Differential regulation by LH and prostaglandins of steroidogenesis in small and large luteal cells of the ewe

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Introduction

In a nonfertile cycle, the corpus luteum of the ewe secretes progesterone for about 2 weeks, and then regresses and is resorbed within the ovary. Luteolysis does not occur, however, if fertilization takes place. In fact, luteolysis must be prevented for pregnancy to be successful. The most important function of the corpus luteum appears to be the secretion of progesterone, since removal of the corpus luteum in early gestation causes abortion, but abortion is prevented by progesterone therapy (Amoroso & Perry, 1977).

Since progesterone plays a crucial role in the establishment and maintenance of early pregnancy, elucidation of the factors which regulate progesterone secretion is important for understanding the mechanisms involved in the maternal recognition of pregnancy. Perhaps the most important factors involved in the regulation of progesterone secretion are luteinizing hormone (LH) and the prostaglandins. LH stimulates progesterone secretion by luteal tissue and can maintain luteal function in hypophysectomized animals (reviewed by Hansel, Concannon & Lukaszewska, 1973; Niswender, Schwall, Fitz, Farin & Sawyer, 1985). Prostaglandins (PG) F-2α and E-2 appear to have opposing effects. Injection of PGF-2α reduces serum concentrations of progesterone and this agent is thought to be responsible for regression of the corpus luteum at the end of the nonfertile cycle (Horton & Poyser, 1976). In contrast, PGE-2 stimulates progesterone secretion (Speroff & Ramwell, 1970; Marsh & LeMaire, 1974), delays the onset of normal luteolysis (Pratt, Butcher & Inskeep, 1979), and counteracts the luteolytic effects of PGF-2α (Henderson, Scaramuzzi & Baird, 1977).

The regulation of progesterone secretion is a complex process. Not only are several hormones involved, but corpora lutea of many animals contain at least two types of steroidogenic luteal cells. Examples of animals in which two types of luteal cells have been identified are listed in Table 1. Although the two types of luteal cells have been referred to by a variety of names (see Table 1), the terms small and large luteal cell are becoming generally accepted because the most obvious difference between them is in size. For example, Rodgers, O’Shea & Bruce (1984), using morphometric techniques, have estimated that in a mature corpus luteum of the ewe the average diameter of a small luteal cell is 15-8 μm and that of a large luteal cell is 29-2 μm.

In tissue sections, large luteal cells are generally spheroidal and small luteal cells have a stellate appearance (Fig. 1). Although small luteal cells outnumber large luteal cells, they comprise 17% of the volume of the corpus luteum, whereas large luteal cells make up 25-35% (Nett, McClellan & Niswender, 1976; Rodgers et al., 1984). At the ultrastructural level, both small and large luteal cells from the ewe have abundant agranular endoplasmic reticulum and numerous mitochondria (Niswender et al., 1985), features which are typical of active steroid-secreting cells (Christensen & Gillim, 1969). Large luteal cells also have features that are consistent with a protein-secreting function, such as numerous Golgi complexes, rough endoplasmic reticulum, and secretory granules. Small luteal cells have a highly in-folded nucleus, and, in appropriate sections, the nucleus may appear to have cytoplasmic inclusions. In contrast, the nucleus of large luteal cells is round. Whereas the cytoplasm of small luteal cells contains lipid droplets, such droplets are rarely
Fig. 1. Thick section of a corpus luteum collected from a ewe on Day 10 after ovulation. Small luteal cells (SLC) are easily distinguished from large luteal cells (LLC). × 1100.

Table 1. Animals in which two types of luteal cells have been identified

<table>
<thead>
<tr>
<th>Animal</th>
<th>Designation of small/large cells</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Pig</td>
<td>Theca lutein/granulosa lutein</td>
<td>Corner (1919)</td>
</tr>
<tr>
<td>Cow</td>
<td>Type I/Type II</td>
<td>Foley &amp; Greenstein (1958)</td>
</tr>
<tr>
<td>Human</td>
<td>Theca lutein/granulosa lutein</td>
<td>Gillim et al. (1969)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Embryonic/mature</td>
<td>Warbritton (1934)</td>
</tr>
<tr>
<td>White-tailed deer</td>
<td>Theca lutein/granulosa lutein</td>
<td>Sinha et al. (1971)</td>
</tr>
<tr>
<td>White whale</td>
<td>Small/large</td>
<td>Mossman &amp; Duke (1973)</td>
</tr>
<tr>
<td>Rat</td>
<td>I/D</td>
<td>Wilkinson et al. (1976)</td>
</tr>
<tr>
<td>Rhesus Monkey</td>
<td>Small/large</td>
<td>Gulyas et al. (1979)</td>
</tr>
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</table>

observed in large luteal cells. The cytoplasm of large luteal cells contains small, electron-dense secretory granules, which are not characteristically found in small luteal cells. For a more detailed description of the ultrastructure of small and large luteal the reader is referred to reports by O'Shea, Cran & Hay (1979), Rodgers & O'Shea (1982), and Gulyas (1984). It should be noted that some of the differences between small and large luteal cells vary with species. For example, the small and large luteal cells of the primate corpus luteum have such similar structure that two cell types were identified only after the tissue was dissociated (Gulyas, Stouffer & Hodgen, 1979).

Separation of small and large luteal cells

Workers in several laboratories have been able partly to purify small and large luteal cells. The techniques that have been used include: (1) sedimentation at unit gravity (Lemon & Loir, 1977; Ursely & Leymarie, 1979; Koos & Hansel, 1981); (2) centrifugal elutriation (Fitz, Mayan, Sawyer & Niswender, 1982; Chegini, Ramani & Rao, 1984); and (3) density, gradient centrifugation (Rodgers & O'Shea, 1982). With these techniques, small luteal cells can be separated from large luteal cells,
but enriched preparations of large luteal cells are invariably contaminated to some degree with clumps of small luteal cells and/or small luteal cells attached to large luteal cells. Nonetheless, the preparations obtained with these techniques have been useful for defining the biochemical characteristics of the two cell types.

**Regulation of progesterone secretion by LH**

The first evidence that small and large luteal cells are biochemically different was obtained in 1977, when Lemon & Loir reported the results of their studies on luteal cells collected from pregnant sows. Using a perifusion system, they found that the basal rate of progesterone secretion by large luteal cells was much higher than that by small luteal cells, and furthermore, that LH readily stimulated the secretion of progesterone by small luteal cells but had little effect on large luteal cells. Similar results have been obtained in subsequent studies of luteal cells from the cow (Koos & Hansel, 1981) and ewe (Fitz *et al.*, 1982; Rodgers, O'Shea & Findlay, 1983a). That small and large luteal cells respond differentially to LH is consistent with the observation that there are numerous receptors for LH on small luteal cells, but few, if any, on large luteal cells (Fitz *et al.*, 1982).

In the pregnant cow, large luteal cells may be more responsive to LH than they are in the ewe and sow, since Ursely & Leymarie (1979) reported that progesterone secretion in both cell types was stimulated by LH. However, small luteal cells responded to doses of LH that were 100–1000 times lower than those that stimulated large cells. More recently, Chegini *et al.* (1984) reported that small and large luteal cells from the pregnant cow secreted progesterone at an equal rate and that both cell types were stimulated to the same extent by hCG. These observations contrast with the lack of responsiveness of large cells collected during the oestrous cycle (Koos & Hansel, 1981). There are several possible explanations for this discrepancy. One is that the preparations of large cells were contaminated by small luteal cells. Ursely & Leymarie (1979) did not comment on the purity of their preparations, but Chegini *et al.* (1984) noted that such contamination amounted to less than 10%. Another possible explanation is that different doses of LH were used. Koos & Hansel (1981) used only one concentration (5 ng/ml), which is slightly below the level (10 ng/ml) at which Ursely & Leymarie (1979) observed any stimulation in large luteal cells.

It is also possible that the corpus luteum of pregnancy is different from the corpus luteum of the cycle. For example, there appear to be two subpopulations of large luteal cells in the cow (Alila & Hansel, 1984). One shares an antigenic determinant with granulosa cells, and hence appears to be granulosa-derived, and one shares an antigenic determinant with theca cells, and so appears to be theca-derived. During pregnancy, the proportion of granulosa-derived large cells decreases as the proportion of theca-derived large cells increases. Since the theca antigen is also present on small luteal cells, the corpus luteum of pregnancy becomes progressively enriched with large cells that share this antigen with small cells.

It is generally accepted that LH stimulates steroidogenesis via the intracellular second messenger, cAMP (Marsh, 1976). This model appears to hold true for the small luteal cell. It has been shown that LH stimulates the production of cAMP in small luteal cells (Hoyer, Fitz & Niswender, 1984) and that progesterone secretion by these cells is stimulated by analogues of cAMP and by factors that activate adenylate cyclase (Rodgers *et al.*, 1983a; Hoyer *et al.*, 1984). However, cyclic AMP appears to have no role in regulating steroidogenesis in large luteal cells. Part of the evidence for this conclusion is that the secretion of progesterone by large luteal cells is not stimulated by dibutyryl-cAMP (Rodgers *et al.*, 1983a; Hoyer *et al.*, 1984). Large luteal cells have adenylate cyclase activity that can be stimulated by cholera toxin and forskolin, but not by LH (Hoyer *et al.*, 1984). In addition, large luteal cells contain a protein that binds cAMP and has been tentatively identified as the type I isoenzyme of cAMP-dependent protein kinase (P. B. Hoyer & G. D. Niswender, unpublished observation), but at present the function of the cAMP system in the large luteal cell remains unknown.
Regulation of progesterone secretion by prostaglandins

Prostaglandins are very important in the regulation of luteal function. There is considerable evidence that PGF-2α plays a key role in luteolysis (Niswender, 1981). The establishment of pregnancy requires that luteolysis be prevented, and in the ewe, PGE-2 is a key factor in this process. The evidence for this conclusion includes the finding that the gravid uterus secretes more PGE-2 than does the nongravid uterus (Ellinwood, Nett & Niswender, 1979; Silvia, Ottobre & Inskeep, 1984), and that infusion of PGE-2 delays normal luteolysis (Pratt et al., 1979) and prevents the luteolytic effects of PGF-2α (Henderson et al., 1977).

The effects of prostaglandins are probably mediated via the large luteal cell. Fitz et al. (1982) reported that receptors for PGE-2 and PGF-2α are abundant on large luteal cells, but are essentially undetectable on small luteal cells. Consistent with this observation, PGE-2 has been shown to stimulate the secretion of progesterone by large, but not small luteal cells (Fitz, Hoyer & Niswender, 1984a). The mechanisms by which PGE-2 stimulates progesterone secretion are not known, but cyclic AMP does not appear to be involved since PGE-2 does not stimulate adenylate cyclase activity or increase intracellular or extracellular levels of cAMP (Fitz et al., 1984a). Perhaps protein kinase C (Nishizuka, 1984) is involved in regulating steroidogenesis in large luteal cells.

Although receptors for PGF-2α are present on large luteal cells (Fitz et al., 1982), and PGF-2α causes luteolysis in vivo (Horton & Poyer, 1976), effects of PGF-2α on the secretion of progesterone by cultured luteal cells have been difficult to demonstrate. PGF-2α has been reported to inhibit progesterone secretion and to have a direct cytotoxic effect on large luteal cells in vitro (Fitz, Mock, Mayan & Niswender, 1984b). This response was not a nonspecific toxicity of the PGF-2α because it could be prevented by coculturing with PGE-2. However, the inhibitory effect of PGF-2α occurs inconsistently (unpublished observations). Koos & Hansel (1981) also tested the effect of PGF-2α on isolated small and large luteal cells and found that it had no effect on basal or LH-stimulated progesterone secretion. In addition, although the prostaglandin analogue cloprostenol stimulates the release of oxytocin from the corpus luteum in vivo (Flint & Sheldrick, 1982), this response has not been observed in cultured cells or luteal slices (R. H. Schwall & G. D. Niswender, unpublished observation). PGF-2α reduces the ability of LH to stimulate progesterone secretion by luteal slices in vitro (Evrard, Leboulleux & Hermier, 1978; Fletcher & Niswender, 1982), but other investigators have observed that PGF-2α stimulates progesterone secretion by dispersed bovine luteal cells (Hixon & Hansel, 1979).

The reasons for the discrepant effects of PGF-2α are not known, but there are several possibilities. (1) PGF-2α may be rapidly metabolized in vitro. (2) If an incubation involved the use of a pool of serum that contained large amounts of prostaglandins, then adding prostaglandins would have no further effect. (3) The actions of PGF-2α may require cell associations that are lost when the tissue is dispersed. These interactions would be maintained in tissue slices, and may be reformed if cells are incubated at a high density. (4) The effects of PGF-2α may require interactions with other factors. For example, Bennegard, Dennefors & Hamberger (1984) have found that noradrenaline is required for PGF-2α to inhibit progesterone secretion by human luteal explants. In addition, factors released by ovarian follicles appear to be required for naturally-occurring (Karsch, Noveroske, Roche, Norton & Nalbandov, 1970) and PGF-2α-induced luteolysis (Hixon, Gengenbach & Hansel, 1974). (5) Handling of the uterus during collection of luteal tissue may cause a release of PGF-2α which may then act on the corpus luteum.

Interaction between small and large luteal cells

LH is a major luteotrophic hormone in the ewe. Infusions of LH maintain luteal function in hypophysectomized ewes (Kaltenbach, Graber, Niswender & Nalbandov, 1968), and prolong the oestrous cycle in intact ewes (Karsch et al., 1971). In addition, administration of an antiserum to
LH reduces the weight and progesterone content of corpora lutea of sheep (Fuller & Hansel, 1970). Since large luteal cells secrete progesterone at a high rate, they must contribute significantly to circulating concentrations of progesterone. Yet receptors for LH appear to be restricted to small luteal cells, and progesterone secretion by large luteal cells is unresponsive to LH (Fitz et al., 1982). This raises some interesting questions about how LH maintains luteal function. Part of the explanation may involve the ability of LH to enhance blood flow to the ovary (Niswender, Reimers, Diekman & Nett, 1976). Additionally, it has been suggested that LH causes small luteal cells to multiply and differentiate into large luteal cells (Donaldson & Hansel, 1965). But if this were the only effect of LH, serum concentrations of progesterone should not fall immediately after hypophysectomy (Hixon & Clegg, 1971).

Lemon & Mauleon (1982) reported that the amount of progesterone secreted by a mixture of small and large pig luteal cells is greater than the sum of the amounts secreted by either preparation alone. This synergism was found to be caused by a factor, produced by small luteal cells, that enhances steroidogenesis in large luteal cells. The experiments of Lemon & Mauleon (1982) were conducted in the absence of gonadotrophins. It would be interesting to know whether LH influences the secretion of this factor since it may be important in the luteotropic actions of LH. The nature of the factor is unknown, but it may be a prostaglandin since LH causes the release of prostaglandins from luteal tissue (Marsh, 1976) and PGE-2 stimulates progesterone release from large luteal cells (see above).

There may also be interactions between the two cell types during luteolysis. Receptors for PGF-2α are present almost exclusively on large luteal cells (Fitz et al., 1982), but the entire corpus luteum regresses after treatment with PGF-2α. There must be some sort of communication between the cell types. One possible candidate for mediating this communication is oxytocin, since: (1) oxytocin has been shown to be present in large luteal cells (Rodgers, O'Shea, Findlay, Flint & Sheldrick, 1983b; Guldenaar, Wathes & Pickering, 1984; Sawyer & Moeller, 1985); (2) cloprostenol, an analogue of PGF-2α, has been shown to stimulate the release of oxytocin by the corpus luteum (Flint & Sheldrick, 1982); and (3) oxytocin has been reported to inhibit the ability of hCG to stimulate progesterone secretion by bovine (Tan, Tweedale & Biggs, 1982a) and human (Tan et al., 1982b) luteal tissue. However, some investigators have found no effect of oxytocin on progesterone secretion by human luteal tissue (Richardson & Masson, 1985). Another possible candidate is neurophysin I, which is secreted in conjunction with oxytocin (Schams, Schallenberger & Legros, 1985).

The interaction between LH and PGF-2α can also occur in the opposite direction. Karsch et al. (1971) have shown that infusion of LH can overcome natural luteolysis, and Bolt (1979) has reported that hCG prevents PGF-2α-induced luteolysis. One factor that determines whether the LH or PGF-2α response predominates may be the relative dose of the hormones. In addition, as noted above, catecholamines may also be important (Bennegard et al., 1984), but as yet the mechanisms involved are unknown.

**Number of small and large luteal cells in the corpus luteum**

Small and large luteal cells have quite different biochemical properties, and therefore, the hormonal responsiveness of the corpus luteum will be determined by the relative number of small and large luteal cells. O'Shea et al. (1979) reported that on Day 10 of the cycle there were 2–3 small luteal cells for every large cell, and that this ratio remained constant throughout pregnancy in sheep. Rodgers et al. (1984) performed a morphometric analysis and concluded that there were 5 small cells for every large cell in mature sheep corpora lutea collected in the middle of the luteal phase.

The studies noted above were performed on luteal tissue collected from mature corpora lutea. To date there is no information about how the number of small and large luteal cells changes over the course of the oestrous cycle. To examine this issue, we have measured the size of steroidogenic
cells in corpora lutea collected on Days 4, 8, 12, and 16 of the sheep oestrous cycle. The corpora lutea were dissociated into single-cell suspensions using collagenase, and the number of cells recovered from each corpus luteum was determined with a haemocytometer. The cells were fixed in 1% paraformaldehyde, washed, dried onto slides, and histochemically stained for 3β-hydroxy steroid dehydrogenase (3β-HSD) activity, as described by Payne, Downing & Wong (1980). Photographs were taken of the preparations stained for 3β-HSD and the diameter of all 3β-HSD-positive cells in each photograph was measured with a Zeiss Videoplan Image Analyzer. Corrections for shrinkage due to fixation were programmed into the Videoplan. Using this approach, it was found that the median diameter of 3β-HSD-positive cells increased about 2 μm every 4 days (Day 4 = 11.2 μm; Day 8 = 12.8 μm; Day 12 = 14.6 μm; Day 16 = 16.8 μm).

The number of small and large luteal cells recovered from each corpus luteum on each day was also determined. Cells less than 18 μm in diameter were classified as small luteal cells, and those with diameters greater than 18 μm, as large luteal cells. The percentages of cells in the appropriate size ranges were pooled and multiplied by the number of 3β-HSD-positive cells per corpus luteum, which was determined by multiplying the percentage of cells that were 3β-HSD-positive by the number of cells recovered from each corpus luteum. As summarized in Table 2, the number of small and large cells increased between Days 4 and 8, and remained elevated through Day 12. By Day 16, the number of small luteal cells was decreased by 60%, but the number of large cells was not significantly different from that on Day 12. The corpora lutea collected on Day 16 fell into 2 distinct groups, based on weight. Three had an average weight of 542 ± 24 (s.e.m.) mg and the other three averaged 260 ± 2 mg. These two groups, which probably resulted from cycles that were of slightly different length, may be representative of corpora lutea during the initial and final phases of luteolysis. Therefore, they were referred to as nonregressed and regressed Day 16 corpora lutea, respectively. The number of small luteal cells in the nonregressed and regressed Day 16 corpora lutea was 7.6 ± 2.4 × 10⁶ and 2.3 ± 0.4 × 10⁶, respectively. Both of these values were less than that obtained for Day 12 corpora lutea (Table 2). The number of large luteal cells in the nonregressed Day 16 corpora lutea (4.6 ± 1.1 × 10⁶) was not different from that for the Day 12 corpora lutea (see Table 2), but in the regressed Day 16 corpora lutea, there were only 1.5 ± 0.3 × 10⁶ large luteal cells.

The results of this study indicate that sheep luteal cells grow in size over the course of the oestrous cycle. If the two groups of corpora lutea collected on Day 16 reflect the early and late stages of luteolysis, then it appears that during luteolysis, small luteal cells are lost before large luteal cells. A preferential loss of small luteal cells has also been observed in the luteolysis

<table>
<thead>
<tr>
<th>Day</th>
<th>Small</th>
<th>Large</th>
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<tr>
<td>4</td>
<td>5.8 ± 1.5a</td>
<td>0.6 ± 0.1a</td>
</tr>
<tr>
<td>8</td>
<td>16.1 ± 2.8b</td>
<td>3.3 ± 0.5b</td>
</tr>
<tr>
<td>12</td>
<td>13.6 ± 2.0b</td>
<td>4.6 ± 0.8b</td>
</tr>
<tr>
<td>16</td>
<td>5.0 ± 1.6a</td>
<td>3.1 ± 0.9b</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. Values within columns with different letters are significantly different (P < 0.05).
associated with parturition in the cow (Archbald, Al-Bagdadi & Godke, 1981). The mechanisms are unknown, but again there must be some communication between the cell types, perhaps via oxytocin or neurophysin, as discussed above.

A word of caution should be mentioned regarding this study. The classification of cells as being small or large luteal cells was based purely on their size, and therefore is somewhat arbitrary. There may be cells that have the ultrastructure of large luteal cells but are smaller than 18 µm, and, conversely, there may be cells that have the ultrastructure of small luteal cells but are larger than 18 µm.

**Future directions**

Much work needs to be done to define further the regulation of steroidogenesis in small and large luteal cells. To date, all of the research in this area has been conducted on tissue collected during the mid-luteal phase of the oestrous cycle. The responsiveness of the cells needs to be characterized throughout the course of the oestrous cycle and pregnancy. And more research needs to be performed with other species. For example, chorionic gonadotrophin, which has LH-like bioactivity, rescues the corpus luteum of the primate during early pregnancy, but PGE-2 appears to serve this function in the ewe. Therefore, it is of interest to determine whether the small and large luteal cells in the primate have hormonal responses that are similar to those in the ewe.

A number of other factors may also be important in the regulation of luteal steroidogenesis. For example, catecholamines have been shown to stimulate progesterone secretion (Condon & Black, 1976), but the effects on purified populations of small and large cells have not been examined. Receptors for oestradiol are more abundant in large luteal cells than in small luteal cells (Glass, Fitz & Niswender, 1984), but their function remains undetermined. Lipoproteins are a major source of cholesterol to be used in steroidogenesis (Gwynne & Strauss, 1982), yet nothing is known about how small and large luteal cells utilize lipoproteins. Angiogenic factors are likely to be important during the growth of the corpus luteum. Ovine trophoblastic protein 1 may also act on the corpus luteum. Receptors for this protein have been found in luteal membranes (Godkin, Bazer & Roberts, 1984). Although in these studies this protein had no effect on progesterone secretion, ovine trophoblastic protein 1 may alter the sensitivity of the corpus luteum to other factors, such as PGE-2. There are probably additional factors that could interplay in regulating luteal function. Currently, the advantage of having two types of steroidogenic luteal cells is unknown, but it may become more apparent as more is learned about their respective functions.

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