Journal of Reproduction and Fertility Supplement **54**, 367–381

Regulation of ovarian extracellular matrix remodelling by metalloproteinases and their tissue inhibitors: effects on follicular development, ovulation and luteal function

M. F. Smith[1], E. W. McIntush[2], W. A. Ricke[1], F. N. Kojima[1] and G. W. Smith[3]

[1]*Department of Animal Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA;* [2]*Animal Reproduction and Biotechnology Laboratory, Colorado State University, Ft Collins, CO 80523, USA; and* [3]*Department of Animal Sciences, Michigan State University, East Lansing, MI 48824, USA*

In most organs, remodelling of tissues after morphogenesis is minimal; however, normal ovarian function depends upon cyclical remodelling of the extracellular matrix (ECM). The ECM has a profound effect on cellular functions and probably plays an important role in the processes of follicular development and atresia, ovulation, and development, maintenance and regression of corpora lutea. Matrix metalloproteinases (MMPs; collagenases, gelatinases, stromelysins and membrane-type MMPs) cleave specific components of the ECM and are inhibited by tissue inhibitors of metalloproteinases (TIMPs). MMPs have been detected at all stages of follicular development and probably modulate follicular expansion or atresia within the ovarian stroma. In addition, increased MMP activity appears to be required for ovulation since follicular rupture occurred in the absence of plasminogen activator activity and inhibitors of MMPs blocked follicular rupture. Development and luteolysis of the corpus luteum are accompanied by extensive remodelling of the ECM. Differentiation and regression of luteal cells are associated with construction and degradation of ECM, respectively. There is increasing evidence that ECM components enhance luteinization; whereas loss of ECM results in luteal cell death. Ovine large luteal cells may be the primary type of cell responsible for controlling the extent of remodelling of luteal ECM since they produce TIMP-1, TIMP-2 and plasminogen activator inhibitor 1. The ratio of active MMPs to TIMPs may be important in maintaining an ECM microenvironment conducive to the differentiation of follicular-derived cells into luteal cells, and maintenance of the phenotype of luteal cells.

Introduction

In female mammals, glandular systems including the cyclic endometrium, mammary gland, ovarian follicle and corpus luteum undergo growth, maturation and involution at various stages in the reproductive cycle or lifespan of the animal. Remodelling of the extracellular matrix (ECM) is required for the dynamic tissue reorganization characteristic of these tissues. The ECM consists of proteinaceous and nonproteinaceous molecules that provide the tissue-specific, extracellular architecture to which cells attach. Furthermore, interaction of cellular receptors (integrins) with proteins of the ECM can regulate cellular structure, second messenger generation and gene expression. Selective synthesis and degradation of proteinaceous components of the ECM are essential for follicular growth, ovulation, luteal formation and luteolysis (reviewed by McIntush

and Smith, 1998). Two families of enzymes that regulate remodelling of ECM are the matrix metalloproteinase (MMP) and the plasminogen activator/plasmin families. The focus of this review is on the role of MMPs and their inhibitors (TIMPs) in remodelling of ovarian ECM and subsequent effects on follicular and luteal function. Emphasis has been given to ruminant species whenever possible.

The Extracellular Matrix and Cellular Function

Within follicles and corpora lutea, cells are exposed to various ECM ligands that bind to integrins (receptors for ECM) as components of ECM are degraded and replaced (Luck, 1994). Proteinaceous and nonproteinaceous components of the ECM vary between and within tissues and, thereby, provide specialised microenvironments for specific cells. The primary proteinaceous components of the ECM have been reviewed elsewhere (Luck, 1994). Components of the ECM actively modulate the function of cells through integrins, which serve as a class of receptor on the cellular surface. Integrins are heterodimers containing α- and β-subunits. With 14 α-subunits and 8 β-subunits that associate in different combinations, integrins can form at least 20 distinct receptors with different ligands. Binding of integrins to their respective ligands modulates the generation of second messengers by hormones, growth factors and cytokines. In addition, interactions between ECM and integrins activate a variety of intracellular signalling molecules including serine–threonine, tyrosine, and lipid kinases and phospholipases. Consequently, the regulation of ECM degradation by MMPs and their natural inhibitors (tissue inhibitors of metalloproteinases; TIMPs) can have a profound influence on the cellular microenvironment and, thereby, modulate the function of follicular and luteal cells.

Matrix Metalloproteinases

Matrix metalloproteinases are zinc- and calcium-dependent enzymes that include collagenases, gelatinases, stromelysins and the membrane bound metalloproteinases (Nagase, 1997). These enzymes share various biochemical properties and are largely responsible for degrading proteinaceous components of the ECM. The family of MMPs currently includes at least 17 members that have different specificities (Table 1); however, new members will undoubtedly be discovered in the future. Activity of MMPs is highly regulated and subject to control at several different points (Fig. 1; Kleiner and Stetler-Stevenson, 1993). Two important points of control include activation of latent enzymes and association with tissue-derived inhibitors (TIMPs; see below). Most MMPs are secreted as proenzymes and are activated by proteolytic cleavage of an N-terminal peptide. Mast cell proteinases (Suzuki *et al.*, 1995), serine proteinases (plasmin and kallikriens; Espey, 1992) and other MMPs including the membrane-bound MMPs (Sato *et al.*, 1994) activate latent MMPs in the extracellular milieu. However, certain MMPs, such as stromelysin-3 and the membrane type-1 metalloproteinase are activated intracellularly. Although most MMPs are secreted, four membrane bound MMPs, which preferentially activate gelatinase A, have been described (Sato *et al.*, 1994; Takino *et al.*, 1995; Will and Hinzmann, 1995). Furthermore, gelatinase A was localized to the cellular surface by association with an integrin (Brooks *et al.*, 1996), which implies another mechanism for temporal and spatial regulation of remodelling of ECM.

Tissue Inhibitors of Metalloproteinases

The TIMPs and the liver-derived, serum-borne α_2 macroglobulin control activity of MMPs in the extracellular microenvironment. Within tissues, a family of TIMPs, including TIMP-1, TIMP-2, TIMP-3 and TIMP-4, regulate activity of MMPs (Greene *et al.*, 1996; Salamonsen 1996; Table 2). All TIMPs share twelve cysteine residues, which are considered to form six disulfide bonds; however,

Table 1. Matrix metalloproteinase (MMP) family of mammalian extracellular matrix proteinases

Family	Enzyme	MMP No.	Matrix substrates of functions
Collagenase	Interstitial collagenase	MMP-1	Collagens I, II, III, VII and X
	Neutrophil collagenase	MMP-8	Collagens I, II and III
	Collagenase 3	MMP-13	Collagens I, II and III
	Collagenase 4 (*Xenopus*)	MMP-18	Collagen I
Gelatinases	Gelatinase A	MMP-2	Gelatins, collagens IV, V, VII, X and XI, fibronectin, laminin
	Gelatinase B	MMP-9	Gelatins, collagens IV, V, XIV, fibronectin
Stromelysins	Stromelysin 1	MMP-3	Gelatins, fibronectin, laminin, collagens III, IV, IX and X, vitronectin: activates proMMP-1
	Stromelysin 2	MMP-10	Fibronectin, collagen IV
	Enamelysin	MMP-20	Amelogenin
Membrane-type MMPs	MT1-MMP	MMP-14	Collagens I, II, III, fibronectin, laminin, vitronectin: activates proMMP-2 and proMMP-13
	MT2-MMP	MMP-15	Gelatin, fibronectin, laminin: activates proMMP-2
	MT3-MMP	MMP-16	Activates proMMP-2
	MT4-MMP	MMP-17	Not known
Others	Matrilysin	MMP-7	Fibronectin, laminin, gelatins, collagen IV
	Stromelysin 3	MMP-11	Weak activity on fibronectin, laminin, collagen IV, gelatins
	Metalloelastase	MMP-12	Elastin
	(Unnamed)	MMP-19	Not known

TIMPs differ in relative molecular size, degree of glycosylation and *in vitro* or *in vivo* expression (Greene *et al.*, 1996; Salamonsen 1996; Table 2). Although they are separate gene products, each TIMP can inhibit most members of the MMP family by noncovalently binding with a 1:1 stoichiometry and high affinity ($K_i < 10^{-9}$ mol l^{-1}; Willenbrock and Murphy, 1994).

Remodelling of the ECM depends upon the ratio of active MMPs to TIMPs. Degradation of the ECM may occur when the ratio favours MMP activity, whereas deposition of ECM may occur when the ratio favours TIMPs. There is a considerable body of literature on the activation of latent MMPs; however, information regarding post-secretory mechanisms for regulating TIMPs is lacking. In particular, few mechanisms have been elucidated for liberating active MMPs from complexes with TIMPs or for the destruction of TIMPs. Potential physiological mechanisms for regulating TIMP-1 include: (1) degradation of TIMP-1 by proteinases (Itoh and Nagase, 1995); (2) degradation of TIMP-1 by peroxynitrite (a product of superoxide radicals and hydrogen peroxide; Frears *et al.*, 1996); and (3) liberation of active MMPs from complexes of TIMP-1 by endothelial stimulating angiogenesis factor (ESAF; McLaughlin *et al.*, 1991). The potential relevance of the preceding mechanisms for inactivating TIMP-1 to luteal function will be described in a subsequent section.

TIMPs are multifunctional molecules that stimulate proliferation of various types of cell (TIMP-1 and -2; Edwards *et al.*, 1996), and promote steroidogenesis (TIMP-1; Boujrad *et al.*, 1995) in addition

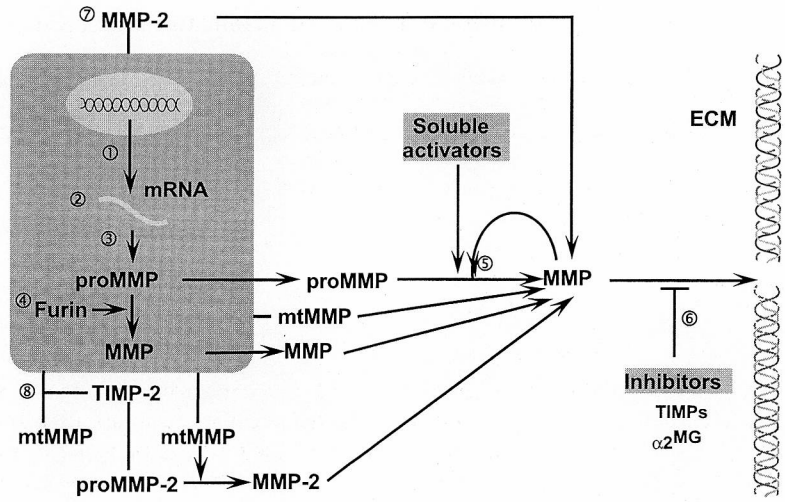

Fig. 1. A schematic representation of the pathways for matrix metalloproteinase (MMP) production, activation and inhibition. Production of MMPs can be regulated at the level of gene activation/transcription (1), mRNA stability (2) and mRNA translation of latent proenzyme (proMMP; 3). Activation of proMMP can occur via intracellular processing by furin (4) or extracellular processing by soluble activators (5; mast cell proteases and serine proteases (plasmin and kallikriens)). Proteolytically active MMPs (MMP) and membrane bound MMP (mtMMP) can also activate proMMP (5). MMPs degrade the proteinaceous components of the extracellular matrix (ECM) and the degree of ECM degradation is primarily regulated by locally produced inhibitors (6) such as tissue inhibitor of metalloproteinases (TIMPs-1, -2, -3 and -4) and possibly the serum derived inhibitor (6) α_2 macroglobulin (α_2MG). ECM degradation can be localized to the cell surface by the association of MMP-2 with an integrin (7). Another mechanism (8) for concentrating MMP activity near the cellular surface involves formation of a tri-molecular (mt-MMP–TIMP-2–MMP-2) complex that activates MMP-2 (progelatinase A). (Redrawn with permission from McIntush and Smith, 1998.)

to inhibiting MMPs. Boujrad and coworkers (1995) identified TIMP-1 as the active component of a TIMP-1–procathepsin L complex that stimulated production of progesterone by various types of steroidogenic cell (including luteinized granulosal cells). Cathepsin L is a lysosomal cysteine proteinase that is involved in intracellular protein metabolism and is found in numerous tissues. However, cathepsin L can be secreted and can cleave a variety of proteins including proteins of the ECM.

Follicular Growth and Atresia

Follicular expansion

Growth of bovine follicles from the primordial to the preovulatory stage is characterized by an approximately 360 000-fold increase in surface area as the follicular basement membrane expands within the limits of the ovarian stroma. Modification of the adjacent ovarian stroma is necessary for a growing follicle to reach a place on the surface of the ovary where the oocyte can be released at ovulation; therefore, remodelling of ECM occurs at all stages of follicular development. Remodelling of ECM also occurs during recruitment of a vascular supply within the thecal layer. The MMPs

Table 2. Tissue inhibitor of metalloproteinase (TIMP) family

Inhibitor	Relative molecular weight	Glycosylation	Extracellular location
TIMP-1	28 000	Glycosylated	Soluble in ECM and body fluids
TIMP-2	21 000	Not glycosylated	Soluble in ECM and body fluids
TIMP-3	24 000	Not glycosylated	Bound to ECM
TIMP-4	22 000	nd	nd

nd = not determined.
ECM: extracellular matrix.
Reproduced with permission from McIntush and Smith (1998).

and TIMPs probably regulate remodelling of ECM accompanying follicular expansion and angiogenesis. In rats, collagenase 3 was localized to thecal cells and interstitial tissue, but not to granulosal cells of antral follicles. Maximal expression of the enzyme occurred during pro-oestrus (Balbin *et al.*, 1996). Hence, collagenase 3 may be involved in remodelling of the ovarian stroma during follicular development to the preovulatory stage. Interstitial collagenase is also present within thecal and granulosal cells of rabbit follicles at all stages of development (Tadakuma *et al.*, 1993).

Bioavailability of growth factors

Follicular development requires significant proliferation of thecal and granulosal cells. The role of growth factors in control of ovarian cell proliferation has been examined extensively. Often growth factors are secreted constitutively and sequestered in the extracellular matrix in an inactive form or in association with specific binding proteins where they can subsequently be liberated by proteolysis of the ECM. Therefore, MMPs probably play a key role in regulating the availability of growth factors and their activities within developing follicles. For example, MMPs can liberate fibroblast growth factor molecules bound to heparin sulfate proteoglycans in the extracellular matrix and hence increase growth factor availability to nearby cells. Other growth factors and growth factor-binding proteins that are capable of binding to components of the ECM include transforming growth factor β, platelet derived growth factor, hepatocyte growth factor, heparin binding-epidermal growth factor, and insulin-like growth factor binding protein 3 (IGFBP-3).

Proteolytic degradation of the insulin-like growth factor binding proteins is rapidly emerging as a physiologically important mechanism for regulating availability of insulin-like growth factor (IGF) within follicles. In general, follicular growth is characterized by a decrease in concentrations of specific IGF-binding proteins in follicular fluid, presumably resulting in increased bioavailability of IGF to healthy follicles. The reduced concentration of binding protein is believed to result, at least in part, from proteolytic degradation. MMPs have been shown to degrade IGFBP-3 in other systems (Fowlkes *et al.*, 1994). However, the biochemical properties of proteinases responsible for degradation of IGF-binding proteins in follicular fluid are inconsistent with those of MMPs (Besnard *et al.*, 1996).

Atresia

Increased expression of specific activities of MMPs may facilitate the process of atresia. MMP activity is linked to involution in numerous tissues, such as the postpartum uterus and the mammary gland. Gelatinolytic activity corresponding to gelatinase A and B is increased within follicular fluid of atretic ovine follicles collected after hypophysectomy (Huet *et al.*, 1997). The increased activity of these enzymes is probably required for the breakdown of the basement membrane characteristic of later stages of atresia. The precise contribution of MMPs and their inhibitors to follicular growth and atresia remains to be determined.

Ovulation

The preovulatory gonadotrophin surge initiates a complex cascade of events resulting in follicular rupture, release of an oocyte, and luteinization of the remaining follicular cells. A growing body of evidence indicates that proteolytic degradation of the ECM at the follicular apex is the rate-limiting step in the ovulatory process. The apex region of the preovulatory follicular wall is composed of granulosal cells, basement membrane, theca interna, theca externa, tunica albuginea, and the surface epithelium with its underlying basement membrane. Distinct morphological changes that are indicative of degradation of ECM occur after exposure to the surge of gonadotrophins (Espey and Lipner, 1994).

Although a role for proteolytic enzymes in the process of ovulation was proposed in 1916, the identification and subsequent characterization of enzymes required for follicular rupture is incomplete. The serine proteinases, tissue plasminogen, activator (tPA) and urokinase plasminogen activator (uPA), convert the inactive zymogen, plasminogen into its active form plasmin, which has broad substrate specificity. Recent studies in mice with targeted mutations in the tPA and uPA genes have shed important insight on the regulation of the ovulatory process and on the potential requirement of MMPs.

Mice with a homozygous null mutation in either the tPA or the uPA gene displayed normal rates of ovulation. However, mice carrying homozygous null mutations in both genes exhibited a 26% reduction in rates of ovulation (Leonardsson *et al.*, 1995). These authors suggested that the plasminogen activator–plasmin enzyme family contributes to proteolysis of the ECM accompanying ovulation, although ovulation still occurs, albeit at a reduced rate, in mice lacking both plasminogen activators. Thus, the activity of this family of enzymes is not solely responsible for the marked proteolysis that occurs before ovulation, as they cannot cleave collagenous components of the ECM. The most important role of the plasminogen activator–plasmin system during the periovulatory period may be to increase the rate of activation of the latent proenzyme form of interstitial collagenase.

A growing body of evidence, primarily in rodents, indicates that MMPs help mediate follicular rupture. Administration of synthetic inhibitors of MMPs disrupted ovulation in rats (Brannstrom *et al.*, 1988). However, the requirement of individual MMPs for the ovulatory process has not been determined. The ovarian capsule, theca externa, and tunica albuginea are rich in type I and III collagens. Mice with a targeted mutation in the type I collagen gene, resulting in a molecule that is resistant to collagenase, display markedly reduced fertility. The reduction in fertility is potentially due to an impairment of the ovulatory process (Liu *et al.*, 1995). Collagenolytic activity, as measured by liberation of ^{3}H hydroxyproline or cleavage of tritiated type I collagen, is increased within rat ovaries (Reich *et al.*, 1985; Curry *et al.*, 1986) and sheep follicles (Murdoch and McCormick, 1992) after the preovulatory LH surge. In sheep, activity is substantially higher within the follicular apex than in the base; however, the mechanisms that accounted for spatial differences in collagenolytic activity within preovulatory follicles are not understood.

Collagenases

Collagenases, including interstitial collagenase and collagenase-3, may be responsible for the initial degradation and unwinding of the triple helical fibres of collagen within the follicular apex before ovulation. Expression of interstitial collagenase during the periovulatory period has been examined in rodents and rabbits. Messenger RNA expression of interstitial collagenase was increased twenty-five fold within rat follicles after exposure to an ovulatory dose of hCG and the mRNA was present within both isolated granulosal cells and the remaining ovarian tissue (Reich *et al.*, 1991). Immunohistochemical analysis of rabbit follicles indicated that this enzyme was present within the thecal and granulosal layers and increased within thecal and granulosal cells after follicular rupture (Tadakuma *et al.*, 1993). Although collagenase 3 is highly expressed by thecal cells/stroma of rat antral follicles (Balbin *et al.*, 1996), regulation of its expression by the preovulatory surge of gonadotrophins has not been demonstrated. The role of collagenase 3 in the ovulatory process is undefined.

Gelatinases

The gelatinases (gelatinase A and gelatinase B) are most noted for their ability to cleave the denatured helix of collagen (gelatin) and type IV collagen, a major component of basement membranes. It has been postulated that during the ovulatory process these enzymes play a key role in facilitating breakdown of the basement membrane and further hydrolysis of the denatured fibrils of collagen after their initial cleavage by collagenase(s).

Gelatinase A: Messenger RNA expression (Reich *et al.*, 1991) and enzymatic activity (Curry *et al.*, 1992) of gelatinase A were increased within rat ovaries after exposure to the LH surge and the mRNA was present within the residual ovarian tissue but not within isolated granulosal cells (Reich *et al.*, 1991). Immunization of ewes against the N-terminal peptide of the 43 kDa subunit of α-N inhibin resulted in reduced concentration of gelatinase A in follicular fluid and an impairment of the ovulatory process (Russell *et al.*, 1995). Immunized animals displayed reduced oviductal recovery of oocytes and abnormal corpora lutea in which the normal tissue remodelling process was partially disrupted. Mice with a targeted mutation in the gelatinase A gene have been generated. Surprisingly, a preliminary report indicated that disruption of the gelatinase A gene did not reduce fertility (Itoh *et al.*, 1997). Elucidation of the role of gelatinase A in the ovulatory process will require further investigation.

Gelatinase B: The role of gelatinase B in the ovulatory process is unclear. Like gelatinase A, gelatinase B can cleave type IV collagen and may play a role in basement membrane breakdown. Evidence in rats indicates that interleukin-1β, an LH regulated-putative paracrine mediator of the ovulatory process, can regulate expression of gelatinase B by preovulatory follicles. Expression of gelatinase B is increased by treatment of whole ovarian dispersates or enriched theca, but not granulosa cells with interleukin 1β (Hurwitz *et al.*, 1993). However, activity of gelatinase B was low or undetectable by gelatin zymographic analysis of rat ovarian extracts (Curry *et al.*, 1992) and ovine follicular fluid (Russell *et al.*, 1995) collected during the periovulatory period. Mice null for the gelatinase B gene were fertile (Vu *et al.*, 1998).

Membrane-bound MMPs

Members of the newly discovered family of membrane-bound metalloproteinases, such as the membrane type 1 metalloproteinase, may also be involved in ovulation. Membrane type-1 metalloproteinase was expressed within bovine follicles and corpora lutea (G. W. Smith *et al.*, unpublished). This enzyme can activate progelatinase A through formation of a tri-molecular complex with TIMP-2 (Strongin *et al.*, 1995). During activation, TIMP-2 first binds to the membrane type 1 metalloproteinase. Next, TIMP-2 binds progelatinase A through its carboxy-terminal hemopexin domain. Binding to TIMP-2 localizes progelatinase A at the cellular surface in immediate proximity to the membrane type 1 metalloproteinase where the propiece of progelatinase A can be cleaved. In addition to activation of progelatinase A, the membrane type-1 metalloproteinase can hydrolyse type I and III collagen, fibronectin, laminin and proteoglycans (Nagase, 1997). Therefore, it may participate in degradation of ECM during ovulation. Espey and Lipner (1994) postulated that proteinases localized on the surface of cells promote dissociation of cells and breakdown of the surrounding fibres of collagen within the theca externa and tunica albuginea during ovulation.

Tissue inhibitors of metalloproteinases

Regulation of MMPs is important for tissue homeostasis. As discussed previously, the activity of MMPs in the extracellular milieu is regulated through production of specific inhibitors. The MMPs and their inhibitors are often secreted in parallel; i.e. agents that stimulate expression of MMPs also increase expression of the inhibitors (Murphy *et al.*, 1985). Therefore, net proteolysis during ovulation may be regulated by the ratio of enzyme to inhibitor.

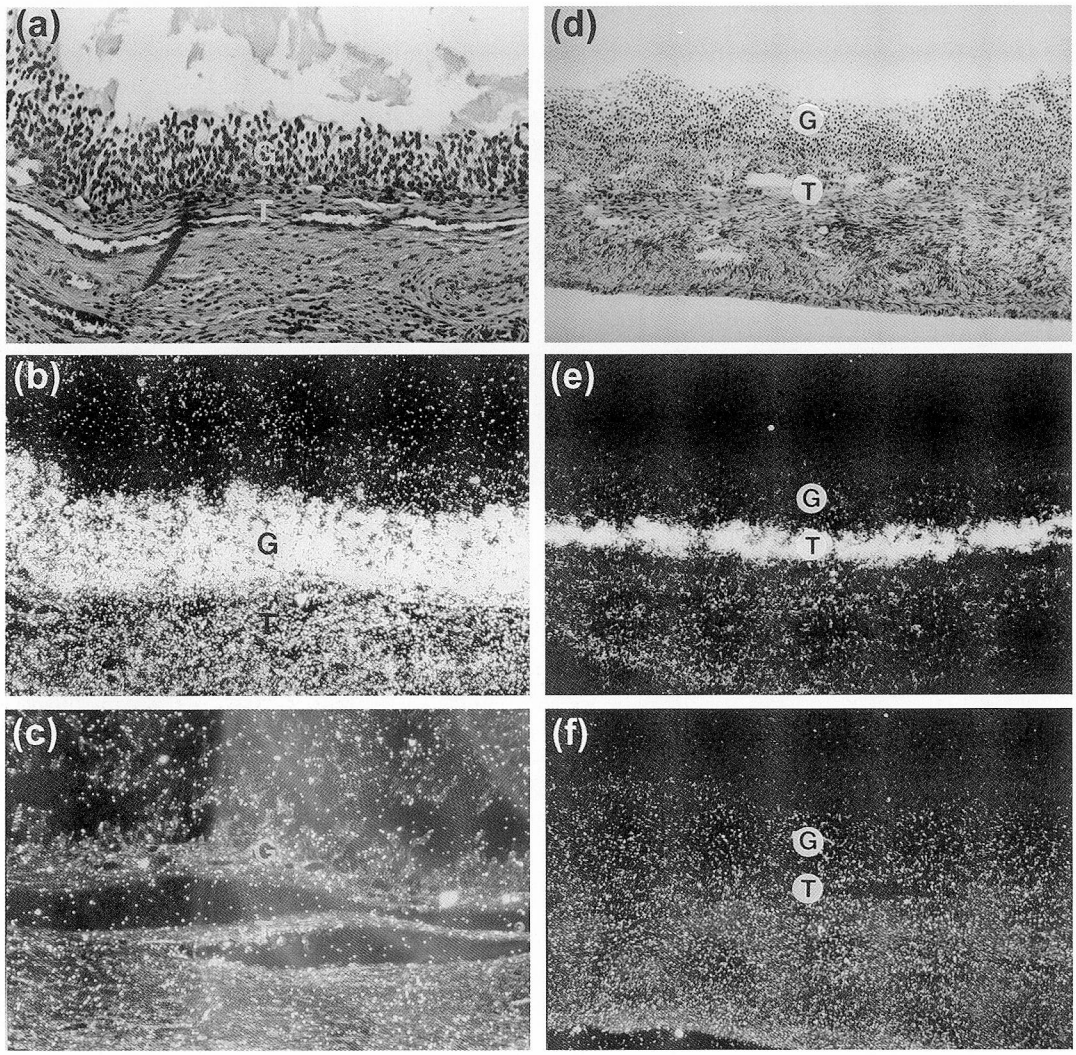

Fig. 2. Differential localization of tissue inhibitor of metalloproteinase 1 (TIMP-1) versus TIMP-2 mRNA within ovine follicles collected 12 h after the LH surge (post-surge). (a,d) Bright field images of sections of post-surge ovine follicles hybridized with ^{35}S antisense TIMP-1 (a) or TIMP-2 (d) cRNA probes. (b,e) Dark field images of sections of post-surge ovine follicles hybridized with ^{35}S antisense TIMP-1 (b) or TIMP-2 (e) cRNA probes. (b) TIMP-1 mRNA was localized to the granulosal layer of ovine post-surge follicles, while (e) TIMP-2 mRNA was localized specifically to the thecal layer. (c,f) Specific hybridization was not detected when sections were hybridized with sense (negative control) TIMP-1 (c) or TIMP-2 (f) cRNA probes. G (granulosal cell layer) T (thecal cell layer). (Reproduced with permission from Smith *et al.*, 1994; 1995; © The Endocrine Society.)

We determined the effect of the preovulatory LH surge on expression of two members of the TIMP family (TIMP-1 and -2) within ovine follicles (Fig. 2). Expression of TIMP-1 mRNA (Smith *et al.*, 1994a) and follicular fluid concentration of TIMP-1 (McIntush *et al.*, 1997) were increased approximately 10-fold within ovine follicles after a preovulatory LH surge. The granulosal cells were the primary source of the increased expression of TIMP-1 (Smith *et al.*, 1994a, Fig. 2, and McIntush *et al.*, 1996). In contrast, expression of TIMP-2 was constitutive within ovine follicles collected at similar time points after the preovulatory LH surge, and TIMP-2 was localized to the thecal layer (Fig. 2;

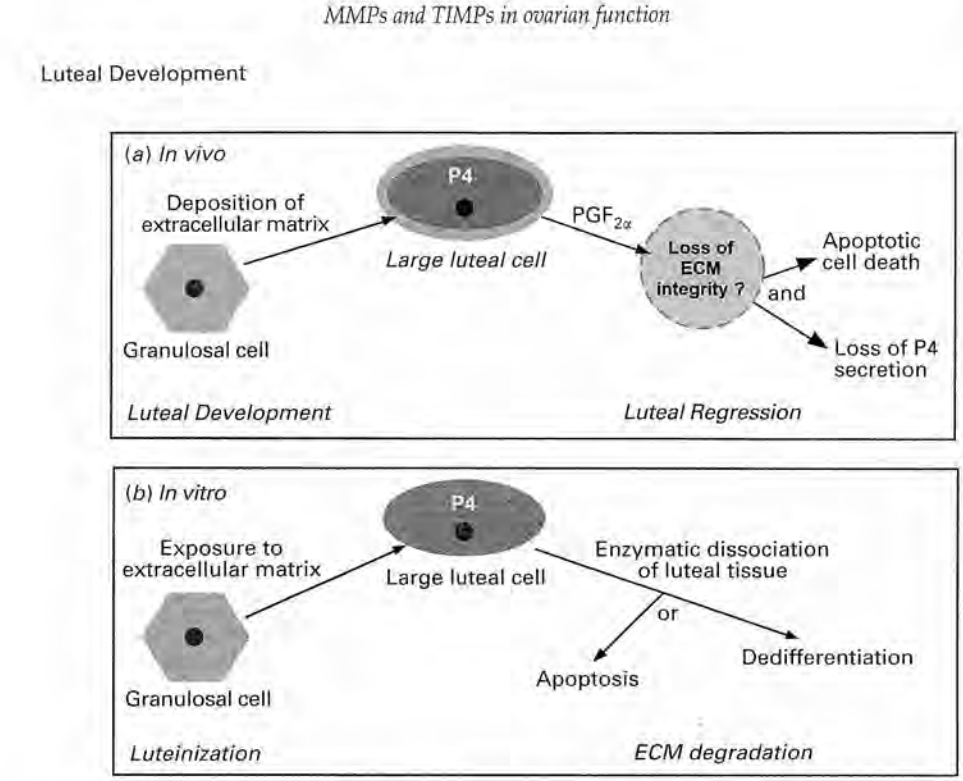

Fig. 3. Relationship between extracellular matrix (ECM) and luteal cell function *in vivo* and *in vitro*. (a) *In vivo*. Relationship of luteal development and regression to changes in ECM. Differentiation of follicular cells into large luteal cells is associated with net accumulation of ECM during luteal development. Likewise, reduction in progesterone (P4) secretion and apoptotic cell death is associated with loss of ECM integrity. (b) *In vitro*. Granulosal cell luteinization is enhanced by exposure to basal lamina components (laminin and fibronectin). In addition, enzymatic dissociation of luteal tissue leads to apoptosis or dedifferentiation of luteal cells. (Reproduced with permission from McIntush and Smith, 1998.)

Smith *et al.*, 1995). The distinct localization and temporal expression of TIMP-1 versus TIMP-2 within ovine follicles indicates complementary yet distinct roles for each inhibitor during the periovulatory period. TIMP-1 probably regulates the extent of proteolysis within the granulosal layer during the ovulatory process. In contrast, TIMP-2 within the thecal layer may enhance proteolysis through localization of progelatinase A at the surface of cells with the membrane type 1-metalloproteinase.

Corpus luteum

The transition of a preovulatory follicle into a corpus luteum is a complex process involving mechanisms similar to wound healing and tumour formation. Corpus luteum development and luteolysis are accompanied by extensive remodelling of ECM, which can modulate specific cellular processes including mitosis, migration, differentiation, apoptosis and gene expression. Presumably, MMPs and their inhibitors play an important role in remodelling of luteal tissue. Indeed, expression of MMP genes and activity of MMPs in corpora lutea have been demonstrated. Likewise, luteal tissue expresses inhibitors of these proteinases ((TIMP-1, TIMP-2, TIMP-3) and plasminogen activator inhibitor-1 (PAI-1)), presumably to control the extent of breakdown of ECM. Although many reproductive and non-reproductive tissues produce TIMP-1, the production of TIMP-1 mRNA (Hampton *et al.*, 1995) and protein (McIntush and Smith, 1997) by ovine corpora lutea was 30 to 3 000

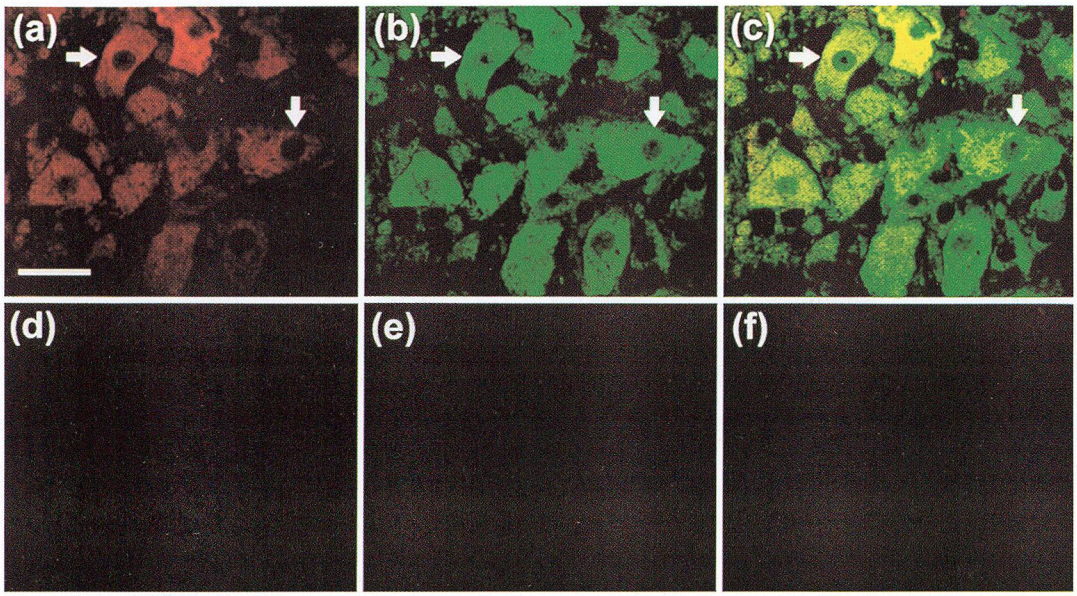

Fig. 4. (a–c) Confocal micrographs illustrating portions of ovine corpora lutea collected on day 10 after oestrus (day 0 = oestrus). Tissue inhibitor of metalloproteinase 1 TIMP-1 (a) and oxytocin (b) were colocalized (c) within day 10 large luteal cells (arrows indicate examples of cells in which TIMP-1 and oxytocin were colocalized). Control tissues treated with second antibody alone (d–f) did not show immunofluorescence. Scale bar represents 25 μm. (Reproduced with permission from McIntush *et al.*, 1996.)

fold greater than that of other tissues. On the basis of reports of growth- (Edwards *et al.*, 1996) and steroidogenesis-promoting (Boujrad *et al.*, 1995) activities of TIMP-1, it may be that this protein modulates several processes within corpora lutea.

There are similarities between luteal development–luteolysis *in vivo* and luteal cell differentiation/dedifferentiation or death of luteal cells *in vitro*. A model for the role of the ECM during differentiation and regression of luteal cells *in vivo* is shown (Fig. 3a). Evidence derived from studies *in vitro* indicates that ECM components enhance luteinization of follicular cells and that loss of ECM results in death or loss of differentiated phenotype of luteal cells (Fig. 3b). Aspects of this model were derived from studies of ovine and rat corpora lutea. Specific details may not apply to all species, but similar principles may apply. Experimental evidence to support this model are provided in subsequent sections.

Luteal development

Luteinization. The process of luteinization involves morphological and biochemical changes that follicular cells undergo during transformation into the steroidogenic cells of a corpus luteum. In sheep and cattle, corpora lutea contain two types of steroidogenic cell (large luteal cells (LLC), presumably derived from follicular granulosal cells and small luteal cells (SLC), presumably derived from follicular thecal cells (reviewed by Smith *et al.*, 1994b)). These cells differ biochemically, physiologically and morphologically (Farin *et al.*, 1986). Ovine large luteal cells appear to be the primary type of cell responsible for controlling the extent of remodelling of luteal ECM as they produced TIMP-1 (Fig. 4; Smith *et al.*, 1994a; McIntush *et al.*, 1996), TIMP-2 (Smith *et al.*, 1995; McIntush *et al.*, 1996), and plasminogen activator inhibitor 1 (G. W. Smith and M. F. Smith, unpublished).

Components of the ECM have an important effect on the differentiation of a variety of types of

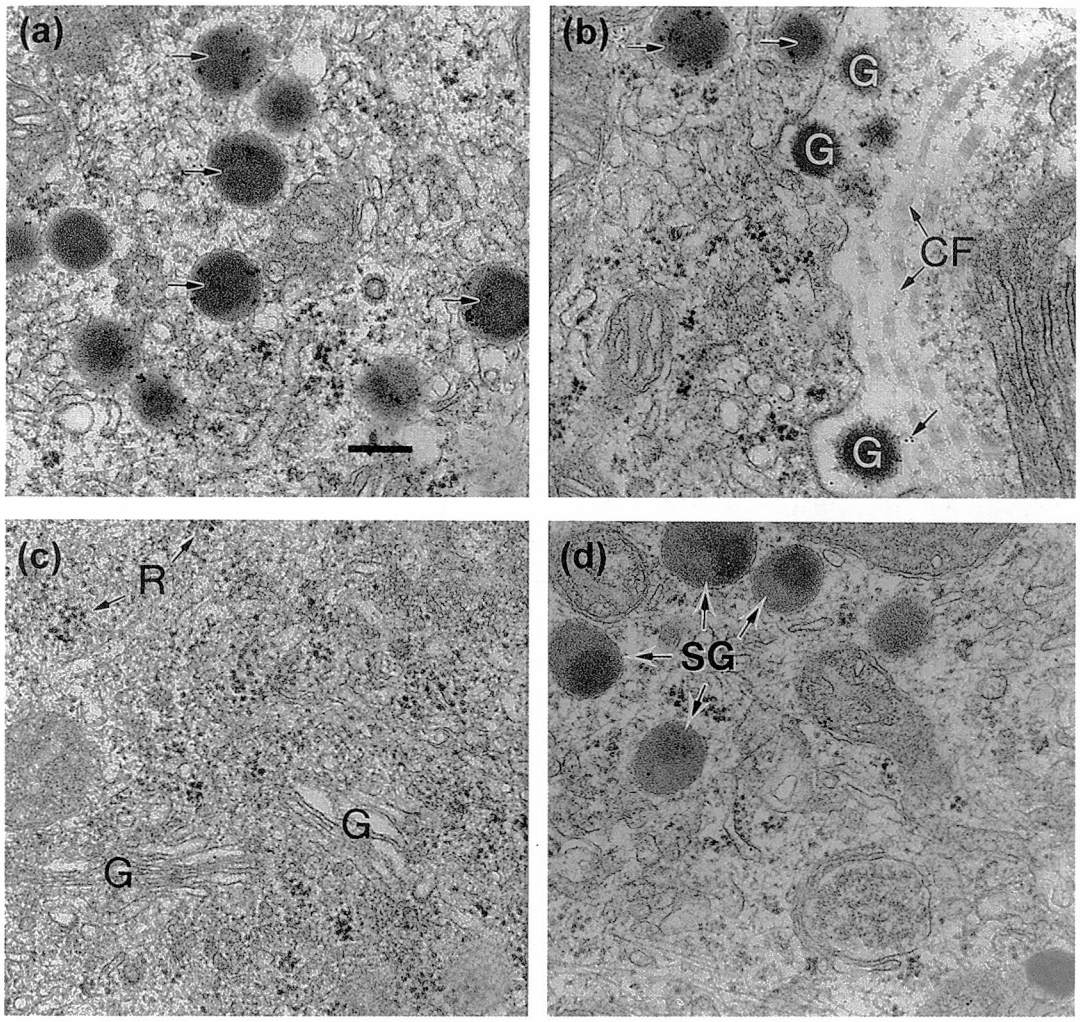

Fig. 5. (a–d) Electron micrographs illustrating portions of luteal cells from ovine corpora lutea collected on day 10 after oestrus (day 0 = oestrus). Scale bar represents 200 nm. (a) Colloidal gold particles indicative of positive immunostaining for tissue inhibitor of metalloproteinase 1 (TIMP-1) were restricted to secretory granules (arrows) dispersed throughout the cytoplasm of large luteal cells. (b) The contents of secretory granules in the cytoplasm of large luteal cells (arrows) consistently stained positive for TIMP-1. After release of secretory granules (G) into the extracellular spaces, particles of colloidal gold were frequently observed in association with granule contents undergoing dissolution. Collagen fibrils (CF) were present in the extracellular matrix. (c) Portion of a small luteal cell located in close proximity to the large luteal cell shown in (a). Aggregates of ribosomes (R) and Golgi cisternae (G) are evident. Note the absence of colloidal gold particles. (d) Negative control. A group of secretory granules (SG) in the cytoplasm of a large luteal cell in a section that was incubated in preimmune serum rather than the primary antiserum (M17W4). In negative control sections, background (nonspecific) staining associated with secretory granules was minimal (compared with positive staining secretory granules in (a)). (Reproduced with permission from McIntush *et al.*, 1996.)

cell, including granulosal cells. Aten and coworkers (1995) demonstrated that fibronectin or laminin (two components of the basal lamina) promote the differentiation of rat granulosal cells into luteal cells, while an antibody to the integrin β_1 subunit inhibits differentiation of granulosal cells. Interaction of luteal cells with the ECM probably changes during the luteal phase as components of

the matrix are degraded and replaced coincident with cell proliferation and migration. Changing contacts between cells and the ECM may even be necessary for differentiation of follicular cells into luteal cells, as is the case for differentiation of other types of cell. Differentiation of granulosal cells into large luteal cells is likely to require sequential exposure to different environments of ECM.

Approximately 80% of the progesterone secreted *in vivo* by ovine corpora lutea is believed to come from LLC (Niswender *et al.*, 1985). The factor(s) responsible for regulating secretion of progesterone by LLC are unknown. Recently, a TIMP-1–procathepsin L complex was shown to stimulate synthesis of progesterone by several steroidogenic cell types including luteinized granulosal cells (Boujrad *et al.*, 1995). TIMP-1 may be an important stimulator of synthesis of progesterone in luteinizing granulosal cells and LLC.

Cellular proliferation/migration. Luteal development has been characterized by cellular proliferation equal to that of rapidly growing tumours (Jablonka-Shariff *et al.*, 1993). Such a rapid rate of proliferation is not surprising since corpora lutea increase in size 10- to 20-fold over a few days (Jablonka-Shariff *et al.*, 1993) and develop into one of the most vascular tissues known. Many cells migrate at this time, as the distinctly compartmentalized tissue of the follicle makes the transition into a corpus luteum consisting of a heterogeneous population of cells. Many of the migrating and proliferating cells are endothelial cells (Jablonka-Shariff *et al.*, 1993). TIMP-1 and -2 promote growth of a variety of types of cell, including fibroblasts and endothelial cells. The mechanism by which TIMPs stimulates cellular proliferation is unclear but may involve membrane receptors (Edwards *et al.*, 1996). Alternatively, MMPs and TIMPs may influence proliferation of cells by regulating the bioavailability of growth factors (see Follicular Growth and Atresia section).

Angiogenesis. During formation of the corpus luteum, the follicular wall containing an avascular granulosal layer undergoes a transition to become one of the most vascular tissues in the body. Two important stages of angiogenesis include breakdown of the basement membrane and migration of endothelial cells. It is likely that MMPs play a significant role in both stages of angiogenesis. Interestingly, endothelial cells within melanomas were found to contain active gelatinase A associated with integrin $\alpha_v\beta_3$ (Brooks *et al.*, 1996). A naturally occurring, noncatalytic fragment of gelatinase A competes with gelatinase A for binding to the $\alpha_v\beta_3$ integrin and may function to limit the extent of migration and invasion of endothelial cells during angiogenesis (Brooks *et al.*, 1998). The activity of gelatinase A localized at the cell surface, as well as that of the secreted and membrane-bound MMPs may facilitate migration of endothelial cells within the developing corpus luteum.

Tissue inhibitor of metalloproteinases 1 is expressed abundantly during ovine luteal development (Smith *et al.*, 1994a) and TIMP-1 has also been shown to inhibit angiogenesis. These results suggest an apparent paradox since TIMP-1 inhibited neovascularization and migration of cells (Khokha, 1994). A possible explanation is that expression of TIMP-1 mRNA is not indicative of the concentration of TIMP-1 in the extracellular milieu. Although TIMP-1 mRNA per unit of DNA decreased (Freudenstein *et al.*, 1990; Smith *et al.*, 1996) from early to mid-luteal phase, TIMP-1 protein per milligram of luteal tissue increased approximately threefold from early to the mid-luteal phase (McIntush and Smith, 1997). The secretory granules to which TIMP-1 was localized (McIntush *et al.*, 1996) fit the morphological criteria for dense core secretory granules (Fig. 5), which are often associated with a regulated pathway of protein secretion. Thus, increasing TIMP-1 protein, decreasing TIMP-1 mRNA, and localization of TIMP-1 to secretory granules are consistent with the hypothesis that, although some TIMP-1 may be secreted, TIMP-1 accumulates within secretory granules of LLC. This possibility helps resolve the paradox of high TIMP-1 expression in the midst of extensive neovascularization.

Luteolysis

Regression of the corpus luteum is marked by loss of adhesion of cells to matrix (Nett *et al.*, 1976), loss of capacity to synthesize progesterone (Niswender and Nett, 1994), and apoptosis (Juengel *et al.*, 1993). Furthermore, changes in luteal gene expression in response to $PGF_{2\alpha}$ (the natural luteolysin in numerous species) are similar to changes in gene expression induced by

enzymatic dissociation of luteal tissue (G. D. Niswender, personal communication). Ovine LLC are the cells that possess receptors for and respond to $PGF_{2\alpha}$. If degradation of matrix is an important component of the physiological response to $PGF_{2\alpha}$, vesting LLC with both maintenance of the ECM of luteal tissue and responsiveness to the luteolysin ($PGF_{2\alpha}$) appears logical. In such a scenario, response to $PGF_{2\alpha}$ may include removal of substances involved in preservation of the ECM. Indeed, TIMP-1 mRNA (Duncan *et al.*, 1996) and protein (McIntush and Smith, 1997) were lower within hours after administration of $PGF_{2\alpha}$. Furthermore, dissolution of microtubules within LLC was noted soon (2 h) after administration of $PGF_{2\alpha}$ (Murdoch, 1996). These data may indicate that contacts between LLC and ECM were disrupted during luteal regression. Thus, maintenance of corpora lutea appears dependent upon establishment and maintenance of proper contact with ECM. Luteal tissue may produce TIMP-1, TIMP-2, TIMP-3 and PAI-1 to preserve the integrity of components of the ECM upon which luteal cells appear dependent.

If inhibitors of ECM proteinases are important for luteal maintenance, destruction of inhibitors may be important for luteal regression. Inactivation of TIMP-1 could be accomplished by phenomena that have been implicated in luteolysis. Leukocytes, which appear to be involved in luteolysis (Murdoch, 1987), could inactivate TIMP-1 by proteolytic degradation (Itoh and Nagase, 1995). In addition, highly reactive peroxynitrite ($ONOO^-$; generated by the reaction of superoxide (O_2^-) with nitric oxide (NO)), which inactivated TIMP-1 (Frears *et al.*, 1996) may be generated by luteal tissue during the generation of oxygen radicals associated with luteolysis. Combining data on development, maintenance and regression of mammary and luteal tissues provides the rationale for the hypothesis that remodelling of the ECM plays a fundamental role in luteal function. However, confirmation of this hypothesis awaits further investigation.

Conclusions

The extracellular matrix is integrally involved in the function of tissues. In adult mammals, few tissues undergo extensive remodelling of the ECM. In fact, remodelling of the ECM in adult tissues is often linked to pathologies such as osteoarthritis and tumour cell metastasis. However, the ovary is one tissue that undergoes remodelling as a continuous, physiological phenomenon. Information on the ability of the ECM to direct the proliferation, differentiation and function of cells implies that breakdown and regeneration of the ECM plays more than a permissive role in normal ovarian function. The ECM probably plays an active part in directing the processes of follicular development and atresia, ovulation, and development, maintenance and regression of corpora lutea. Matrix metalloproteinases appear to be key enzymes that determine which molecules of the ECM are degraded. The extent of degradation of ECM molecules by MMPs is controlled to a large extent by availability of TIMPs. To date, most studies have focused upon correlating expression of MMPs and TIMPs with various stages of the reproductive cycle. From these studies, many key molecules have been identified. However, a better understanding of the role of individual MMPs and TIMPs in ovarian function awaits additional studies that characterize the temporal and spatial expression of these proteins more thoroughly. Subsequently, creative manipulative studies must be undertaken to determine the complex interactions of these molecules if a clear understanding of their function is to be obtained.

This manuscript is a contribution of the Missouri Agricultural Experiment Station. Journal Series Number 12,770. This work was partially funded by USDA Grant, USDA CSRS 92-37203-7950. The Michigan Agricultural Experiment Station is gratefully acknowledged for their support (GWS).

References

Aten RF, Kolodecik TR and Berhman HR (1995) A cell adhesion receptor antiserum abolishes, whereas laminin and fibronectin promote, luteinization of cultured rat granulosa cells *Endocrinology* **136** 1753–1758

Balbin M, Fueyo A, Lopez JM, Diez-Itza I, Velasco G and Lopez-Otin C (1996) Expression of collagenase-3 in the rat ovary during the ovulatory process *Journal of Endocrinology* **149** 405–415

Besnard N, Pisselet C, Zapf J, Hornebeck W, Monniaux D and Monget P (1996) Proteolytic activity is involved in changes in intrafollicular insulin-like growth factor-binding

protein levels during growth and atresia of ovine ovarian follicles *Endocrinology* **137** 1599–1607

Boujrad N, Ogwuegbu SO, Garnier M, Lee C, Martin BM and Papadopoulos V (1995) Identification of a stimulator of steroid hormone synthesis isolated from testis *Science* **268** 1609–1612

Brannstrom M, Woessner JF, Jr, Koos RD, Sear CH and LeMaire WJ (1988) Inhibitors of mammalian tissue collagenase and metalloproteinases suppress ovulation in the perfused rat ovary *Endocrinology* **122** 1715–1721

Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP and Cheresh DA (1996) Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin $\alpha_v \beta_3$ *Cell* **85** 683–693

Brooks PC, Silletti S, von Schalscha TL, Friedlander M and Cheresh D (1998) Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity *Cell* **92** 391–400

Curry TE, Jr, Clark MR, Dean DD, Woessner JF, Jr and LeMaire WJ (1986) The preovulatory increase in ovarian collagenase activity in the rat is independent of prostaglandin production *Endocrinology* **118** 1823–1828

Curry TE, Jr, Mann JS, Huang MH and Keeble SC (1992) Gelatinase and proteoglycanase activity during the periovulatory period in the rat *Biology of Reproduction* **46** 256–264

Duncan WC, Illingworth PJ and Fraser HM (1996) Expression of tissue inhibitor of metalloproteinases-1 in the primate ovary during induced luteal regression *Journal of Endocrinology* **151** 203–213

Edwards DR, Beaudry PP, Laing TD, Kowal V, Leco KJ, Leco PA and Lim SA (1996) The roles of tissue inhibitors of metalloproteinases in tissue remodelling and cell growth. *International Journal of Obesity* **20** 9–15

Espey LL (1992) Ovulation as an inflammatory process. In *Local Regulation of Ovarian Function* pp 183–200 Eds N Sjoberg, L Hamberger, P Janson, C Owman and H Coelingh-Bennink. Parthenon Publishing Group, Park Ridge

Espey LL and Lipner H (1994) Ovulation. In *The Physiology of Reproduction* pp 725–780 Eds E Knobil and JD Neill. Raven Press, New York

Farin CE, Moeller CL, Sawyer HR, Gamboni F and Niswender GD (1986) Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle *Biology of Reproduction* **35** 1299–1308

Fowlkes JL, Enghild JJ, Suzuki K and Nagase H (1994) Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast culture *Journal of Biological Chemistry* **269** 25742–25746

Frears ER, Zhang Z, Blake DR, O'Connell JP and Winyard PG (1996) Inactivation of tissue inhibitor of metalloproteinase-1 by peroxynitrite *FEBS Letters* **381** 21–24

Freudenstein J, Wagner S, Luck MR, Einspanier R and Scheit KH (1990) mRNA of bovine tissue inhibitor of metalloproteinases: sequence and expression in bovine ovarian tissue *Biochemical and Biophysical Research Communications* **171** 250–256

Greene J, Wang M, Liu YE, Raymond LA, Rosen C and Shi YE (1996) Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4 *Journal of Biological Chemistry* **271** 30375–30380

Hampton AL, Butt AR, Riley SC and Salamonsen LA (1995) Tissue inhibitor of metalloproteinases in endometrium of ovariectomized steroid-treated ewes and during the estrous cycle and early pregnancy *Biology of Reproduction* **53** 302–311

Huet C, Monget P, Pisselet C and Monniaux D (1997) Changes in extracellular matrix components and steroidogenic enzymes during growth and atresia of antral ovarian follicles in the sheep *Biology of Reproduction* **56** 1025–1034

Hurwitz A, Dushnik M, Solomon H, Ben-Chetrit A, Finci-Yeheskel Z, Milwidsky A, Mayer M, Adashi EY and Yagel S (1993) Cytokine-mediated regulation of rat ovarian function: interleukin-1 stimulates the accumulation of a 92-kilodalton gelatinase *Endocrinology* **132** 2709–2714

Itoh Y and Nagase H (1995) Preferential inactivation of tissue inhibitor of metalloproteinases-1 that is bound to the precursor of matrix metalloproteinase 9 (progelatinase B) by human neutrophil elastase *Journal of Biological Chemistry* **270** 16518–16521

Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T and Itohara S (1997) Unaltered secretion of beta-amyloid percursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice *Journal of Biological Chemistry* **272** 22389–22392

Jablonka-Shariff A, Grazul-Bilska AT, Redmer DA and Reynolds LP (1993) Growth and cellular proliferation of ovine corpora lutea throughout the estrous cycle *Endocrinology* **133** 1871–1879

Juengel JL, Garverick HA, Johnson AL, Youngquist RS and Smith MF (1993) Apoptosis during luteal regression in cattle *Endocrinology* **132** 249–254

Khokha R (1994) Suppression of tumorigenic and metastatic abilities of murine B16-F10 melanoma cells *in vivo* by overexpression of the tissue inhibitor of metalloproteinases-1 *Journal of the National Cancer Institute* **86** 299–304

Kleiner DE and Stetler-Stevenson WG (1993) Structural biochemistry and activation of the matrix metalloproteinases *Current Opinion in Cell Biology* **5** 891–897

Leonardsson G, Peng XR, Liu K, Nordstrom L, Carmeliet P, Mulligan R, Collen D and Ny T (1995) Ovulation efficiency is reduced in mice that lack plasminogen activator gene function: functional redundancy among physiological plasminogen activators *Proceedings of the National Academy of Sciences USA* **92** 12445–12450

Liu X, Wu H, Byrne M, Jeffrey J, Krane S and Jaenisch R (1995) A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodelling *Journal of Cell Biology* **130** 227–237

Luck MR (1994) The gonadal extracellular matrix *Oxford Reviews of Reproductive Biology* **16** 34–85

McIntush EM and Smith MF (1997) Concentration of tissue inhibitor of metalloproteinases (TIMP-1) in ovine follicular and luteal tissues *Biology of Reproduction* **56** (Supplement 1) 123

McIntush EM and Smith MF (1998) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in ovarian function *Reviews in Reproduction* **3** 23–30

McIntush EW, Pletz JD, Smith GW, Long DK, Sawyer HR and Smith MF (1996) Immunolocalization of tissue inhibitor of metalloproteinase-1 within ovine periovulatory follicular and luteal tissues *Biology of Reproduction* **54** 871–878

McIntush EW, Keisler DH and Smith MF (1997) Concentration of tissue inhibitor of metalloproteinases (TIMP)-1 in ovine follicular fluid and serum *Journal of Animal Science* **75** 3255–3261

McLaughlin B, Cawston T and Weiss JB (1991) Activation of the matrix metalloproteinase inhibitor complex by a low

molecular weight angiogenic factor *Biochimica et Biophysica Acta* **1073** 295–298

Murdoch WJ (1987) Treatment of sheep with prostaglandin $F_2\alpha$ enhances production of a luteal chemoattractant for eosinophils *American Journal of Reproductive Immunology* **15** 52–56

Murdoch WJ (1996) Microtubular dynamics in granulosa cells of periovulatory follicles and granulosa-derived (large) lutein cells of sheep: relationships to the steroidogenic folliculo-luteal shift and functional luteolysis *Biology of Reproduction* **54** 1135–1140

Murdoch WJ and McCormick RJ (1992) Enhanced degradation of collagen within apical vs. basal wall of ovulatory ovine follicle *American Journal of Physiology* **263** E221–225

Murphy G, Reynolds JJ and Werb Z (1985) Biosynthesis of tissue inhibitor of metalloproteinases by human fibroblasts in culture: stimulation by 12-*O*-tetradecanoylphorbol 13-acetate and interleukin 1 in parallel with collagenase *Journal of Biological Chemistry* **260** 3079–3083

Nagase H (1997) Activation mechanisms of matrix metalloproteinases *Biological Chemistry* **378** 151–160

Nett TM, McClellan MC and Niswender GD (1976) Effects of prostaglandins on the ovarian corpus luteum: blood flow, secretion of progesterone and morphology *Biology of Reproduction* **15** 66–78

Niswender GD and Nett TM (1994) The corpus luteum and its control in infraprimate species. In *The Physiology of Reproduction* pp 781–816 Ed. E Knobil and JD Neill. 2nd Edn. Raven Press, New York

Niswender GD, Schwall RH, Fitz TA, Farin CE and Saywer HR (1985) Regulation of luteal function in domestic ruminants: new concepts *Recent Progress in Hormone Research* **41** 101–151

Reich R, Tsafriri A and Mechanic GL (1985) The involvement of collagenolysis in ovulation in the rat *Endocrinology* **116** 522–527

Reich R, Daphna Iken D, Chun SY, Popliker M, Slager R, Adelmann Grill BC and Tsafriri A (1991) Preovulatory changes in ovarian expression of collagenases and tissue metalloproteinase inhibitor messenger ribonucleic acid: role of eicosanoids *Endocrinology* **129** 1869–1875

Russell DL, Salamonsen LA and Findlay JK (1995) Immunization against the N-terminal peptide of the inhibin alpha 43-subunit (alpha N) disrupts tissue remodelling and the increase in matrix metalloproteinase-2 during ovulation *Endocrinology* **136** 3657–3664

Salamonsen LA (1996) Matrix metalloproteinases and their tissue inhibitors in endocrinology *Trends in Endocrinology and Metabolism* **7** 28–34

Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E and Seiki M (1994) A matrix metalloproteinase expressed on the surface of invasive tumor cells *Nature* **370** 61–65

Smith GW, Goetz TL, Anthony RV and Smith MF (1994a) Molecular cloning of an ovine ovarian tissue inhibitor of metalloproteinases: ontogeny of messenger ribonucleic acid expression and *in situ* localization within preovulatory follicles and luteal tissue *Endocrinology* **134** 344–352

Smith MF, McIntush EW and Smith GW (1994b) Mechanisms associated with corpus luteum development *Journal of Animal Science* **72** 1857–1872

Smith GW, McCrone S, Petersen SL and Smith MF (1995) Expression of messenger ribonucleic acid encoding tissue inhibitor of metalloproteinases-2 within ovine follicles and corpora lutea *Endocrinology* **136** 570–576

Smith GW, Juengel JL, McIntush EW, Youngquist RS, Garverick HA and Smith MF (1996) Ontogenies of messenger RNA encoding tissue inhibitor of metalloproteinases 1 and 2 within bovine periovulatory follicles and luteal tissue *Domestic Animal Endocrinology* **13** 151–160

Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA and Goldberg GI (1995) Mechanism of cell surface activation of 72-kDa type IV collagenase: isolation of the activated form of the membrane metalloproteinase *Journal of Biological Chemistry* **270** 5331–5338

Suzuki K, Lees M, Newlands G, Nagase H and Woolley DE (1995) Activation of precursors for matrix metalloproteinases 1 (interstitial collagenase) and 3 (stromelysin) by rat mast-cell proteinases I and II *Biochemical Journal* **305** 301–306

Tadakuma H, Okamura H, Kitaoka M, Iyama K and Usuku G (1993) Association of immunolocalization of matrix metalloproteinase-1 with ovulation in hCG-treated rabbit ovary *Journal of Reproduction and Fertility* **98** 503–508

Takino T, Sato H, Shinagawa A and Seiki M (1995) Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library: MT-MMP form a unique membrane type sub-class in the MMP family *Journal of Biological Chemistry* **270** 23013–23020

Vu TH, Shipley JM, Bergers G, Gergers JE, Helms JA, Hanahan D, Shapiro SD, Senior RM and Werb Z (1998) MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes *Cell* **93** 411–422

Will H and Hinzmann B (1995) cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment *European Journal of Biochemistry* **231** 602–608

Willenbrock F and Murphy G (1994) Structure–function relationships in the tissue inhibitors of metalloproteinases *American Journal of Respiration and Critical Medicine* **150** 165–170