[Frontiers in Bioscience 9, 2474-2483, September 1, 2004]

MMPS AND TIMPS IN OVARIAN PHYSIOLOGY AND PATHOPHYSIOLOGY

Shlomit Goldman [1] and Eliezer Shalev [1, 2]

[1] Laboratory for Research in Reproductive Sciences, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula,
[2] Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

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1. ABSTRACT

The matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been postulated to play a critical role in the extracellular matrix (ECM) remodeling associated with follicular development. The gelatinases were localized to the theca of developing follicles and in the stroma of the rodent ovary. Gelatinolytic activity corresponded with the localization of MMP-2 and MMP-9 around the developing follicles and at the apex of preovulatory follicles. The TIMPs-1, -2, and -3 were localized to the stroma and theca of developing follicles and correlation between MMPs and the quality of the developing follicles was found.

During the process of ovulation, MMP-1 protein was found in the theca interna and externa, interstitial glands, and germinal epithelium. Synthetic inhibitor of mammalian tissue collagenases was documented to be inhibitory to ovulation in perfused rat ovaries. MMP-19 and TIMP-1 messenger RNA were localized to the granulosa and thecal-interstitial cells of large preovulatory and ovulating follicles. Both were induced and upregulated 5-10 fold by human chorionic gonadotropin (hCG). MMP-2 mRNA found in theca-interstitial cells and membrane-type (MT) 1-MMP mRNA found in granulosa and theca-interstitial cells were both induced after stimulation with pregnant mare's serum gonadotropin (PMSG).

Gelatinolytic activity was observed throughout the formation of the corpus luteum. At 12 h after hCG, luteinizing granulosa cells expressed TIMP-1 and TIMP-3 mRNA. In the newly forming corpus luteum at 24 h after hCG administration, the luteal cells expressed TIMP-1, -2, and -3 mRNA with unique pattern of cellular expression for each of the TIMPs. Regression of the corpus luteum is associated with a significant increase in the activity of the metalloproteinases.

In luteinized granulosa cells from women with polycystic ovarian syndrome (PCOS) the MMP-TIMP balance is shifted towards greater MMP activity. Cultured luteinized granulosa cells obtained from PCOS patients secrete higher levels of MMP-9 and MMP-2 compared to granulosa cells from normal ovulatory patients whereas the secreted basal level of TIMP-1 was similar in both types of granulosa cells. These results indicate a higher net gelatinolytic activity within the luteinizing granulosa cells of patients with PCOS. It has been shown that in sheep, diversion of normal follicles to atresia by hypophysectomy is followed by a significant increase of intrafollicular levels of MMP-2 and MMP-9 and the disappearance of connexin-43. It is therefore reasonable to speculate that MMP-9 and MMP-2 may be associated with inappropriate atresia in PCOS.

2. INTRODUCTION

The matrix metalloproteinases (MMPs) are part of an expanded family of proteins called the astacin family of zinc metalloproteinases. The human MMP family can be subdivided according to their structural and functional properties into five groups: collagenases, gelatinases, stromelysins, membrane type (MT), and a fifth heterogeneous subgroup. These five subgroups of extracellular matrix (ECM) – degrading enzymes share common functional domains and activation mechanisms. They are synthesized and secreted (except the MT-MMPs) as a latent pro-form (zymogens) which requires activation to achieve proteolytic activity. Their active site contains Zn^{2+}, and they need calcium to maintain stability. They function at neutral pH and they are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs). The gelatinases, MMP-2 and MMP-9, differ from other MMPs in that the catalytic domain is separated from the haemopexin-like domain by a fibronectin-like domain (1).

MMPs synthesis is regulated primarily at the transcriptional level. MMP gene expression is regulated by numerous stimulatory and suppressive factors, driven via multiple signaling pathways. In addition to cell stress, change in cell shape and the proteases themselves; the long list of factors includes: phorbol esters, integrin-

derived signals, cytokines and growth factors such as interleukins, interferon, EGF, KGF, NGF, basic FGF, VEGF, PDGF, TNF-α, TGF-β, and the extracellular matrix metalloproteinases inducer (EMMPRIN). Post-transcriptional regulation has also been documented. Cytokines, growth factors and hormones can modulate the stability of the mRNA transcript. The transcript can either be stabilized, or destabilized (2).

The synthesized and secreted pro-peptide is thought to preserve the enzyme in its latent form by the interaction of a cysteine residue with the zinc ion on the catalytic domain. The activation of the enzyme proceeds via three different mechanisms. The primary one is a stepwise mechanism, which starts with proteinases such as plasmin, attacking the susceptible propeptide domain, exposing other (down stream) cleavage sites for a second proteinase. Destroying the Cys-catalytic zinc interaction ends this stepwise mechanism. Disruption of this triggers the cysteine switch mechanism (on the propeptide) and results in activation of the enzyme. A second activation mechanism occurs at the cell surface by MT-MMPs and the third less clear mechanism, involves intracellular activation by Golgi-associated, furin-like proteases (3).

The natural inhibitors of MMPs are α_2-macroglobulin and the TIMPs. Whereas the first are primarily fluid phase regulators, the TIMPs are considered to be the key inhibitors in tissue. The four known mammalian TIMP proteins (TIMP-1 to 4) are two domain molecules sharing 30-40% identity at the amino acid level. The longest N-terminal domain of about 125 residues is responsible for the inhibitory activity against MMPs through complex interaction with the conserved zinc-binding site of the active MMPs. The smaller C-terminal domain has about 65 amino acid residues and interacts with the zymogen pro-peptide. The relatively small two domain TIMPs have been found to exhibit several biochemical and biological functions. This includes inhibition of the active MMP and pro MMP activation, cell growth promotion, inhibition of angiogenesis and the induction of apoptosis. Whereas TIMP-1, -2 and 4 are secreted in soluble form, TIMP-3 is associated with ECM. The first two TIMPs (TIMP-1 and TIMP-2) exhibit inhibitory activity against the active forms of all known MMPs (4).

MMPs, probably balanced by their tissue inhibitors, are essential effectors of developmental processes. The MMPs fulfill an important role in endothelial cell invasion, angiogenesis and in tumor progression. They participate in the processes of cell migration, cell proliferation, apoptosis and tissue morphogenesis regulating the function of biologically active molecules. Fundamental aspect of ovarian function (secretion of hormones and production of oocytes), require dynamic, extensive, cyclic tissue remodeling. That is why MMPs are expected to have a role during the ovarian cycle, including follicular development, ovulation, and subsequent formation and regression of the corpus luteum, while derangement in their balance may contribute to ovarian pathology, which is characterized by improper follicular development and atresia, such as the polycystic ovarian syndrome (PCOS).

3. MMPS AND TIMPS IN FOLLICULAR DEVELOPMENT

Initiation of follicular growth at the primordial follicle stage begins with activation and proliferation of the granulosa cells, differentiation of the theca cell layer from the ovarian stroma, and deposition of a basement membrane between the granulosa and theca compartments. With continued growth and development, the resulting mature Graafian follicle is approximately 400-fold larger than its original primordial follicle progenitor. The follicle rests in an extracellular environment composed, in part, of collagen, laminin, and fibronectin (5–8). To accommodate the dynamic and extensive growth of the follicle, there must be concomitant remodeling of the granulosa cell basement membrane and the extracellular matrix surrounding the follicle. During the ovulatory process, extensive remodeling is again apparent in the dissolution of the granulosa cell basement membrane (9, 10) and fragmentation of the extracellular matrix of the follicular wall (11–14), allowing the process of oocyte release to take place. In the ovary, MMPs and TIMPs have been postulated to play a critical role in extracellular matrix remodeling associated with follicular development (15-19). Nevertheless, little is known about the cellular localization of the MMP system during this periovulatory period.

In rodents, gelatinase mRNA (MMP-2 and MMP-9) has been localized to the theca of developing follicles, and to the stroma. During this period, MMP-9 mRNA remains predominately in the theca. *In situ* zymography for gelatinolytic activity demonstrated a pattern of activity that corresponded with the localization of MMP-2 and MMP-9 mRNA around the developing follicles. Gelatinolytic activity was observed at the apex of preovulatory follicles. The mRNA for TIMP-1, -2, and -3 has been localized to the stroma and theca of developing follicles. TIMP-3 mRNA was present in the granulosa cells of certain follicles but was absent in granulosa cells of adjacent follicles (15, 18, 19). In other species, such as the goat, collagenase activity has been noted to increase with increasing follicular size (19). Although little is known about the MMPs during follicular growth in the human, in other species, the MMPs are present within the follicle, are stimulated by the events associated with follicular development, and have localization and expression patterns that coincide with the changes in follicular remodeling of the granulosa and theca cell layers.

Of particular interest is the possible role of the MMPs in follicular atresia. A correlation between MMP-9 secretion and the quality of the developing follicles was found (15). When the MMP was secreted, the probability of follicles having at the end of the culture period, healthy granulosa or theca cells was 0.85 and 0.60, respectively. If TIMP-1 was released, the probability of follicles having healthy somatic cells was 0.79. When TIMP-2 was detected, the probability of granulosa and theca cell health was 0.78 and 0.67, respectively. MMP-9 and TIMPs were

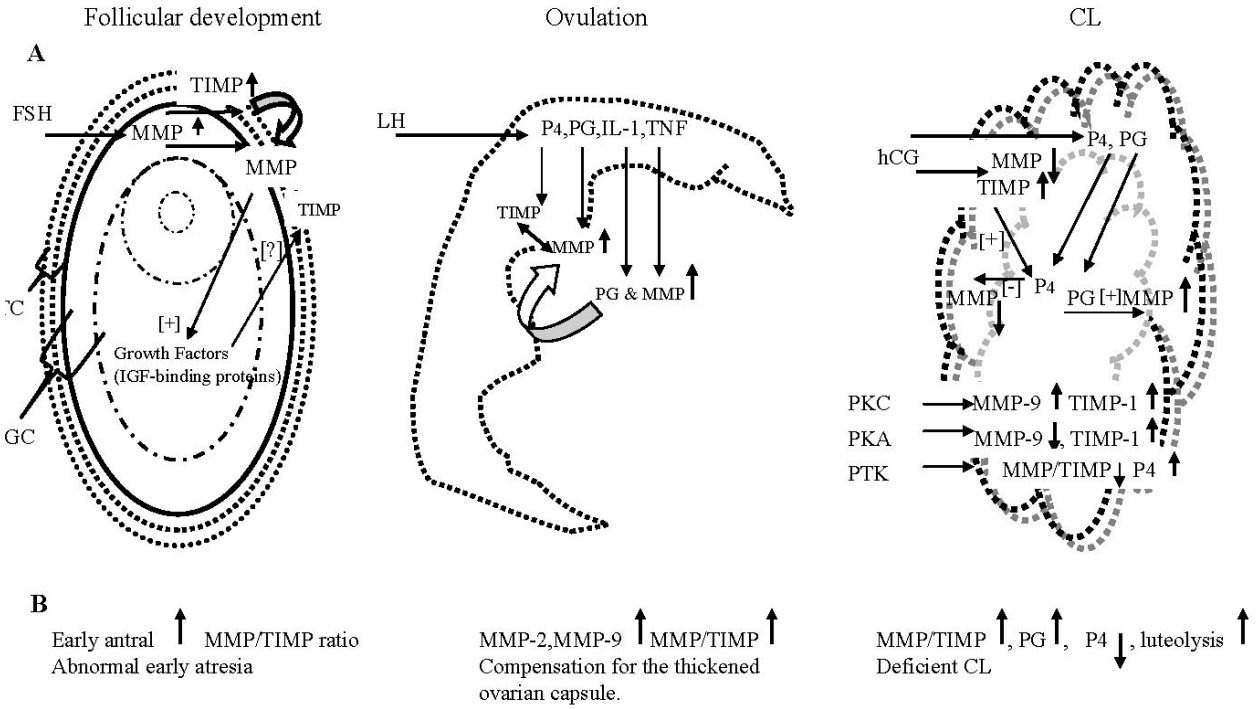

Figure 1. Regulation of the MMP system in the normal ovary (A) with suggested imbalance in the MMP/TIMP ratio having morphologic and functional impact in PCOS (B). An increase in MMP-2 and in the MMP/TIMP ratio seems to be the reason for early selection of follicles destinated for atresia in PCOS. Exposure to phorbol 12-myristate 13-acetate (TPA), acting via protein kinase C (PKC) elicited more pronounced increase in MMP-9 and TIMP-1 secretion in normal control group. TPA apparently did not affect progesterone secretion in the normal or in the PCOS group. Epidermal growth factor (EGF), acting via protein tyrosine kinas (PTK), did not significantly change the secretion of MMP-9 or TIMP-1. However, EGF dose-dependently, decreased the MMP-9/TIMP-1 ratio, and increased progesterone secretion in the PCOS group. Forskolin, acting via protein kinas A (PKA) inhibited MMP-9 activity more pronouncedly in the normal ovulatory group. Forskolin increased TIMP-1 and progesterone secretion in the normal ovulatory group. Progesterone production was inversely related to the ratio of MMP-9/TIMP-1 regardless of cell origin. The abnormally increased net gelatinolytic activity within the luteinizing granulosa cells of patients with PCOS is probably the reason for insufficient luteal function observed in these women.

found to be related to follicular health and therefore, can be used as markers of follicular development. The active form of MMP-2 and an additional band of proMMP-9 were detected only in atretic follicular fluid. Gelatinase activity was recorded in both granulosa cells (GCs) and theca cells (TCs) but was found in comparatively higher numbers in those follicles that exhibited a thinned and partially detached granulosa layer. Huet *et al.* (20) also found that in sheep, diversion of normal follicles to atresia by hypophysectomy is followed by a significant increase of intrafollicular levels of MMP-2 and MMP-9. The process of atresia was followed by comparing healthy follicles from intact ewes, with early atretic follicles recovered 24 h after hypophysectomy clearly atretic follicles recovered 36 h after hypophysectomy and late atretic follicles recovered 72 h after hypophysectomy. Intrafollicular levels of matrix metalloproteinase-2 and -9 were shown to increase as early as 24 h after hypophysectomy and a further increase was observed until 72 h after hypophysectomy. In bovine ovaries (21), proMMP-2 was detected in all follicles regardless of their size. The abundance of proMMP-2 varied with follicular size. There was a positive and negative correlation between estradiol (E_2) and progesterone (P_4) concentrations with an abundance of proMMP-2, respectively. Follicles of diameter over 25 mm had greater proMMP-9 activity than other follicles. Similar to the observed changes in the expression of TIMP-1, which parallels the changes in the MMPs, TIMP-4 levels of mRNA that basically are extremely low, increases following gonadotropin stimulation (22).

Regulation of the MMP system in the follicular phase is probably FSH dependent (Figure 1). FSH stimulates follicular development via the granulosa or theca compartments to increase the MMPs and regulate TIMP expression either directly by inducing the PKA (Protein Kinase A) signal transduction pathway or indirectly through several growth factors such as IGF-I (Insulin Like Growth Factor-I). Insulin-like growth factors, and particularly IGF-I, have been identified as being important paracrine regulators of ovarian function. However, the precise role of the IGF system during the earlier stages of follicular development is poorly understood and its role on MMP modulation is not yet clear. In addition to the possible effect of IGF-I on MMP it was postulated that MMPs might act to regulate the availability of IGF by cleaving IGFBP (IGF-binding proteins) releasing IGF to further enhanced follicular growth (15, 22, 23).

Table 1. Changes, in the MMPs and their TIMPs during follicular stage, based on Curry & Osteen 2003 (22

Group	Collagenase			Gelatinase		Stromelysin			Membrane type			Mat	TIMP			
No.	1	8	13	2	9	3	10	11	14	17	19	7	1	2	3	4
Mouse								I								
Rat			I	I	I				NC		NC		I	NC	D	I
Ovine				I	I											
Bovine					I											
Porcine				I	I											

Mat: matrilysin; I: Increased; D: decreased; NC: No change in expression; Ab: Absence of MMP or TIMP expression

A summary of the changes in the ovary of the MMPs and their TIMPs during the follicular stage is presented in table 1.

4. MMPS AND TIMPS IN THE OVULATION PROCESS

Outgrowth of the pre-ovulatory follicle brings it into close contact with the ovarian surface epithelium while the epithelium within the immediate vicinity of the ovulatory stigma become apoptotic (24). The process of ovulation, which starts with the surge of the luteinizing hormone (LH), ends with the oocyte released from the mature pre-ovulatory follicle. Already in 1991, a synthetic inhibitor of mammalian tissue collagenase and related metalloproteinases (SC 44463) was documented to be inhibitory to ovulation in perfused rat ovaries (25). During ovulation, there are structural changes within the extracellular matrix, where regulated dissolution of matrix proteins occurs. Proteolytic degradation of the follicular wall is required to release the mature oocyte (26). In the process of ovulation, MMP-1 protein was found in the theca interna and externa, interstitial glands, and germinal epithelium. The presence and the activity of this protease were increased in the capillary lamina at the apex of the follicle, as ovulation approached (27). Hagglund *et al.*, (26) have examined the regulation of 11 MMPs and 3 tissue inhibitors of metalloproteinases (TIMPs) during gonadotrophin-induced ovulation in the mouse. The majority of the examined MMPs were undetected (MMP-9,-8,-13,-3,-10,-7 and –12), while others (MMP-2, -11, and –14) and TIMP-2 and TIMP-3 were expressed during the periovulatory period at a constitutive level. However, MMP-19 and TIMP-1 were both induced and upregulated 5-10 fold by hCG. Both reached their maximum levels at 12 h after hCG treatment. At this time point, corresponding to the time of ovulation, MMP-19 and TIMP-1 messenger RNA were localized to the granulosa and thecal-interstitial cells of large preovulatory and ovulating follicles. This regulation pattern suggests that MMP-19 might be involved in the tissue degradation that occurs during follicular rupture and that TIMP-1 could have a role in terminating MMPs activity after ovulation (28). Liu *et al*., (27) used *in situ* hybridization to study the regulation and distribution of mRNA coding for MMP-2 (gelatinase A), and its cell surface activator membrane-type MMP1 (MT1-MMP) during induced ovulation. The levels of both MT1-MMP and MMP-2 mRNA were low in the ovaries of untreated immature rats. MMP-2 mRNA found in theca-interstitial cells and MT1-MMP mRNA found in granulosa and theca-interstitial cells were both induced after stimulation with PMSG. Following stimulation, the expression of MMP-2 and MT1-MMP remained and appeared to be up regulated, together in the theca-interstitial cells surrounding the large preovulatory follicles. However, the expression of MT1-MMP was extensively down regulated in the granulosa cell layers of these large preovulatory follicles. The expression kinetics and tissue distribution support the notion that MT1-MMP may have dual functions in the ovary. Initially, MT1-MMP may act as a matrix degrading protease inside the follicle during follicular development and later, just prior to ovulation, as an activator of proMMP-2 in theca-interstitial cells surrounding preovulatory follicles (29). Additional support for the role of the MMPs in follicle rupture during ovulation comes from the study of Kimura *et al*., (30) in which bradykinin B2 receptor was localized in the follicles of the porcine ovary, and massive expression of MMP-3 and -20 was induced in cultured granulosa cells by bradykinin treatment. In contrast to the observed increased expression of TIMP-1, decreased levels of TIMP-4 were reported following hCG administration (22).

A new human matrix metalloproteinase, tentatively called MMP-23, has been cloned from an ovary cDNA library by Velasco *et al* (31). This protein exhibits sequence similarity with MMPs, but displays a different domain structure. Northern blot analysis demonstrated that MMP-23 is predominantly expressed in ovary, testis, and prostate, suggesting that this new MMP may play a specialized role in reproductive processes.

Regulation of the MMP system during ovulation starts with LH secretion (Figure 1). LH initiates and synchronizes a series of biochemical events that culminate in the breakdown of the follicle wall and extrusion of the oocyte. These biochemical events include synthesis and secretion of progesterone, prostaglandin (PG), growth factors and cytokines, which activates the expression and secretion of several MMPs and TIMPs (22,32). PGs, which are involved in the follicular rupture, increase MMP production. Inhibition of PG synthesis blocks the periovulatory increase in the mRNA for MMP-1 and MMP-13 as well as collagenase activity (22, 33). LH stimulates an increase in cytokines, such as interleukins (IL) and tumor necrosis factor (TNF), which may act independently or in concert with other signal pathways to regulate MMPs and their inhibitors. The ovary has a complete, compartmentalized, and hormonally regulated IL-1 system that has been postulated to play a role in ovulation. The preovulatory LH surge increases levels of IL-1. IL-1 stimulates PG biosynthesis, and induces ovulation in perfused ovaries. An IL-1 receptor antagonist inhibits oocyte release, and IL-1 stimulates MMPs in numerous tissues. Support for the concept that interleukins may regulate

Table 2. Changes, in the MMPs and their TIMPs during ovulation, based on Curry & Osteen 2003 (22)

Group	Collagenase			Gelatinase		Stromelysin			Membrane type			Mat	TIMP			
No.	1	8	13	2	9	3	10	11	14	17	19	7	1	2	3	4
Mouse		Ab	Ab	NC	I	Ab	Ab	NC	NC	Ab	I		I	NC	I	
Rat	I		I	I	NC				I		I		I	NC	I	D
Ovine													I	NC		
Bovine				NC					I					I		
Porcine													I			
Primate	I			I	I							I	I	I		
Human													I			

Mat: matrilysin; I: Increased; D: decreased; NC: No change in expression; Ab: Absence of MMP or TIMP expression

the ovarian MMP system is forthcoming from studies demonstrating that IL-1 stimulates MMP-9 activity (34, 35).

A summary of the changes in the ovary of the MMPs and their TIMPs during ovulation is presented in table 2.

5. MMPS AND TIMPS IN THE CORPUS LUTEUM

Following ovulation, the ruptured follicle is transformed into a corpus luteum (CL) by extensive cellular reorganization and neovascularization. The degree of reorganization and intermixing of the thecal and granulosa cell layers during luteal formation differs among species. In nonprimate mammals, extensive cellular migration and reorganization occur. In primates, the intermixing of the different follicular cells is less extensive than in other species, such that a distinct granulosa-lutein and theca-lutein layer are formed (36). With formation of the CL, progesterone production increases dramatically, yet negligible changes take place in the luteal connective tissue matrix during this period. At the end of the CL life span, two phases of luteolysis occur. First, a functional luteolysis is marked by a rapid decline in progesterone production. This is followed by a slower, prolonged structural luteolysis of the CL. The structural changes occur, in part, by an apoptotic mechanism involving proteolysis and phagocytosis (37). Further degradation is accomplished by invading macrophages. Macrophages, which contain MMPs or can stimulate enzyme production in other tissues, increase in number in the involuting CL and phagocytize the luteal cells. Following an hCG stimulus, MMP-2 mRNA increases as the granulosa cells of preovulatory follicles undergo luteinization during formation of the corpus luteum (CL) (38). Gelatinolytic activity was observed throughout the formation of the CL. At 12 h after hCG, luteinizing granulosa cells expressed TIMP-1 and TIMP-3 mRNA, but TIMP-2 mRNA was equivalent to the background levels. In the newly formed CL at 24 h after hCG administration, the luteal cells expressed TIMP-1, -2, and -3 mRNA, although the pattern of cellular expression was unique for each of the TIMPs. For TIMP-4, there was a trend toward higher level of expression on the day of estrus with no changes observed across the cycle in the mouse or the rat. In both, background levels of mRNA were observed in the new CL, with punctate distribution observed within the older CL (22).

A fundamental aspect of corpus luteum (CL) formation and regression is dynamic and extensive tissue remodeling which is modulated via MMP enzymes (39). It is well known from studies in other mammals, that regression of the corpus luteum is associated with a significant increase in the activity of the matrix metalloproteinases (40). Stamouli *et al*. (37), have shown that hCG decreases MMP-9 expression and increases TIMP expression and have suggested that the rescue of the corpus luteum in early pregnancy involves the maintenance of cellular function through the stabilization of the ECM by increasing TIMP levels. Moreover, it has been shown that in porcine luteinized granulosa cells, MMPs are expressed and released in high amounts and that this is essential for the structural regression of the corpus luteum (38). While there are several types of TIMPs and it is possible that one of the other TIMPs could play a modulatory role in the collagenolytic balance in the corpus luteum, it is TIMP-1 that has been most consistently demonstrated.

TIMP–1 has been described has a multifunctional protein with tissue specific activities. The protein was found to be responsible for activation of steroidogenesis in the testis (40) as well as in the ovary (41). TIMP-1 was suggested to play a pivotal role in maintaining the production of progesterone (P_4) in the early corpus luteum. Immature wild type and TIMP-1 null female mice were primed with gnadotropins. Serum progesterone concentration was found to be significantly lower in the TIMP-1 null mice (42).

Normal regulation of the MMP system in the CL is directed first for rescue and then, if pregnancy has not occurred, for regression. A reduction in the mRNA expression and activity of the MMPs, and increased TIMP expression has been observed following hCG (Figure 1). Progesterone has a similar effect on the MMP system. It is documented that progesterone inhibits MMP secretion while the PG are responsible for the luteolysis process induced by increased MMP production (22, 32, and 43).

Summary of changes in MMPs and TIMPs during corpus luteum formation and regression are presented in table 3 and table 4, respectively.

6. THE POLYCYSTIC OVARY SYNDROME

Since the first description by Stein and Leventhal (1935), the entity of PCOS has been extensively investigated, nevertheless, its etiology still remains a subject of much speculation. About half of infertility cases are attributable to female factors (44, 45), of which

Table 3. Changes, in the MMPs and their TIMPs during corpus luteum formation, based on Curry & Osteen 2003 (22)

Group	Collagenase			Gelatinase		Stromelysin			Membrane type			Mat	TIMP			
No.	1	8	13	2	9	3	10	11	14	17	19	7	1	2	3	4
Mouse																
Rat			NC	I					NC				I	I/NC	I/D	
Ovine									I				NC	I		
Bovine				NC	I								I	NC		
Porcine	NC			NC	NC								I	I		
Primate																
Human	NC			NC	NC								NC	NC		

Mat: matrilysin; I: Increased; D: decreased; NC: No change in expression; Ab: Absence of MMP or TIMP expression

Table 4. Changes, in the MMPs and their TIMPs during corpus luteum regression, based on Curry & Osteen 2003 (22)

Group	Collagenase			Gelatinase		Stromelysin			Membrane type			Mat	TIMP			
No.	1	8	13	2	9	3	10	11	14	17	19	7	1	2	3	4
Mouse													I	I		I
Rat			I	I/NC	NC				I				I	I	I	I
Ovine				I									D	D	I	
Bovine									I/NC				I	NC		
Porcine	I			I	I								NC	NC		
Primate													D			
Human	NC												NC	NC		

Mat: matrilysin; I: Increased; D: decreased; NC: No change in expression; Ab: Absence of MMP or TIMP expression

anovulation is the leading cause (46). Most cases of anovulation, i.e. improper follicular development and steroidogenic functions are due to the polycystic ovarian syndrome (PCOS) (47, 48). Clinically, PCOS is presented in 5% of all women, causing major health and cosmetic problems (49).

The polycystic ovary syndrome consists of the following major constituting clinical features (48,50): Oligomenorhea, anovulation, hyperandrogenism (i.e. acne and hirsutism), increased serum LH levels along with normal or low follicular stimulating hormone (FSH) leading to an elevated LH/FSH ratio compared to normally cycling woman and a typical polycystic appearance of the ovaries on ultrasonography. There are multiple small pre-antral and antral follicles in a peripheral location. These follicles are generally atretic on histologic examination and the volume of stroma is typically increased. Homburg (2002) (47) formulated the diagnostic requirement for PCOS. Ultrasonographical typical findings are the most significant and some evidence of hyperandrogenism is needed to make the diagnosis. Nevertheless, hyperandrogenic anovulation is still within the boundaries of the syndrome, even in the absence of the ultrasonographic findings. Infertility was included in the original description of PCOS, but in women who have not attempted conception it may not be recorded. Many studies also describe an increased miscarriage rate in PCOS (48). At least one-half of women with PCOS are obese (50). About 50-60 % of women with PCOS are also insulin resistant and hyperinsulinemic, independent of obesity, compared to normal women (51). The direct cause to this insulin resistance is still enigmatic (52).

Familial clustering of PCOS patients occurs in some cases. Some studies suggested an autosomal dominant inheritance (53) whereas other studies did not confirm this finding. These differences may be attributable to differences in the populations' genetic background. Other studies have shown that sisters to PCOS patients are hyperandrogenic and if overweight, drift towards full-blown PCOS (54). As of today, no solid genetic foundation for PCOS has been described which would reconcile all previous findings. It is likely that the eventual verdict would be some form of a complex trait. Accordingly, at the tissue level of events, several systems of paracrine and autocrine effectors were investigated in an attempt to define the underlying pathological mechanism responsible for PCOS. Cells from PCOS patients differ functionally from normal cells as reflected by IGF-I and IGF-II production by GCs (55) and in the apoptosis rate (56), as well as higher MMP-2 and MMP-9 production (57, 58). PCOS theca cells have a gene expression profile that is distinct from normal theca cells. Transcripts that are increased include aldehyde dehydrogenase 6 and retinol dehydrogenase 2, which play a role in all-trans-retinoic acid biosynthesis and the transcription factor GATA6. Retinoic acid and GATA6 increase the expression of 17alpha-hydroxylase, which can explain androgen overproduction. The number of suggestions as to the exact mechanism, leading to the final clinical picture, seems to equal the number of defects described. A fundamental aspect of follicular development, ovulation and subsequent formation and regression of the corpus luteum is dynamic and extensive tissue remodeling (59). As hinted by the landmark of PCOS, the formation of multiple mid-sized ovarian cysts and by the frequently insufficient luteal function, the mechanism(s) involved in tissue remodeling during the life cycle of an ovarian follicle may be affected in this pathology.

7. MMPS AND TIMPS IN PCOS

In follicular fluid from women with PCOS, levels of MMP-9 and MMP-2 were higher than the

normal group, as was the basal production of these proteins by cultured cells. Basal production of TIMP-1 by cultured cells was not different between PCOS and normal controls. A time-dependent increase in the production of MMP-9 was observed in cells from both normal and PCOS women, although the increase was more pronounced in the latter. Thus the MMP-TIMP balance is shifted toward greater MMP activity in luteinized granulosa cells from women with PCOS. We found that cultured luteinized granulosa cells obtained from PCOS patients secrete higher levels of MMP-9 and MMP-2 compared to granulosa cells from normal ovulatory patients. The same trend was observed in the follicular fluid, indicating that the in-vitro finding is consistent with the in-vivo situation. In contrast to MMP-2 and MMP-9 activity, the secreted basal level of TIMP-1 was similar in both types of granulosa cells. Together these results indicate a higher net gelatinolytic activity within the luteinizing granulosa cells of patients with PCOS (57). One could therefore suggest that a high gelatinolytic activity could contribute to a rapid regression of the corpus luteum and consequently lead to insufficient luteal function, resulting in a higher than normal abortion rate. It is well known that together with anovulation and increased androgen production, patients with PCOS portray a typical morphology of multiple mid-size ovarian cysts. These are believed to be follicles that have undergone early antral stage atresia but for some unknown reason did not shrink and disappear (60). It is conceivable that to a certain extent tissue remodeling in women with PCOS is different from normal since in normal ovulatory women, non-dominant follicles undergo atresia only at the pre-ovulatory stage. It has been shown that in sheep, diversion of normal follicles to atresia by hypophysectomy is followed by a significant increase of intrafollicular levels of MMP-2 and MMP-9 and the disappearance of connexin-43 (20). It is therefore reasonable to speculate that MMP-9 and MMP-2 may be associated with inappropriate atresia in PCOS.

Little is known of the expression of ECM genes in polycystic ovaries. Oksjoki *et al*. (61) studied and compared the expression levels of genes coding for collagens, matrix metalloproteinases (MMP), their inhibitors (TIMP) and cathepsins in polycystic ovaries using fertile and post-menopausal ovaries as controls. In Northern analyses, the gene expression profiles of type I and III collagen of PCOS samples resembled those observed in normal follicular phase ovaries, while mRNA levels of proalpha1 (IV) collagen and TIMP-3 mRNA were significantly lower in polycystic than control ovaries. During the normal menstrual cycle, an increase was observed in MMP-9 gene expression during the luteal phase. In post-menopausal ovaries, mRNA levels for type I, III and IV collagens and osteonectin were reduced, while the MMP, TIMP (excluding TIMP-3) and cathepsins did not reflect this metabolic down-regulation. Immunohistochemical staining for MMP-9 and TIMP-4 suggested differences between polycystic and normally functioning ovaries. These data demonstrate that normal ovarian functions are associated with changes in production and degradation of ECM. The alterations observed in the production and/or distribution of type IV collagen, TIMP-3 and TIMP-4, suggest involvement of basement membranes in the pathogenesis of PCOS. An imbalance between the activity of MMPs and their inhibitors, the TIMPs, may be one of the mechanisms responsible for dysfunction and early regression of the corpus luteum. Progesterone secretion was described to be lower in cells from PCOS patients (62).

The regulation of MMP-9, TIMP-1 and progesterone was investigated in our laboratory via three signal transduction pathways in normal ovulatory versus PCOS human luteinized granulosa cells. Exposure of these cells to phorbol 12-myristate 13-acetate (TPA, 10^{-7}M-10^{-9}M), acting via protein kinase C elicited an increase in MMP-9 and TIMP-1 secretion in both groups but more pronouncedly in the normal control group. TPA apparently did not affect progesterone secretion in the normal or in the PCOS group. Epidermal growth factor (EGF, 20-100ng/ml), acting via protein tyrosine kinase, did not significantly change the secretion of either MMP-9 or TIMP-1. However, EGF dose-dependently decreased the MMP-9/TIMP-1 ratio and increased progesterone secretion in the PCOS group. Forskolin (1-100 μM), acting via protein kinase A inhibited MMP-9 activity in both groups but more pronouncedly in the normal ovulatory group. In contrast, forskolin increased TIMP-1 and progesterone secretion. Progesterone production was inversely related to the ratio of MMP-9/TIMP-1 regardless of cell origin (58). These findings (Figure 1) raised the possibility, at least *in vitro*, that repressing the MMP-9/TIMP-1 ratio may have an important modulatory effect on progesterone secretion. An imbalance in the TIMP/MMP ratio that favors net MMP activity contributes to dampened progesterone production during the early stage of corpus luteum development (42). This phenomena seems to be exaggerated in time and magnitude in the PCOS ovary.

8. SUMMARY AND PERSPECTIVE

Adaptive cyclic changes in the ovary are the main features of human reproduction. These changes are accompanied and modulated by dynamic changes in the expression of the MMPs. These proteins are acting not only as ECM degrading enzymes but, balanced by their tissue inhibitors, the MMPs are essential effectors of developmental processes. In the mammalian ovary, other than the human, an increase in collagenase activity with unique spatial and temporal distribution is observed prior to ovulation. Although direct data in the human have not yet been elaborated, it seems that the MMPs are playing a pivotal role in the human ovulation process. The extensive tissue remodeling taking place during formation and regression of the corpus luteum is mainly modulated via the MMPs. In order to maintain corpus luteum survival, hCG both regulates the activity of the MMPs by suppression of their production, and enhancement of the production of their TIMPs. Further delineation of the functions of these proteins, first in normal human ovarian physiology and then in pathology such as the PCOS, will allow pharmacological intervention and management of the

pathological conditions associated with abnormal expression of these proteins.

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Abbreviations*:* CL: corpus luteum; D: decreased; ECM: extracellular matrix; E_2: estradiol; FGF: fibroblast growth factor; FSH: follicular stimulating hormone; GCs: granulosa cells; hCG: human chorionic gonadotropin; I: increased; IGF: insulin-like growth factor; IL: interleukins; KGF: keratinocyte growth factor; LH: luteinizing hormone; MMPs*:* matrix metalloproteinases; MT: membrane-type; NGF: nerve growth factor; P_4: progesterone; PG: prostaglandin; PDGF: platelet-derived growth factor ;PMSG: pregnant mare's serum gonadotropin; PCOS: polycystic ovarian syndrome; PKA: protein kinas A; PKC: protein kinase C; PTK: protein tyrosine kinas; TCs: theca cells; TPA: tumor promotion activator; TIMPs; tissue inhibitors of matrix metalloproteinases; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

Key Words: MMP, TIMP, PCOS, Ovary, Review

Send correspondence to: Eliezer Shalev MD, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula 18101, Tel: 972-4-6494031, Fax: 972-4-6494032, E-mail: shaleve@tx.technion.ac.il