs [117]. Nevertheless, in rhesus macaques, although MMP-1 mRNA expression was elevated in the progressing corpus luteum, it underwent a substantial reduction during the process of luteolysis [118]. In humans, MMP-1 mRNA expression remains consistent throughout corpus luteum formation and subsequent regression [114]. MMP-13 has also been implicated in luteal regression [119]. These results are in alignment with the findings reported by Nothnick et al. [120], who reported a similar augmentation 12 days after hCG administration in rats.

4.5.2 | Gelatinases

The higher expression levels of gelatinase have been observed in the peripheral regions of the forming corpus luteum [63]. In pigs, transcripts of MMP-2 and MMP-9 exhibit an upward trend from the early to the late corpus luteum [121]. MMP-2 maintains consistent basal activity during the early and middle luteal phases and plays a significant role in corpus luteum formation in humans, as suggested by a study with a small sample size [37]. Furthermore, MMP-9 exhibited a pronounced peak during the initial stages of corpus luteum formation and during luteolysis [122].

Research in rat ovaries revealed a notable surge in MMP-2 transcripts as they shifted from ovulation to luteogenesis, with MMP-9 expression reaching its peak during luteinization. However, transcripts of both gelatinases diminished during corpus luteum regression [95]. In contrast, the mRNA expression of MMP-2 and MMP-9 increased during the regression of the corpus luteum in both bovine and porcine [121, 123]. In ewes, MMP-9 mRNA expression is elevated during the development of luteal cells, whereas MMP-2 enzymatic activity rises during luteolysis [124]. Furthermore, the involvement of MMP-2 and MMP-9 in the regression of postovulatory follicles might occur through mechanisms associated with apoptosis [125]. However, Kliem et al. [57] did not observe any alterations in MMP-2 transcripts throughout the luteal development process.

It appears that MMP-2 assumes a pivotal role during the initial stages of regression of postovulatory follicles in avian species, whereas MMP-9 becomes more significant during the later stages of regression [126]. The preceding observations suggest the significant involvement of the MMP system in the regulatory processes of postovulatory follicle regression in chickens [126].

4.5.3 | Other MMPs and TIMPs

In bovines, MMP-14 expression remains unchanged during corpus luteum formation but increases during luteal regression [57, 123]. TIMP-2 locally regulated the MMP-1 and MMP-2 activity in the corpus luteum [114]. A decrease in TIMP-1 mRNA expression has been evidenced throughout the luteal phase in cows, spanning from early to late stages [127]. Similar trends have been documented in other species, such as ovine [124]. High gene expression of TIMP-1 and TIMP-2 in the functional corpus luteum, along with its diminished levels in the regressing corpus luteum, has been observed in bovine and porcine [121, 123]. Additionally, a decrease in TIMP-2 and TIMP-3 mRNA expression has been noted in postovulatory follicles in avian species [126]. Furthermore, TIMP-3 exhibited an ascending trend during the luteal phase, from its initial formation in early pseudopregnancy through luteal maintenance and eventual regression, reaching its peak levels in rats [120].

4.6 | Association of MMPs and TIMPs With Steroidogenesis

There is evidence indicating a bidirectional interplay between ovarian steroids and MMPs, where steroids regulate the expression of MMPs and their inhibitors, while MMPs may also influence steroidogenesis and thus modulate steroid levels, collectively impacting the structural dynamics of the ovary [15]. A negative correlation has been observed between progesterone levels and MMP-9/TIMP-1 levels in luteinized granulosa cells in both healthy women and those with PCOS undergoing ovarian stimulation for IVF/ICSI [128]. This suggests that high gelatinolytic activity contributes to decreased progesterone secretion, thereby promoting luteal regression. Therefore, it is reasonable to hypothesize that luteal dysfunction observed in patients with PCOS may be attributed to an imbalance in MMPs activity. The reduction in progesterone and estradiol levels may mitigate the negative feedback on LH

secretion, which shows high levels in PCOS patients [129]. The inverse correlation between MMP-9 and progesterone has been consistently documented during oocyte maturation [130]. Conversely, others have highlighted the role of MMP-2 or MMP-9 in progesterone synthesis and LH-induced steroidogenesis [94, 131]. The findings are supported by the observed attenuating effects of MMP inhibitors on LH-mediated processes, and some studies suggest ADAM-17 as a significant regulator of LH-mediated cumulus cell expansion and oocyte maturation [132, 133]. Notably, MMP inhibitors like doxycycline impede LH-induced steroidogenesis by obstructing the release of EGF-like ligands, a process mediated by MMP [94].

In addition, both broad-spectrum MMP inhibitors and those with partial effects have been observed to disrupt LH-stimulated steroid production [131, 134]. Furthermore, a significant positive correlation was observed between MMP-2 and estradiol levels in the follicular fluid of patients undergoing ICSI [135].

The stimulatory effects of TIMP-3 on progesterone secretion have been reported in goat granulosa cells, where it influences the expression of steroidogenic enzymes, including StAR, p450scc, and HSD3B [23]. Nevertheless, the low expression of LH receptors in cumulus-oocyte complexes or Leydig cells, which do not require MMPs for steroidogenesis, undermines these findings [94, 136]. Moreover, the treatment of bovine cumulus-oocyte complexes during the maturation period with human MMP-9 did not significantly alter progesterone levels [137]. In addition to the effects of MMPs on steroidogenesis, it is noteworthy that steroidogenic hormones can also influence MMP expression.

4.6.1 | Progesterone and Estrogen

Progesterone has been shown to upregulate the levels of MMP-1, TIMP-1, and ADAMTS-1 in the primate ovary [65]. Progesterone and estrogen-stimulated MMP-1 and MMP-3 expression, while MMP-9 expression remained unaffected in chicken ovary [41]. These findings align with previous studies in hens, which have elucidated the stimulatory effects of progesterone on collagenase activity in vitro [138]. Furthermore, the administration of the estrogen receptor inhibitor tamoxifen has been shown to impact the expression and activity of MMPs, specifically MMP-2, MMP-9, MMP-10, MMP-13, as well as TIMPs such as TIMP-2 and TIMP-3, within hen ovarian follicles [139]. Moreover, estrogen receptor β can modulate MMP-19 downstream, as evidenced by a decrease in MMP expression observed in ERKO mouse ovaries [140].

4.6.2 | Androgens

The association between MMPs and steroidogenesis has been increasingly recognized in the context of hyperandrogenism and PCOS. González et al. [141] demonstrated that short-term elevation of circulating androgens, similar to levels seen in PCOS, leads to an increase in MMP-2 protein expression in plasma, suggesting a link between androgen excess and MMP activity in the reproductive system. Supporting this, the study by Rezvanfar et al. [142] found that hyperandrogenism in a rat model of PCOS leads to a significant increase in ovarian MMP-2 levels, which is associated with oxidative stress, inflammation, and an ovarian aging-like phenotype characterized by disrupted folliculogenesis and increased follicular atresia. These findings highlight the potential role of elevated MMP-2 activity in the premature ovarian aging observed in PCOS. Furthermore, another research study has suggested that altered expression of MMPs in the theca cells may contribute to androgen-induced ovarian dysfunction [143], emphasizing the need for further exploration into the role of MMPs in the pathophysiology of reproductive disorders.

A comparable correlation between MMP-2 protein and circular androgens was noted in PCOS patients, both during fasting and following glucose stimulation [144, 145]. These findings suggest that androgen excess plays a significant role in disrupting the balance between MMPs and TIMPs, key drivers of fibrosis in the pathogenesis of PCOS.

After testosterone propionate treatment, MMP-2 and MMP-9 exhibited upregulation in granulosa cells compared to weak expression in thecal cells, indicating ovarian remodeling in granulosa cells as a consequence of hyperandrogenism [52]. Administration of testosterone to sheep resulted in an upregulation of MMP-9 and a downregulation of TIMP-1 protein expression within the theca cells of preantral follicles [47]. Nevertheless, the diminished progesterone levels and increased estradiol concentrations observed in prenatal testosterone-treated sheep could potentially serve as additional factors contributing to the altered MMP activity. While a positive relationship has been observed with the MMP-9/TIMP-1 ratio, an inverse correlation between testosterone levels and TIMP-2 has also been documented [146]. Collectively, these studies suggest that MMPs may play a crucial role in the disruption of ovarian function under conditions of hyperandrogenism. The effects of different gonadotropin and steroid hormones on the expression of MMPs or their inhibitors across various species are summarized in Table 1.

5 | MMPs and Pregnancy Outcomes

Evidence suggests a correlation between MMPs and pregnancy outcomes. Research indicates that GCs from infertile, older, and poor responder patients exhibit high expression levels of MMP-2 [100]. Elevated levels of pro-MMP-9 in follicular fluid are considered crucial for successful implantation and favorable pregnancy outcomes in IVF cycles [149]. This assertion is supported by observations in MMP-9-deficient mice, which exhibited a lower number of pups per litter compared to their counterparts with intact MMP-9 production [150]. Moreover, a notable correlation was observed between MMP-9 mRNA levels and the number of oocytes at metaphase II in GCs [100]. In accordance with these findings, bovine GCs isolated from follicles yielding competent oocytes exhibit elevated levels of MMP-9 [151].

In humans, individuals harboring a single-nucleotide polymorphism (SNP) within the MMP-9 promoter exhibit an increased susceptibility to idiopathic recurrent spontaneous abortion [100]. While elevated serum MMP-9 levels in PCOS patients may reflect prolonged infertility duration [152], their intrafollicular concentrations, along with those of MMP-2, do not appear to

TABLE 1 | Steroid- and gonadotropin-induced modulation of MMPs and inhibitors in ovaries of different species.

Hormone	Type of effect	Type of MMP or inhibitor	Species	Examined cells	References
LH/FSH	Stimulatory	MMP-9 (mRNA)	Porcine	GCs	[91]
LH	Stimulatory	MT2-MMP (mRNA and protein)	Medaka	Preovulatory follicles	[103]
LH	Stimulatory	TIMP-2 (mRNA)	Brook trout	Preovulatory follicles	[93]
		MMP-2 (mRNA)			
		MMP-19 (mRNA)			
		ADAMTS (mRNA)			
LH	Stimulatory	MMP-15 (mRNA and protein)	Medaka	Preovulatory follicles	[103]
LH	Stimulatory	MMP-1 (mRNA)	Monkey	GCs of preovulatory follicles	[85]
		MMP-9 (mRNA)			
		TIMP-1 (mRNA)			
LH	Stimulatory Inhibitory	TIMP-2 (mRNA)	Monkey	Corpus luteum	[92]
		TIMP-1 (mRNA and protein)			
		MMP-1 (mRNA and protein)			
		MMP-2 (protein)			
LH	No effect	MMP-2 and MMP-9 (mRNA and protein)	Mouse	Primary GCs	[94]
FSH LH	Stimulatory	MMP-1 and MMP-3 (mRNA)	Chicken	GCs	[41]
Progesterone Estrogen	No effect	MMP-9 (mRNA)			
Progesterone	Stimulatory	MMP-1 (mRNA)	Macaque	Periovulatory CGs	[65]
		TIMP-1 (mRNA)			
FSH	Inhibitory	MMP-23 (mRNA)	Rat	GCs	[147]
FSH	Inhibitory	MMP-2 & MMP-9 (mRNA)	Bovine	GCs	[148]
hCG	Inhibitory	MMP-2 and MMP-9 (activity)	Human	GCs	[97]
	Stimulatory	TIMP-1 (activity)			
Testosterone	Stimulatory	MMP-2 and MMP-9 (protein)	Guinea pigs	GCs	[52]
Testosterone	Stimulatory	MMP-9 (protein)	Sheep	Theca cells of preantral follicles	[47]
	Inhibitory	TIMP-1 (protein)			

Abbreviations: FSH, follicle-stimulating hormone; GCs, granulosa cells; hCG, human chorionic gonadotropin; LH, luteinizing hormone; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinases.

influence pregnancy outcomes during IVF [153], suggesting a complex yet non-predictive role for these enzymes in reproductive success. Among patients diagnosed with PCOS, those displaying elevated levels of TIMP-1 have been observed to achieve more favorable pregnancy outcomes [62]. However, studies have also documented upregulation of TIMP-1 in cumulus cells among older patients and those categorized as poor responders [100].

Recent studies have demonstrated that endometrial stromal fibroblasts from women with PCOS exhibit impaired decidualization and a pro-inflammatory cytokine profile, which are

critical factors influencing implantation and early pregnancy maintenance [154]. The dysregulation of MMP-2 and MMP-9 in a progesterone-resistant, inflamed endometrial environment could further compromise receptivity [155]. These findings are consistent with the subfertility and elevated miscarriage rates observed in PCOS [156] and highlight the need for further research into the role of MMPs in the endometrial dysfunctions associated with the condition.

6 | Alteration of MMPs and TIMPs in Polycystic Ovaries

Recent research has increasingly focused on exploring the role of fibrotic factors, such as MMPs and TIMPs, in the pathogenesis of PCOS, as these factors are known to influence the production of ovarian stromal elements and contribute to follicular atresia [157]. It appears that increased MMP activity, through the disruption of tissue remodeling, perturbations in gap junction communications, and alterations in growth factor availability, contributes to ovarian dysfunction observed in individuals with PCOS [47]. For instance, it has been documented that there is a high expression of gap junction alpha-1 protein in granulosa and theca cells. Consequently, any disruption in its functionality caused by changes in MMP activity may hinder follicular development [158]. Moreover, elevated MMP levels, through their influence on the proteolytic processing of TNF, can adversely impact folliculogenesis [159, 160]. Heightened levels of MMP and the MMP/TIMP ratio could contribute to the early selection of follicles for atresia in polycystic ovaries [129, 161].

It has been demonstrated that transgenic mice with constitutive secretion of active plasminogen activator inhibitor-1 (PAI-1) exhibit numerous cystic ovarian structures accompanied by elevated plasma testosterone levels [162]. Increased PAI-1 levels inhibit tissue plasminogen activator, subsequently suppressing MMP activity. However, the observed elevation in PAI-1 levels may represent a secondary response to increased MMP activity and fibrosis [163]. PAI-1 also plays a significant role in preventing follicular wall rupture and inducing anovulation, as observed in patients with PCOS [164].

Considering the pivotal role of MMP-9 in follicular development, several studies in animal models have investigated the alterations in MMPs under cystic conditions (Figure 2). MMP-2

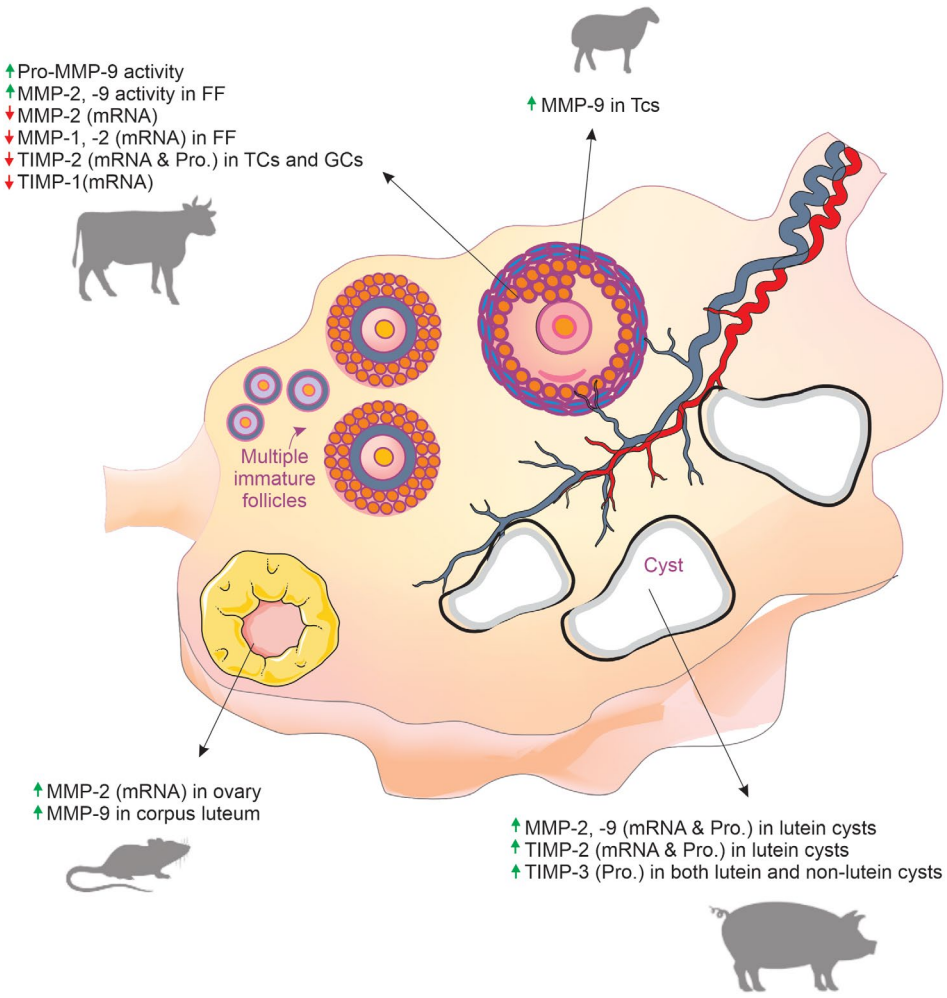

FIGURE 2 | Alterations in matrix metalloproteinases (MMPs) and their inhibitors in various regions of the ovary in animal models of PCOS or cystic ovaries. FF, follicular fluid; Pro, protein; TCs, Theca cells; TIMP, tissue inhibitor of metalloproteinases. Green arrows indicate high levels and red arrows indicate low levels.

protein expression was also observed to be higher in polycystic rat ovaries compared to superovulated ones during ovulation [165]. Interestingly, metformin inhibits PCOS pathogenesis in a rat model by downregulating MMP-2 and MMP-9 via the AKT/mTOR/autophagy pathway, emphasizing the critical role of MMPs in PCOS pathogenesis [166]. Another investigation concerning sows explored the alterations in MMP components across different types of cysts, revealing increased mRNA and protein expression of MMP-2 and MMP-9 within follicular lutein cysts. Furthermore, follicular cysts demonstrated heightened protein levels of both MMP-2 and MMP-9 compared to preovulatory follicles [61].

The elevated protein expression of MMP-9 within the theca cells of preantral follicles may contribute to the multi-follicular morphology observed following preantral testosterone treatment in sheep [47]. Furthermore, an association has been identified between an elevation in proMMP-9 levels and the development of follicular cysts in cattle [53]. When comparing MMP activity in the follicular fluid of cysts and preovulatory bovine follicles, increased protein levels of MMP-2 and MMP-9 were noted in follicular cysts [167].

However, MMP-2 mRNA expression is downregulated in preovulatory follicles of cows with cystic ovaries compared to the normal ones [167]. This finding corroborates the outcomes reported by Mutlag et al. [168], indicating reduced mRNA expression of MMP-1 and MMP-2 in follicular cysts. Hentze et al. [169] also observed a higher ratio of TIMP-1/MMP-9 and TIMP-2/MMP-2 in the plasma of cows with cysts compared to those without cysts.

TIMP-1 was found to be downregulated in the ovarian stroma of women diagnosed with PCOS [11]. In bovine cystic ovaries, a reduction in both mRNA and protein expression levels of TIMP-2 was detected in the theca and granulosa cells. Compared to preovulatory follicles, cystic follicles showed a notable reduction of TIMP-1 mRNA expression, although protein levels remained unaffected [167]. TIMP-2 expression, both at the mRNA and protein levels, was found to be higher in lutein cysts, while the increase in TIMP-3 was predominantly observed at the protein level [61]. In contrast, TIMP-3 mRNA levels were lower in whole ovary samples from PCOS patients [170].

The destructive effects of elevated MMP activity can contribute to the regression of cellular mass in the follicles of polycystic ovaries [165]. The findings indicate more pronounced ECM remodeling in cysts compared to preovulatory follicles, a difference that may vary depending on the degree of luteinization [61]. Shalev et al. [171] reported significantly higher gelatinolytic activity, reflected by elevated MMP-2 and MMP-9 levels, in luteinized granulosa cells from PCOS patients compared to those from normal ovulatory women. In a study using a rat model of PCOS, it was found that while MMP-9 protein expression significantly increased in the corpus luteum, it decreased in the follicular basement membrane [172].

Several studies have found no significant correlation between serum levels of MMP-9 or MMP-2 and their respective inhibitors TIMP-1 and TIMP-2 in PCOS patients [146, 173–175], while certain limited investigations have reported a notable correlation between MMP-2 and TIMP-2 levels in both serum and follicular fluid [62]. The absence of correlation between MMPs and their inhibitors may be attributed to resistance to TIMP or varying levels of MMP regulation.

Alternative mechanisms proposed for MMP regulation, aside from TIMPs, include transcriptional and post-transcriptional regulation via steroid hormones, cytokines, and growth factors, as well as the activation of proenzymes [176]. Furthermore, MT1-MMP acts as a regulatory protein capable of cleaving and subsequently activating MMP-2. The increased MMP activity observed in the PCO model may be attributed to the upregulation of MT1-MMP. This hypothesis is supported by the co-localization of MT1-MMP and MMP-2 expression within the follicles of the PCO model [165].

Studies in human PCOS patients, as summarized in Table 2, have shown elevated levels and activity of MMP-2 and MMP-9 in the follicular fluid and cultured granulosa cells of individuals with PCOS compared to those with normal ovulation [62, 129, 171]. Moreover, other studies indicated increased circulating levels of MMP-2 and MMP-9 among individuals with PCOS [173, 174, 179]. In PCOS offspring, circulating MMP-9 concentrations were significantly elevated compared to age- and BMI-matched controls, suggesting early-life alterations in extracellular matrix remodeling [181]. Although total MMP-9 levels in follicular fluid did not significantly differ between PCOS and non-PCOS women undergoing ICSI, the significantly lower MMP-9 per oocyte in the PCOS group suggests altered MMP-9 activity and potential dysregulation in the follicular environment of these patients [180].

Elevated levels of MMP-8 and the MMP-8/TIMP-1 ratio in serum and saliva, along with increased MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios in plasma, have been reported in patients with PCOS [146, 178]. These findings contrast with the reported role of MMPs in the growth and maturation of bovine follicles. Previous research indicated that pro-MMP-2 and active MMP-2 in the follicular fluid could reflect follicle health [15, 53]. The role of elevated MMP levels in follicular cysts remains unclear, as it is uncertain whether it signifies a primary cause or is a secondary response to overcome cortex fibrosis in PCOS patients. However, some studies did not observe differences in MMP-2 and MMP-9 concentrations between PCOS patients and healthy women [146, 175], and analysis of MMP-2 and MMP-9 activity in follicular fluid revealed no significant differences [58]. These discrepancies may be due to variations in sample sizes across studies, as well as differences in the use of serum or plasma in the study protocols.

The expression of ADAMTS-1 has been found to decrease in the granulosa cells of women with PCOS [182]. Moreover, in PCOS patients undergoing IVF, cumulus cells derived from oocytes at the metaphase II (MII) stage exhibit increased ADAMTS-9 mRNA expression compared to those associated with oocytes at the metaphase I (MI) stage [183]. Additionally, an association between ADAMTS-19 polymorphisms and PCOS has been established, further confirming the involvement of ECM remodeling in early ovarian development in PCOS pathogenesis and suggesting ADAMTS-19 as a potential diagnostic marker for this syndrome [184, 185].

TABLE 2 | Alterations in MMPs and their inhibitors in PCOS patients across various sample types.

Sample source	MMP-1	MMP-2	MMP-8 and MMP-8/ TIMP-1	MMP-9	TIMP-1	TIMP-2	MMP-2/ TIMP1	MMP-9/ TIMP2	Ref.
Salivary				Increased levels					[177]
Salivary and serum			Increased levels		Levels NC				[178]
Serum				Increased levels					[179]
Serum				Increased levels					[152]
Serum	Conc. NC	Activity or levels NC		Increased activity/ levels NC	Conc. NC			Increased ratio	[175]
Serum		Increased levels		Increased levels	Increased levels	Levels NC			[173]
Serum				Increased levels				Increased ratio	[174]
Plasma		Activity NC		Levels NC	Levels NC	Decreased levels	Increased ratio	Increased ratio	[146]
FF	Protein expression NC	Activity NC		Activity NC	Decreased protein expression				[58]
FF/IVF		Increased levels		Increased levels	Increased levels	Increased levels			[62]
FF and luteinized GCs/IVF		Increased activity in FF/ Higher activity and protein expression (in cells)		Increased activity in FF/Higher activity and protein expression (in cells)	Protein expression NC (in cells)				[171]
FF Oocyte				Levels NC Increased levels					[180]
Stroma/GCs	mRNA expression NC	mRNA expression NC		mRNA expression NC	mRNA expression decreased (in stroma)				[11]

Note: MMP levels, enzymatic activity, mRNA expression, and protein expression were assessed using ELISA, zymography, qPCR, and Western blot techniques, respectively.
Abbreviations: FF, follicular fluid; GC, granulosa cell; IVF, in vitro fertilization; MMP, matrix metalloproteinase; NC, no change; TIMP, tissue inhibitors of metalloproteinases.

7 | Concluding Remarks and Future Perspectives

Investigating the specific alterations in MMP expression and activity in PCOS is essential for understanding the pathophysiology of this syndrome. Elucidating how these changes in MMPs and ECM composition contribute to abnormalities in folliculogenesis may facilitate the development of novel therapeutic strategies. Targeted treatments aimed at modulating MMP activity could restore normal ECM remodeling, potentially alleviating PCOS symptoms and improving fertility outcomes in affected women.

Future research should focus on unraveling the precise molecular mechanisms through which ECM and MMP alterations drive the pathological features of PCOS. Advanced bioengineering approaches, such as the development of in vitro models that accurately mimic the ovarian microenvironment, could provide valuable insights into the dynamic interactions between follicular cells and their ECM. Continued exploration in this field is essential for designing innovative treatments that address the underlying causes of PCOS, rather than merely managing its clinical manifestations.

Author Contributions

Saba Nikanfar: conceptualization, data curation, funding acquisition, visualization, writing – original draft. **Christiani A. Amorim:** project administration, resources, validation, writing – review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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