The human corpus luteum: remodelling during luteolysis and maternal recognition of pregnancy

W. Colin Duncan

Department of Reproductive and Developmental Sciences, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK

The marked tissue remodelling associated with luteolysis involves increased expression and activity of matrix metalloproteinases (MMPs) and an influx of immune cells, notably macrophages. Since the corpus luteum expresses high concentrations of specific tissue inhibitors of MMPs, it is clear that it is not only the increased activity of MMPs that is important, but also their tissue localization. Human chorionic gonadotrophin inhibits both MMP expression and macrophage influx in the rescued corpus luteum of early pregnancy. However, macrophages and the main cellular sources of MMPs in the corpus luteum do not express LH–hCG receptors. Therefore, it is likely that products of the steroidogenic cells, which do express LH–hCG receptors, are involved in the differential paracrine regulation of MMP expression and macrophage influx during luteolysis and maternal recognition of pregnancy.

The human corpus luteum has a large capacity for hormone synthesis and secretion. Indeed, weight for weight, it is the most active endocrine gland in the body. At the peak of its activity in the mid-luteal phase, the corpus luteum measures up to 2 cm in diameter, produces up to 25 mg progesterone each day and has a blood supply per unit mass of about eight times that of the kidney. However, 7 days later, at the time of menstruation, progesterone secretion has ceased and the corpus luteum has become a small, relatively avascular fibrous remnant. However, in a cycle in which conception occurs, the structure and function of the corpus luteum has been maintained by logarithmically increasing concentrations of hCG (Behrman et al., 1993). Although the corpus luteum has a fundamental role in the menstrual cycle and the establishment of pregnancy, the molecular and cellular mechanisms of luteolysis and the method by which hCG prevents these during maternal recognition of pregnancy are not fully understood.

The process of luteolysis involves marked tissue remodelling. One of the major features of tissue remodelling is the continued synthesis and breakdown of the extracellular matrix (ECM). Indeed, the integrity of the ECM is crucial to both cell function and survival (Pullan et al., 1996). The key proteolytic enzymes involved in the degradation of ECM proteins during tissue remodelling are the matrix metalloproteinases (MMPs) (Hulboy et al., 1997). Of the 17 MMPs so far described, interstitial collagenase (MMP-1), gelatinase A (MMP-2) and gelatinase B (MMP-9), which degrade the major structural collagens, gelatines and elastins, have received most attention.

As would be expected, the activity of these powerful enzymes is tightly regulated at several levels: transcriptional control of their expression; their synthesis as inactive pro-enzymes that require proteolytic activation; and the presence of specific tissue inhibitors (Birkedal-Hansen, 1995). Tissue inhibitors of metalloproteinases (TIMPs) are frequently regulated in coordination with MMPs. They bind to, and specifically inhibit, MMPs with a one-to-one stoichiometry. So far, four TIMPs have been described, of which the secreted proteins, TIMP-1 and TIMP-2, have been most widely scrutinized (Hulboy et al., 1997).

Tissue inhibitors of metalloproteinases

Smith and co-workers showed that the MMP remodelling system was important in the corpus luteum by demonstrating that TIMP-1 was produced in large amounts by the corpus luteum of pigs, cows and sheep (Smith et al., 1994, 1996; McIntush and Smith, 1998). Subsequently, TIMP-1 has been shown to be one of the major products of the corpus luteum of all species investigated, including non-human primates (Duncan et al., 1996a) and humans (Duncan et al., 1996b). It is likely that TIMP-1 plays an important role in the regulation of tissue remodelling and MMP action in the corpus luteum. However, investigation of the expression of TIMP-1 at different stages of the luteal lifespan has produced some conflicting results.

If regulation of TIMP-1 expression were fundamental to luteal remodelling, it would be expected that TIMP-1 expression would decrease during luteolysis and increase during luteal rescue. Indeed, there is some supporting evidence for this hypothesis. Induced luteolysis resulted in decreased TIMP-1 expression in the corpus luteum of non-human primates (Duncan et al., 1996a) and hCG increased TIMP-1 expression in luteinized granulosa cells in vitro (O’Sullivan et al., 1997). However, in cows, induced luteolysis increased the concentrations of TIMP-1, in contrast to the normal luteal phase, where expression tended to decrease (Smith et al., 1996). In sheep, TIMP-1 concentrations were relatively stable throughout the luteal phase (Smith et al., 1994). In pseudopregnant rats, luteal TIMP-1 expression decreased initially and then increased (Nothnick et al., 1995). There are clearly species differences in these findings. Therefore, the hypothesis that regulation of TIMP-1 expression is fundamental to luteal remodelling was tested on human corpora lutea at different stages of the luteal phase.
Human corpora lutea were collected at different stages of a normal functional luteal phase and dated on the basis of the LH surge (Duncan et al., 1996b). In addition, corpora lutea were collected after administration of logarithmically increasing concentrations of hCG that mimicked the hormonal changes of early pregnancy and resulted in luteal rescue (Fig. 1). TIMP-1 was highly expressed in the human corpus luteum as expected, but its expression did not change throughout the normal luteal phase or after luteal rescue with exogenous hCG (Duncan et al., 1996b). These observations do not support the hypothesis that the remodelling associated with luteolysis and luteal rescue is modulated by changes in TIMP-1 expression during the functional luteal phase.

Indeed, when other members of the TIMP family were studied, there was no evidence that their expression changes in such a way to be responsible for the control of tissue remodelling in the corpus luteum (Duncan et al., 1996a). What then is the function of the large amounts of TIMP-1 synthesized by the corpus luteum? Does TIMP-1 have any role in addition to protecting the corpus luteum against the ravages of unregulated proteolysis? The answer to these questions is not yet known, but TIMP-1 may have additional functions. It has been shown that TIMP-1 can promote the proliferation of fibroblasts and endothelial cells in vitro, and may function as a paracrine or autocrine growth factor (Hayakawa et al., 1992).

Some authors have suggested that TIMP-1 expression may facilitate steroidogenesis. The TIMP-1–procathepsin-L complex is a locally produced FSH-responsive factor that stimulates both Leydig and granulosa cell steroidogenesis. Both TIMP-1 and this complex stimulate steroidogenesis in a cAMP-independent manner with a bioactivity similar to saturating amounts of hCG (Boujrad et al., 1995). In addition, TIMP-1 is expressed in other steroidogenic tissues not involved in extensive tissue remodelling (Duncan et al., 1996a). However, the lack of specific problems in TIMP-1 knockout mice means that, at present, the luteal role of TIMP-1 remains uncertain (Nothnick et al., 1997). However, it does not appear to be the main regulator of differential tissue remodelling during the functional luteal phase.

**Matrix metalloproteinases**

If TIMPs do not consistently change over the luteal phase, an alternative hypothesis is that remodelling is regulated by changes in the expression and activity of MMPs. There is certainly evidence for the involvement of MMPs in the remodelling of the corpus luteum (McIntush and Smith, 1998). MMPs, notably MMP-1, MMP-2 and MMP-9, are expressed in the luteal cells of rodents, ruminants and women (Tsang et al., 1995; Nothnick et al., 1996; Aston et al., 1996). If MMPs are involved in the regulation of luteal remodelling, their expression and activity should increase during luteolysis and decrease during luteal rescue. There is some evidence in support of this hypothesis. In rats, prolactin-induced structural luteolysis was associated with increased MMP-2 activity (Endo et al., 1993). MMP-2 activity also increased with duration of culture in bovine luteal cells (Tsang et al., 1995) and human luteinized granulosa cells (Aston et al., 1996). In cultures of luteinized granulosa cells, hCG reduced the expression of MMP-2 and MMP-9 (Stamouli et al., 1996).

The human corpus luteum was investigated using the luteal rescue model shown (Fig. 1) to confirm the hypothesis that MMPs were involved in luteal remodelling. As expected, MMP-2 expression and activity were maximal in the late luteal phase (Duncan et al., 1998a) (Fig. 2). Indeed, high concentrations of MMP-9 were also detected in the late luteal phase. However, unlike MMP-2 expression, MMP-9 expression was also increased at the time of luteinization. This finding indicates that MMP-9 has an additional role in the remodelling associated with the formation of the corpus luteum. There is some other evidence that supports this concept. MMP-9 is the main MMP detected in luteinized granulosa cell cultures (Aston et al., 1996) and follicular explants at the time of luteinization (Russell et al., 1995).

The evidence that MMPs are involved in luteolysis has been strengthened by the observation that MMP expression is reduced by exposure to hCG in the rescued corpus luteum of early pregnancy (Duncan et al., 1998a). Exposure of the corpus luteum to hCG during luteal rescue results in lower expression and activity of MMP-2 than in the late luteal phase (Duncan et al., 1998a) (Fig. 2). Therefore, it is likely that the remodelling associated with luteolysis involves increased expression and activity of MMPs, notably MMP-2, and that hCG inhibits this during maternal recognition of pregnancy.

**Localization of matrix metalloproteinases**

It was not immediately clear how the MMPs involved in luteolysis could function in an environment that contains a large...
excess of specific tissue inhibitor. The answer to this conundrum came to light when the cellular localization of these proteins was reported. MMPs can function in a corpus luteum expressing large amounts of TIMPs because they have a different cellular localization (Duncan et al., 1998a). MMPs are expressed in the stroma cells around the granulosa–lutein cells that express large amounts of TIMP-1 (Duncan et al., 1998a) (Fig. 3). Where MMPs are expressed in the granulosa–lutein cell layer, the expression is localized to the foci of individual cells. It seems that remodelling occurs from the outside in and in hotspots within the steroidogenic cell layer (Fig. 3). The factors involved in the control of MMP expression and the exact nature of the cells expressing them is not entirely clear. However, the differential localization of MMP and TIMP expression is a key factor in the activity of MMPs during luteolysis (Bagavandoss, 1998; Duncan et al., 1998a).

Tissue remodelling results in increased tissue and cellular debris. It is likely that immune cells have essential roles in the remodelling associated with luteolysis and the removal of debris. Immune cells and their cytokine products appear to be normal constituents of the corpus luteum (Norman and Brännström, 1994) and luteolysis is associated with an immune cell infiltration, notably of macrophages. This mechanism has been documented extensively in several species, including humans (Brännström et al., 1994; Best et al., 1996). Macrophages increase in number during the luteal phase and are particularly present in the regressing corpus luteum at the time of menstruation.

The role of macrophage accumulation in the late luteal phase is not fully established. Although macrophages can have a pro-steroidogenic effect, many macrophage products, including
The human corpus luteum

(a) 

(b) 

Corpus luteum

LH receptor expression

MMP-1 expression

MMP-2 expression

MMP-9 expression

TIMP-1 expression

TIMP-2 expression
reactive oxygen species and tumour necrosis factor α, inhibit the steroidogenic pathway (Brännström and Norman, 1993). In addition, macrophage products can promote cell death by apoptosis. Macrophages can clear cellular debris and apoptotic bodies by phagocytosis and can activate and secrete MMPs. It is likely that macrophages have a negative effect on the structure and function of the corpus luteum.

If the roles of macrophages were primarily luteolytic, it would be expected that the number of macrophages would be reduced in the rescued corpus luteum of early pregnancy. Macrophages were localized and counted in human corpora lutea collected using the luteal rescue model (Fig. 1) and this study confirmed that macrophages accumulate during the functional luteal phase to a maximum in the late luteal phase at the time of luteolysis (Fig. 2) (Duncan et al., 1998b). In addition, there is a clear reduction in the number of macrophages during luteal rescue (Duncan et al., 1998b). The accumulation of macrophages in the corpus luteum as it ages does not occur in the presence of exogenous hCG. Therefore, it appears that macrophages have a significant role in the luteolytic process.

Control of tissue remodelling

The rescued human luteal model has demonstrated that the tissue remodelling associated with luteolysis is associated with increased expression and activity of MMP-2 and an influx of macrophages. It is likely that these processes are of major importance, as hCG inhibits them in the rescued corpus luteum of early pregnancy (Duncan et al., 1998a,b). The nature of the factors controlling MMP-2 expression and macrophage influx in the human corpus luteum are not yet known.

It is clear that the localization of MMP-2 and macrophages in the human corpus luteum is different from that of the LH-hCG receptors (Duncan et al., 1996c). The main cellular sources of MMPs and macrophages do not respond directly to hCG. Since LH-hCG receptors are localized to the steroidogenic cells of the corpus luteum (Duncan et al., 1996c), it is likely that steroidogenic cell products regulate their expression. At present, it is not known whether it is an increase in certain luteal-cell products, a reduction in certain inhibitory products, or a combination of both, that stimulates MMP-2 expression and macrophage recruitment. Likewise, it is not known whether hCG increases the expression of inhibiting molecules or reduces the expression of stimulating molecules.

The search for the factors involved in controlling MMP expression and macrophage influx in the corpus luteum is now on. So far, there are excellent candidate molecules in the form of monocyte chemoattractant protein 1 (MCP-1) (Townson et al., 1996), prostaglandins and various cytokines. Progesterone is a candidate molecule of particular note. The corpus luteum expresses specific progesterone receptors (Suzuki et al., 1994), and the inhibition of luteal progestosterone synthesis results in marked structural changes (Duffy et al., 1994). It is increasingly clear that there is a marked similarity between MMP expression and immune cell influx that occur during luteolysis and those that occur during menstruation. It is likely that progesterone withdrawal is of crucial importance in both of these pathways. It is anticipated that there will be many common factors involved in the regulation of luteolysis and menstruation.

References

Key references are identified by asterisks.


Brännström M and Norman RJ (1993) Involvement of leukocytes and cytokines in the ovulatory process and corpus luteum function Human Reproduction 8 1762–1775

Brännström M, Pascoe V, Norman RJ and McClure N (1994) Localization of leukocyte subsets in the follicle wall and in the corpus luteum throughout the human menstrual cycle Fertility and Sterility 61 488–495


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


Endo T, Aten RF, Wang F and Behrman HR (1993) Co-ordinate induction and activation of metalloproteinase and ascorbate depletion in structural luteolysis Endocrinology 133 690–698


Norman RJ and Brännström M (1994) White cells and the ovary – incidental invaders or essential effectors Klinische Wochenschrift 72 1181–1188

References
Russell DL, Salamonsen LA and Findlay JK (1995) Immunization against the N-terminal peptide of the inhibin α43-subunit (αN) disrupts tissue remodeling and the increase in matrix metalloproteinase 2 during ovulation Endocrinology 136 2657–2664