Structural and functional properties of the corpus luteum of pregnancy

H. R. Sawyer

Animal Reproduction and Biotechnology Laboratory, Department of Physiology, Colorado State University, Fort Collins, CO 80523, USA

In domestic ruminants the parenchyma of the corpus luteum consists of two subpopulations of steroidogenic cells commonly referred to as small and large luteal cells. These cells differ not only in size and structural characteristics, but also in functional properties. During the mid-luteal phase of the oestrous cycle approximately 60% of the corpus luteum is occupied by steroidogenic cells. Although the steroidogenic capacity of these cells declines as pregnancy advances, the cells persist as distinct populations throughout pregnancy and for several days following parturition. In general, structural changes typically observed at the end of the oestrous cycle also occur after parturition, but over a more extended period. These include deletion of endothelial cells and occlusion of capillary lumina with cellular debris and apoptotic bodies, an infiltration of eosinophils and macrophages, and fragmentation and lysis of parenchymal cells. However, not all parenchymal cells undergo lysis, nor are they rapidly phagocytosed by macrophages. Instead, many fuse to form what appear to be large syncytia that contain numerous lipid droplets, tightly packed mitochondria and multiple nuclei with condensed chromatin. Fusion of parenchymal cells to form syncytial profiles begins 2–3 days after parturition and the syncytia persist for at least 22 days post partum. By day 35 post partum transformation of the corpus luteum into a corpus albicans is essentially complete.

Introduction

In domestic ruminants, as in other animals, the corpus luteum of the oestrous cycle and pregnancy comprises three distinct cell types: parenchymal (i.e. steroidogenic) cells, fibroblasts and capillary endothelial cells. On the basis of differences in size, morphology and biochemistry, cells that form the parenchymal component of the corpus luteum can be further subdivided into at least two subpopulations (Ursely and Leymarie, 1979; Koos and Hansel, 1981; Fitz *et al.*, 1982; Rodgers *et al.*, 1983a; Niswender *et al.*, 1985; O'Shea, 1987). One population consists of cells that are known as small luteal cells. These cells are presumed to be derived from cells of the theca interna of the follicle and have also been referred to as theca-lutein, paralutein and type I cells. Cells comprising the second population of parenchymal cells are derived from granulosa cells (O'Shea *et al.*, 1987) and are frequently referred to as large luteal cells. Alternative terms for large luteal cells include blossom, granulosa-lutein and type II cells. After ovulation, and as the corpus luteum matures, small luteal cells increase in number but not size, whereas large luteal cells increase in size but not number (Farin *et al.*, 1986). Consequently, as the corpus luteum increases in mass and becomes functionally mature, the relative volume occupied by small and large luteal cells remains stable until luteolysis is initiated, either at the end of the oestrous cycle in nonpregnant animals, or near the time of parturition in pregnant animals. Although both cell types synthesize and secrete progesterone and possess receptors for LH, differences in basal and LH-stimulated

secretion of progesterone between small and large luteal cells have been consistently observed (Niswender *et al.*, 1985). Small luteal cells isolated from ovine (Fitz *et al.*, 1982; Rodgers *et al.*, 1983a; Harrison *et al.*, 1987) and bovine (Ursely and Leymarie, 1979; Koos and Hansel, 1981; Weber *et al.*, 1987) corpora lutea during the mid-luteal phase of the oestrous cycle show marked increases (about 4–20 fold) in progesterone secretion when incubated in the presence of LH. In contrast, secretion of progesterone by large luteal cells is unaffected by LH. During pregnancy, small and large luteal cells retain the ability to synthesize and secrete progesterone, but the ability of small luteal cells to respond to LH appears to diminish gradually (Harrison *et al.*, 1987; Weber *et al.*, 1987; Farin *et al.*, 1989). The underlying cause for this reduced responsiveness is not clear, as there is no concomitant reduction in the number of receptors for LH (Harrison *et al.*, 1987; Farin *et al.*, 1989).

At the end of the oestrous cycle, PGF_{2a} is released from the uterus in discrete pulses and causes the corpus luteum to regress. Regression of the corpus luteum at this time is characterized by a reduction in blood flow (Nett *et al.*, 1976), an infiltration of eosinophils and macrophages (Murdoch, 1987), an increase in autophagosomes within luteal cells (Gemmell *et al.*, 1976; McClellan *et al.*, 1977), and cytoplasmic and nuclear changes consistent with the process of apoptosis (O'Shea *et al.*, 1977; Sawyer *et al.*, 1990). Concomitant with these changes, there is a precipitous decline in circulating concentrations of progesterone. In contrast, during fertile cycles, the presence of a viable conceptus within the uterus prevents luteolysis, and serum concentrations of progesterone remain similar to those measured during the mid-luteal phase of the cycle. The corpus luteum is maintained for the duration of pregnancy in both sheep and cattle; however, in sheep the corpus luteum is not required for maintenance of pregnancy beyond day 50 of gestation, whereas removal or lysis of the corpus luteum in cattle anytime before day 200 of gestation results in termination of pregnancy.

Structural Features of Small and Large Luteal Cells

On the basis of differences in size and morphology, small and large luteal cells can be identified at the light microscope level at all stages of the oestrous cycle and throughout pregnancy. Not only do small and large luteal cells persist as distinct populations throughout pregnancy (O'Shea *et al.*, 1979; O'Shea and McCoy, 1988), but they can also be discerned for several days after parturition (O'Shea and Wright, 1985). Structural changes that occur in the corpus luteum following parturition resemble those that occur at the end of the oestrous cycle; however, the overall process is much slower (O'Shea and Wright, 1985; O'Shea and McCoy, 1988). Structural characteristics of ovine parenchymal cells discernible by light microscopy at selected times during the reproductive cycle from day 12 after oestrus to day 35 post partum are illustrated in Fig. 1(a–f).

Light microscopy

In most studies the diameters of small luteal cells in sheep (Rodgers *et al.*, 1984; Farin *et al.*, 1986; O'Shea *et al.*, 1986) and cattle (Ursely and Leymarie, 1979; Weber *et al.*, 1987; O'Shea *et al.*, 1989; Lei *et al.*, 1991) have been reported to range from 12 to 22 µm. The cytoplasm of these stellate cells contains lipid droplets and usually stains darker than does the cytoplasm of large luteal cells (Fig. 1a, b). However, near the end of pregnancy and following parturition, the cytoplasmic density of large luteal cells appears similar to that for small luteal cells (Fig. 1c, d). In addition to size, shape and cytoplasmic features, a unique structural characteristic that can be used to identify ovine small luteal cells, both by light and electron microscopy, is the presence of nuclei with conspicuous cytoplasmic inclusions (Fig. 1c). Approximately 9% of ovine small luteal cells contain these inclusions (O'Shea and McCoy, 1988). Similar inclusions are not normally observed in nuclei of large luteal cells.

The mean diameter of large luteal cells in sheep (Farin *et al.*, 1986) and cattle (O'Shea *et al.*, 1989) during the mid-luteal phase of the oestrous cycle were 31 and 38 µm, respectively. In contrast to small luteal cells, large luteal cells are usually spherical or polyhedral and contain a large spherical nucleus with prominent nucleoli. The cytoplasm of large luteal cells through midpregnancy appears pale compared to that of small luteal cells, and in sheep generally lacks lipid droplets until the onset of luteolysis. Although
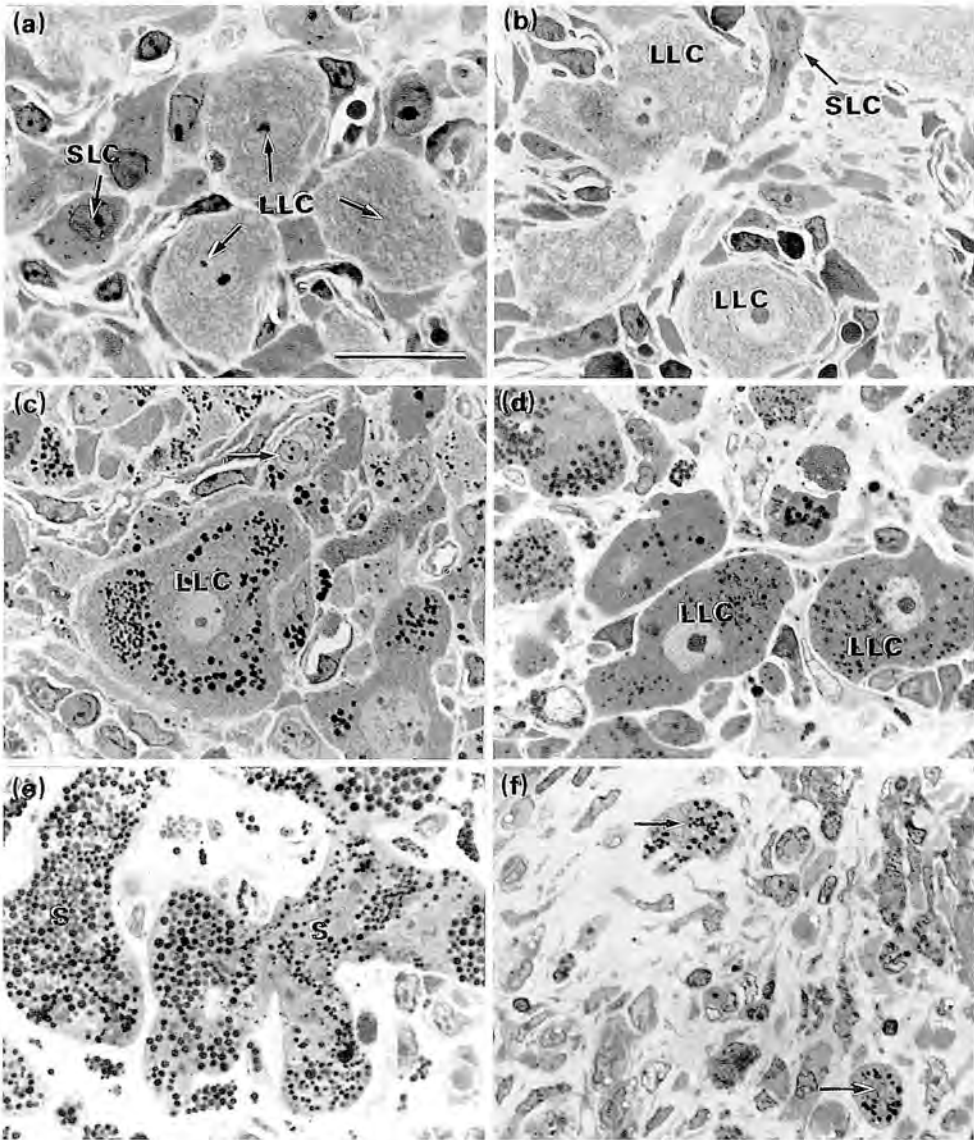
Fig. 1. Light micrographs illustrating the appearance of small (SLC) and large (LLC) luteal cells in corpora lutea during the oestrous cycle, pregnancy and post partum. All photomicrographs were prepared from luteal tissues that had been immersion fixed in a solution of 4% glutaraldehyde and 0.1 mol cacodylate buffer l^{-1} (pH 7.4), postfixed in 1% osmium tetroxide, embedded in Polybed 812 resin, sectioned at a thickness of 1 µm, and stained with Toluidine blue. (a) Day 12 of the oestrous cycle; (b) day 16 of pregnancy; (c) 1 h post partum. The cytoplasmic density of small and large luteal cells is similar at this time and substantial amounts of lipid are present in the cytoplasm of both cell types. Small luteal cells can easily be distinguished by the presence of nuclear inclusions of cytoplasm (arrow); (d) day 3 post partum. In many areas of the corpus luteum large luteal cells appear healthy, and except for the accumulation of lipid there are no overt signs of degeneration. In other areas some cells appear to be degenerating, and in others cells appear to fuse to give rise to syncytia; (e) day 22 post partum. Beginning as early as day 2 post partum some luteal cells appear to fuse and give rise to large syncytia (S); (f) day 35 post partum. Transformation of the corpus luteum into a corpus albicans is essentially complete, but remnants of luteal cells containing lipid droplets (arrows) are still detectable. Scale bar represents 30 µm.
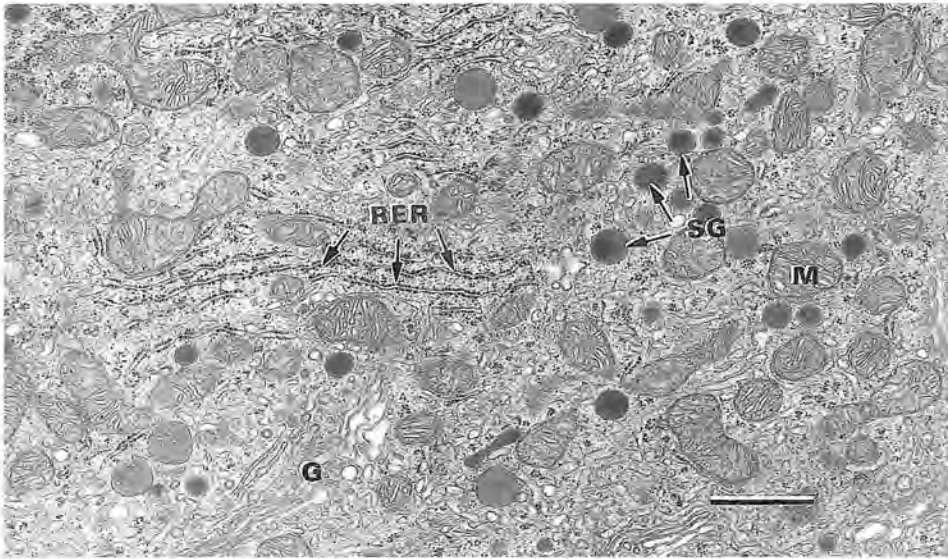
Fig. 2. Electron micrograph of a portion of the cytoplasm of an ovine large luteal cell on day 12 of the oestrous cycle. The cytoplasm contains numerous secretory granules (SG), mitochondria (M), isolated stacks of rough endoplasmic reticulum (RER) and an elaborate Golgi apparatus (G). Scale bar represents 1 μm.

it is relatively easy to identify ovine small and large luteal cells under the light microscope (Fig. 1a–c), the task is more difficult in cattle (O'Shea, 1987) because lipid droplets are present in both small and large luteal cells in cattle. Since the presence or absence of cytoplasmic lipid droplets cannot be used to distinguish between bovine small and large luteal cells accurately (O'Shea et al., 1990), identification of these cells using bright field optics and conventional staining techniques is primarily limited to size, shape and cytoplasmic density. With the exception of the accumulation of lipid in ovine large luteal cells at the end of pregnancy (Fig. 1c, d), the morphology of steroidogenic luteal cells during gestation is very similar to that typically observed during the mid-luteal phase of the oestrous cycle (Weber et al., 1987; O'Shea et al., 1989).

Electron microscopy

The ultrastructure of small and large luteal cells were examined extensively in sheep (O'Shea et al., 1979; Paavola and Christensen, 1981) and cattle (Fields and Fields, 1985; O'Shea et al., 1990). Consistent with a steroidogenic function, the cytoplasm of these cells is characterized by an abundance of smooth endoplasmic reticulum and numerous mitochondria. The volume density and number of mitochondria are greater in large cells than in small cells and may account for the higher basal production of progesterone by large luteal cells (Kenny et al., 1989). A variety of ultrastructural characteristics can be used to distinguish between small and large luteal cells (Box 1), but the most obvious and reliable is the presence of electron-dense secretory granules in large luteal cells (Fig. 2). These granules are approximately 0.2 μm in diameter (Gemmell et al., 1974; Paavola and Christensen, 1981) and have been shown to contain oxytocin (Theodosis et al., 1986; Fields et al., 1992). In sheep, secretory granules are dispersed throughout the cytoplasm of large luteal cells (Fig. 2), whereas in cattle they often occur in clusters (Fields et al., 1985; O'Shea et al., 1990) (Fig. 3). The cytoplasm of small luteal cells (Fig. 4) typically lacks secretory granules, but contains an abundance of smooth endoplasmic reticulum, numerous lipid droplets and what appear to be secondary lysosomes and multivesicular bodies. Since the shape of the nuclei in small luteal cells is very irregular in outline and appears to be folded in some areas, characteristic 'nuclear

Box 1. Ultrastructural characteristics of parenchymal cells in sheep and cattle

Structure/Organelle	Characteristics
Nucleus	The nuclear membrane in ovine small cells is often irregular in shape and characterized by conspicuous infoldings. As a result, and depending upon the plane of section, nuclei in approximately 9% of small cells appear to have cytoplasmic inclusions. The nucleus in large cells is usually spherical and lacks cytoplasmic inclusions.
Plasma membrane	In contrast to small cells, many areas of the plasma membrane in large cells are highly folded giving rise to what appear to be microvilli. Many of the microvillus extensions penetrate the surrounding basal lamina and make contact with neighbouring cells, whereas others fold back and make contact with the plasma membrane or adjacent microvilli of the same cell. Adherens and septate-like junctions are present, but observed more frequently in cattle than in sheep. There is no morphological evidence for classical gap junctions between parenchymal cells.
Basal lamina	Large cells are surrounded by a prominent basal lamina that in most cases appears to be continuous. The basal lamina associated with small cells is less well defined and is absent along some regions of the cell surface.
Secretory granules	A major distinguishing feature between small and large cells is the presence of numerous, electron-dense secretory granules in the cytoplasm of large cells. These granules are about 0.2 μm in diameter and contain oxytocin. Although small cells occasionally have similar granules, they are very rare and do not appear to be released by exocytosis. In cattle, secretory granules often occur in conspicuous clusters.
Lysosomes	Lysosomes, as well as multivesicular bodies, are present in both cell types. Before the onset of luteolysis they appear to be more numerous in small luteal cells than in large cells.
Lipid droplets	Regardless of the stage of the cycle examined, small luteal cells contain many large lipid droplets. Ovine large cells have very few lipid droplets until the onset of luteolysis, at which time lipid droplets begin to accumulate. Bovine large luteal cells have lipid droplets at all stages examined.
Mitochondria	Rod-shaped crystalline inclusions of unknown composition are sometimes present in bovine mitochondria. Small matrix granules have been observed at all stages in both species. During the later stages of pregnancy, large electron-dense granules accumulate within the matrix of mitochondria in large luteal cells.
Golgi apparatus	Both cell types have elaborate and multiple Golgi networks. In large cells it is apparent that the Golgi apparatus is involved in the formation of secretory granules. This is the primary distinguishing feature with respect to the Golgi apparatus, since there are numerous vesicles (both smooth and coated) associated with the *cis* and *trans* elements in both cell types.

Descriptions based on personal observations and electron micrographs published by O'Shea (1979, 1987); Fields *et al.* (1985, 1992) and O'Shea *et al.* (1990).

inclusions' of cytoplasm (Fig. 4) probably reflect a plane of section through a finger-like projection of cytoplasm into a fold in the nuclear membrane. Consistent with this interpretation, O'Shea (1987) has provided evidence for direct continuity between profiles of cytoplasm observed within the nucleus and the cytoplasm proper.

Although small and large luteal cells in sheep and cattle have many features in common, it should be noted that bovine parenchymal cells have several unique features (Fields and Fields, 1985; O'Shea *et al.*, 1990; Fields *et al.*, 1992): (i) both cell types possess numerous lipid droplets during all stages of the
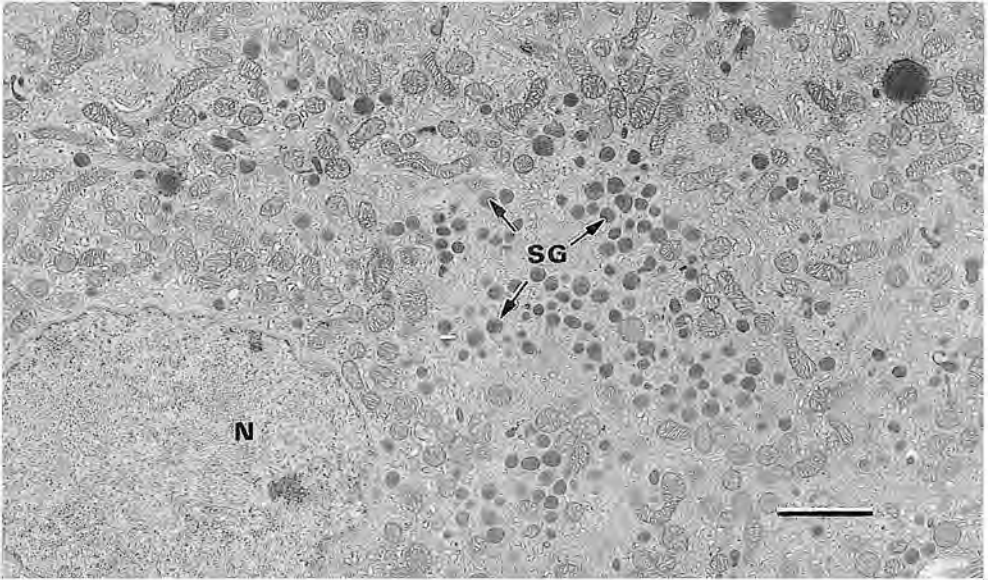
Fig. 3. Electron micrograph of a portion of a bovine large luteal cell on day 12 of the oestrous cycle. A unique feature of bovine large luteal cells is the arrangement of secretory granules (SG) into clusters. These clusters are often in close proximity to the nucleus (N). Scale bar represents 2 μm.
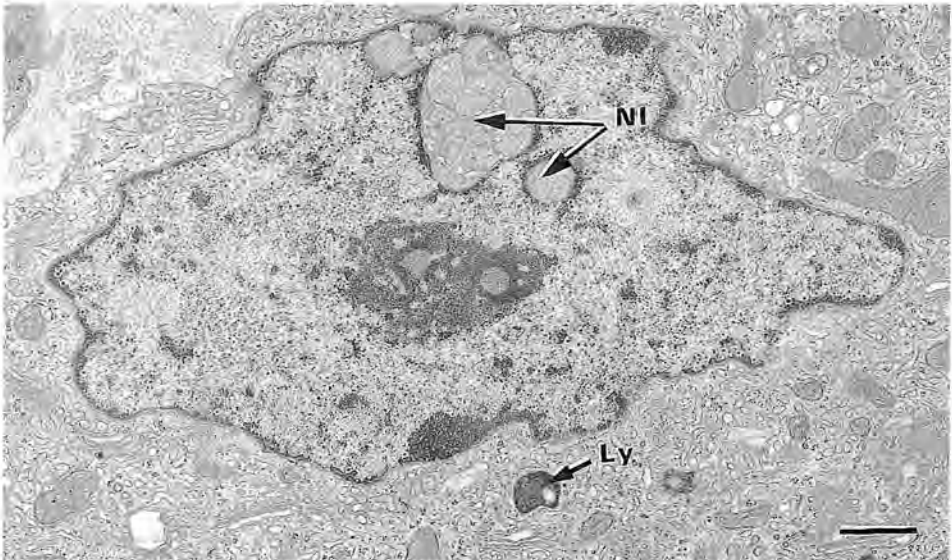

Fig. 4. A portion of an ovine small luteal cell showing inclusions (NI) of cytoplasm within the nucleus. The cytoplasm of small luteal cells contains lysosomal structures (Ly) but lacks secretory granules. Scale bar represents 1 μm.

reproductive cycle; (ii) elements of the smooth endoplasmic reticulum are occasionally arranged in whorls, sometimes enveloping lipid droplets; (iii) ring-shaped mitochondria are frequently observed; (iv) some mitochondria contain crystalline inclusions; and (v) secretory granules present in large luteal cells are sometimes arranged in discrete clusters.
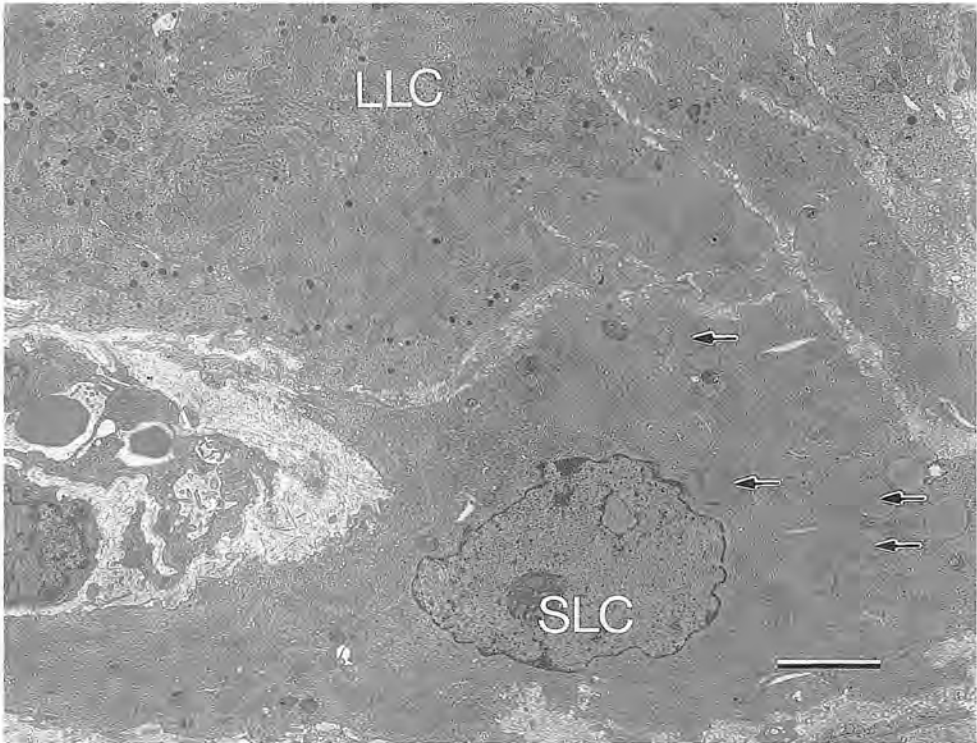
Fig. 5. Electron micrograph illustrating the general ultrastructural features of small (SLC) and large (LLC) luteal cells during the mid-luteal phase of the oestrous cycle. Large luteal cells contain numerous secretory granules and are usually devoid of lipid droplets. Conversely, small luteal cells lack secretory granules but contain numerous lipid droplets (arrows). Scale bar represents 4 μm.

Changes in the Corpus Luteum During Pregnancy

Structure of parenchymal cells

Most of the structural characteristics of small and large luteal cells during the mid-luteal phase of the oestrous cycle (Fig. 5) are maintained throughout pregnancy (O'Shea *et al.*, 1979; Weber *et al.*, 1987; O'Shea and McCoy, 1988). Although signs of involution (for example weight loss, small focal areas with degenerating cells) are detectable before parturition (O'Shea and McCoy, 1988), parenchymal cells in many areas appear healthy and exhibit no overt signs of degeneration several days after parturition (Fig. 6).

Cellular composition

Morphometric techniques have been used in several electron microscope studies to obtain quantitative data regarding the cellular composition of corpora lutea in sheep (Rodgers *et al.*, 1984; Farin *et al.*, 1986; O'Shea *et al.*, 1986) and cattle (O'Shea *et al.*, 1989) during selected stages of the oestrous cycle, but application of these techniques to evaluate the cellular composition during defined stages of pregnancy is limited to one study in sheep (O'Shea and McCoy, 1988). On the basis of results obtained by Farin *et al.* (1986), it is evident that after ovulation small luteal cells increase in number but not size, whereas large luteal cells increase in size but not number. Although volume densities and diameters of parenchymal cells remain relatively stable from day 12 to day 142 of pregnancy, there appears to be a
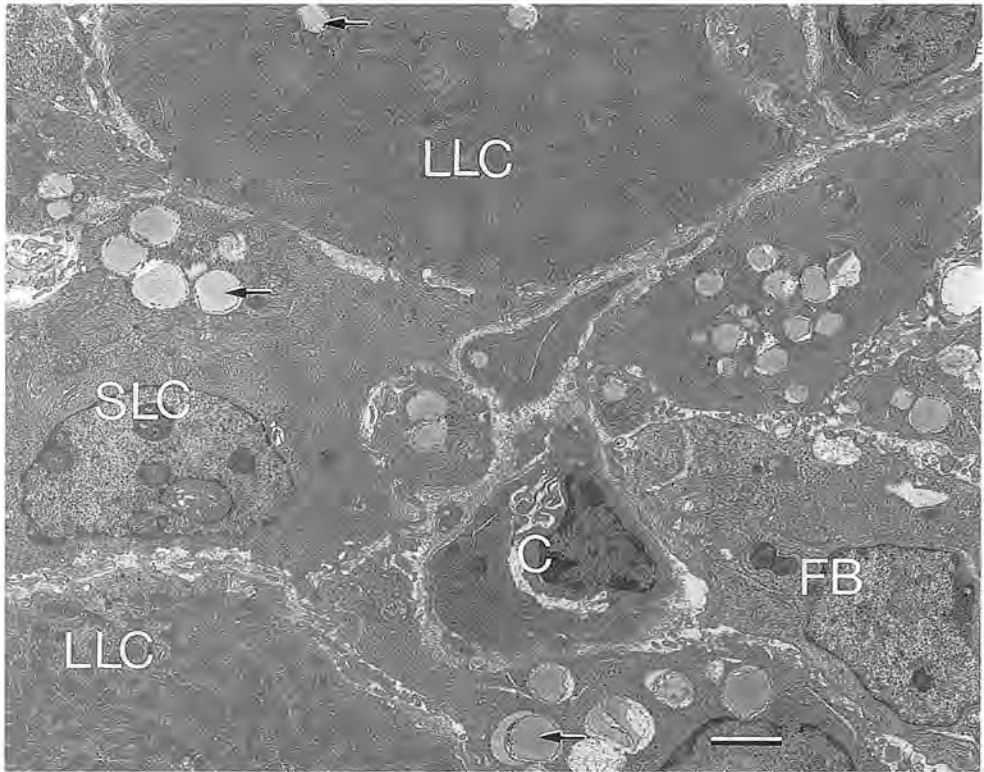
Fig. 6. Portion of an ovine corpus luteum 1 h after parturition. All of the normal cellular components present during the mid-luteal phase of the oestrous cycle are present (small luteal cell, SLC; large luteal cell, LLC; capillary, C; fibroblast, FB). Minor signs of involution include the accumulation of lipid droplets (arrows) and compact arrangement of mitochondria within the cytoplasm of large luteal cells. Scale bar represents 2 µm.

slight increase in the diameter of ovine large luteal cells after day 25 (Farin *et al.*, 1989) (Table 1). During the mid-luteal phase of the cycle, approximately 55% of the corpus luteum is occupied by steroidogenic cells, compared with approximately 61% (range 59–62%) from day 30 to day 142 of pregnancy. Thus, even though in sheep the corpus luteum is not required after day 50 of pregnancy, there is very little change in the parenchymal component and small and large luteal cells are maintained as distinct populations. Data regarding changes in the cellular composition of the bovine corpus luteum during pregnancy are not available, but on day 12 of the oestrous cycle the volume densities of small and large luteal cells were found to be 27.7 ± 6.3 and $40.2 \pm 7.0\%$, respectively (O'Shea *et al.*, 1989).

Hypotheses regarding the origin and fate of parenchymal cells that comprise the corpus luteum have been thoroughly reviewed by Niswender *et al.* (1985) and O'Shea (1987). There is general agreement that small and large luteal cells are initially derived from theca and granulosa cells, respectively; however, there is controversy regarding the suggestion that small luteal cells subsequently differentiate, or transform, into large luteal cells during the oestrous cycle and pregnancy. The strongest evidence in favour of this concept is that provided by Alila and Hansel (1984). Using monoclonal antibodies specific for theca and granulosa cells, respectively, they found that after ovulation the percentage of large luteal cells to which the granulosa-specific antibody bound progressively declined through day 100 of pregnancy. Conversely, the percentage of large luteal cells that bound the theca-specific antibody increased up to day 12 and remained unchanged through midpregnancy. On the basis of these findings, they concluded that as the corpus luteum matures, large luteal cells originally derived from granulosa

Table 1. Volume density, number and diameter (mean ± SEM) of ovine steroidogenic luteal cells during the oestrous cycle and pregnancy

Cell type	Day of cycle[a]		Day of pregnancy[b]			
	4	12	30	60	100	142
Small luteal cells						
Volume density (%)	15 ± 1	23 ± 2	18 ± 2	22 ± 3	22 ± 3	18 ± 7
Number ($\times 10^6$)	10 ± 3	46 ± 6	—	—	—	—
Diameter (μm)	17 ± 1	18 ± 1	17	18	19	18
Large luteal cells						
Volume density (%)	39 ± 4	33 ± 3	41 ± 5	40 ± 6	40 ± 4	41 ± 4
Number ($\times 10^6$)	12 ± 2	15 ± 3	—	—	—	—
Diameter (μm)	22 ± 1	31 ± 1	37	37	38	36

[a]Data summarized from Farin *et al.* (1986). [b]Data summarized from O'Shea and McCoy (1988). Diameters were calculated from data reported for cell volume.

cells are replaced by small luteal cells that differentiate into large luteal cells. Additional evidence to support this concept comes from a study performed by Farin *et al.* (1988) in which the numbers and volume densities of small and large luteal cells in normally cyclic ewes were compared with those for normally cyclic ewes that had been treated with LH. Compared with untreated ewes, the number and volume density of large luteal cells in LH-treated ewes increased and was paralleled by a concomitant decrease in the number and volume density of small luteal cells. These observations strongly suggest that small luteal cells do in fact have the potential to develop into large luteal cells. In addition, there is also evidence suggesting that there is a unique population of cells (Type 5 cells) within bovine corpora lutea that may play a role in renewal of luteal cells (Spanel-Borowski *et al.*, 1994). However, since the number of large luteal cells present at all stages during the lifespan of the corpus luteum can be accounted for on the basis of cells present at the onset of luteogenesis (O'Shea *et al.*, 1987; O'Shea and McCoy, 1988), and in the absence of corroborative evidence for the presence of degenerating large luteal cells (i.e. those to be replaced), the concept that small luteal cells transform into large luteal cells remains controversial.

Functional properties

Progesterone secretion in vitro.　Development and application of methods to dissociate corpora lutea into single cell suspensions and obtain relatively pure populations of small and large luteal cells by unit gravity sedimentation (Ursely and Leymarie, 1979; Koos and Hansel, 1981) and centrifugal elutriation (Fitz *et al.*, 1982; Chegini *et al.*, 1984) provided a means to evaluate individual functional properties of the two parenchymal cell types. Initially, efforts focused on the respective abilities of isolated small and large luteal cells to secrete progesterone in the presence or absence of LH and other secretagogues. Two consistent findings using parenchymal cells isolated from corpora lutea of ruminants during the oestrous cycle are (i) basal secretion of progesterone by large luteal cells is several times more than basal secretion by small luteal cells; and (ii) when small luteal cells are incubated in the presence of LH, secretion of progesterone increases by 4–20 times, whereas LH does not alter secretion of progesterone by large luteal cells (Ursely and Leymarie, 1979; Koos and Hansel, 1981; Fitz *et al.*, 1982; Rodgers *et al.*, 1983a; Weber *et al.*, 1987). Direct comparisons between parenchymal cells isolated during the oestrous cycle versus pregnancy are limited, but it appears that during late pregnancy basal production of progesterone by bovine parenchymal cells is less than that for cells obtained during the oestrous cycle (Weber *et al.*, 1987). In addition, secretion of progesterone by small luteal cells isolated from corpora lutea obtained

during late pregnancy is not substantially enhanced in response to LH as is typical for small cells obtained during the mid-luteal phase of the oestrous cycle (Koos and Hansel, 1981). Basal secretion of progesterone by small and large luteal cells of sheep on day 25 and days 40–50 of pregnancy was not different from that of cells on day 10 of the oestrous cycle; however, the ability of small luteal cells to respond to LH had diminished (Harrison *et al.*, 1987; Farin *et al.*, 1989). The reason for this apparent reduction in sensitivity of small luteal cells to LH during pregnancy does not appear to be due to a reduction in the number of receptors for LH. These data are consistent with the reported reduction in quantities of cytochrome $P450_{scc}$ and adrenodoxin in corpora lutea during late pregnancy (Rodgers *et al.*, 1988) and provide evidence that as pregnancy progresses there is a reduction in steroidogenic capabilities.

Secretory granules, oxytocin and relaxin. In ruminants, it has been conclusively demonstrated that the corpus luteum produces and secretes oxytocin during the oestrous cycle (Wathes *et al.*, 1983) and that oxytocin plays an important role in initiating luteolysis by stimulating the release of $PGF_{2\alpha}$ from the uterus (Schams, 1989). On the basis of results from immunohistochemical (Guldenaar *et al.*, 1984; Sawyer *et al.*, 1986) and *in vitro* (Rodgers *et al.*, 1983b; Harrison *et al.*, 1987) studies, it is also apparent that production and secretion of oxytocin by the corpus luteum is restricted to large luteal cells. Similar to the synthesis, storage and release of oxytocin by hypothalamic neurones, in large luteal cells oxytocin is also synthesized as a prohormone (Ivell and Richter, 1984), packaged and stored in secretory granules and released at the cell surface via exocytosis (Theodosis *et al.*, 1986; Fields *et al.*, 1992) (Figs 2, 3).

Changes in populations of secretory granules in large luteal cells during the oestrous cycle and pregnancy of cattle have been examined (Fields *et al.*, 1985, 1992). Not only are there stage-specific changes in the number of secretory granules, but there are also changes with respect to content. During the oestrous cycle, the number of oxytocin-containing secretory granules is greatest at approximately day 11. Thereafter, granules containing oxytocin are progressively depleted concomitant with involutive changes beginning at about day 14 after oestrus. During the early stages of pregnancy, granules containing oxytocin can be detected until day 25, at which time they are depleted. From about day 45, a second population of granules emerges. This population gradually increases and maximum numbers are detected between day 180 and day 210 of pregnancy. Although the content(s) and function of granules in this second population are not known, they do not contain oxytocin (Fields *et al.*, 1992). Similar granules in large luteal cells of pigs contain relaxin (Kendall *et al.*, 1978; Fields and Fields, 1985) and relaxin immunoreactivity has been detected in bovine large luteal cells during late pregnancy (Fields *et al.*, 1980). However, there are no studies that demonstrate the presence of relaxin in secretory granules in sheep or cattle, and efforts to detect a specific mRNA encoding relaxin in these ruminants have not been successful (Roche *et al.*, 1993; Roche *et al.*, 1994). On the basis of the reported effects of administration of porcine relaxin on induction of parturition in heifers (Musah *et al.*, 1988), as well as effects on progesterone production by dispersed bovine luteal cells obtained at different stages of pregnancy (Musah *et al.*, 1990), it appears that uterine and luteal receptors that can recognize relaxin are present. None the less, definitive evidence for a role of the corpus luteum in the production and secretion of relaxin in ruminants is not available.

Luteolysis

Luteolysis is a complex process by which the corpus luteum degenerates and is transformed into a nonfunctional corpus albicans. There are two well defined periods when luteolysis occurs. One occurs at the end of the oestrous cycle. Structural and functional changes at this time have been characterized and it has been demonstrated that $PGF_{2\alpha}$ is the initial luteolytic signal. The other period is at the end of gestation. Although the exact signal(s) for initiating luteolysis at this time is not known, changes in the structure and function of the corpus luteum are similar to those observed at the end of the oestrous cycle, except that the overall degenerative process proceeds at a much slower rate.
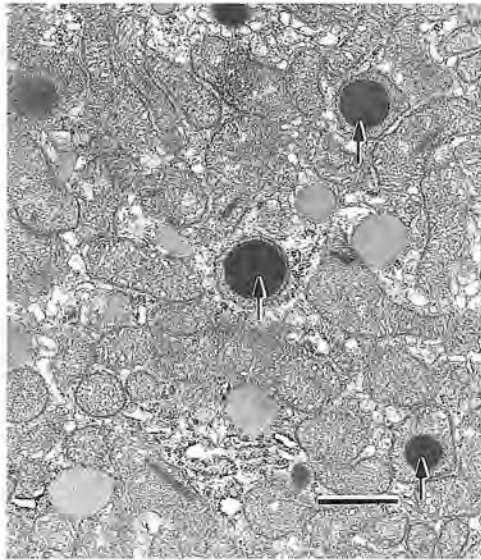
Fig. 7. Electron micrograph of a portion of an ovine large luteal cell on day 140 of pregnancy showing the presence of electron-dense inclusions (arrows) within the matrix of mitochondria. The appearance of matrix inclusions and compact arrangement of mitochondria serve as early signs that degenerative changes have been initiated. Scale bar represents 1 μm.

Luteal regression during the oestrous cycle versus late pregnancy and post partum

Oestrous cycle. Since the early 1970s substantial evidence has accrued to indicate that the primary luteolysin in domestic ruminants is $PGF_{2\alpha}$ (Knickerbocker *et al.*, 1988). Although the precise cellular mechanisms and sequelae that result in the demise of the corpus luteum are not completely understood, it is evident that during nonfertile cycles $PGF_{2\alpha}$ from the uterus reaches the ovary via a veno–arterial transfer (Mapletoft and Ginther, 1975). In response to $PGF_{2\alpha}$ there is a reduction in blood flow to the corpus luteum (Nett *et al.*, 1976), an increase in autophagosomes within luteal cells (McClellan *et al.*, 1977), an infiltration of eosinophils and macrophages (Murdoch, 1987), a reduction in mRNA for 3β-hydroxysteroid dehydrogenase (Couet *et al.*, 1990; Hawkins *et al.*, 1993); degeneration of capillary endothelial cells and obstruction of capillary lumina (O'Shea *et al.*, 1977) and general changes in the cytoplasm and nuclei of luteal cells that are consistent with the process of apoptosis (O'Shea *et al.*, 1977; Sawyer *et al.*, 1990). Thus, the process of luteolysis is complex and involves numerous changes, both structural and functional, all of which can contribute to the rapid decline in the production and secretion of progesterone.

Pregnancy and post partum. Luteal regression begins during late pregnancy as evidenced by a gradual decline in luteal mass and the presence of small numbers of degenerating cells in focal areas (O'Shea *et al.*, 1979; O'Shea and McCoy, 1988; Weber *et al.*, 1987). Quantitative data are not available, but there also appears to be a gradual increase in the number of mitochondria in parenchymal cells that contain large electron dense inclusions (Fig. 7). The composition of these mitochondrial granules is not known; however, since cytochrome P450 and adrenodoxin are substantially lower in corpora lutea during late pregnancy than during the mid-luteal phase of the oestrous cycle (Rodgers *et al.*, 1988), it is possible that they simply reflect accumulation of cholesterol within individual mitochondria in which there has been a reduction in, or a total loss of, steroidogenic capabilities.
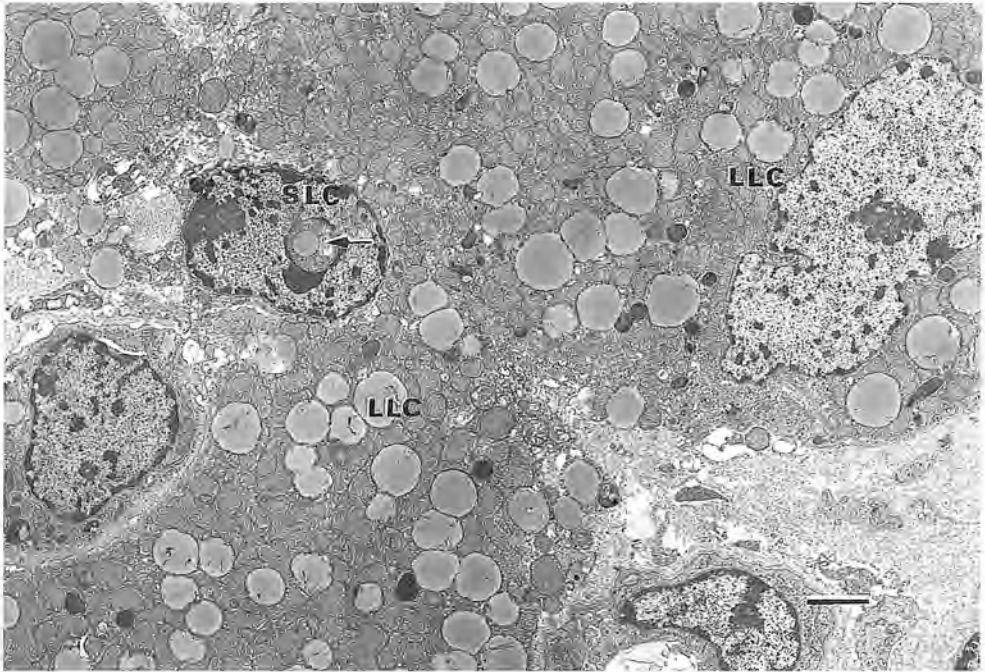
Fig. 8. Electron micrograph of a corpus luteum obtained 2 days after parturition showing what appears to be the fusion of two large luteal cells (LLC) and one small luteal cell (SLC). Note the inclusion of cytoplasm (arrow) within the nucleus of the small luteal cell. Scale bar represents 2 μm.

In general, all structural changes observed during luteolysis at the end of the oestrous cycle also occur following parturition, including deletion of capillary endothelial cells and occlusion of capillary lumina with cellular debris and apoptotic bodies, an infiltration of eosinophils and macrophages, fragmentation and lysis of parenchymal cells, vesiculation of the smooth endoplasmic reticulum, appearance of electron dense granules within the matrix of many mitochondria and depletion of secretory granules (O'Shea and Wright, 1985). However, there are at least two notable differences. First, the overall demise of the corpus luteum during late pregnancy and following parturition is much slower. Second, the formation of what appear to be syncytia (Fig. 1e), which often contain five or more nuclei and are up to 300 μm in length. All of the syncytial profiles contain numerous lipid droplets, tightly packed mitochondria, multiple nuclei and appear to form as a result of fusion of parenchymal cells. Other organelles and derivatives of the endomembrane system are usually absent. Fusion of luteal cells to form these conspicuous syncytial profiles begins approximately 2–3 days after parturition (Fig. 8) and appears to continue for at least 22 days (Fig. 1e). By day 35 remnants of luteal cells are still detectable but most of the remaining tissue consists of fibrillar connective tissue components and blood vessels with thickened walls (Fig. 1f).

Because of the extended time required for the corpus luteum of pregnancy to undergo complete regression following parturition, the potential functions of the corpus luteum of pregnancy on subsequent reproductive function have been examined. Although removal of the corpus luteum before parturition does not shorten the interval to first oestrus after parturition (Campbell *et al.*, 1994; Sawyer, unpublished), there is evidence that the corpus luteum of pregnancy contains a nonsteroidal aromatase inhibitor (Al-Gubory *et al.*, 1994), can reduce follicular development and ovulation in response to hCG (Hall *et al.*, 1993), and has an inhibitory effect on the pulsatile release of LH during the postpartum period (Al-Gubory *et al.*, 1989). However, since there is no evidence that lysis or removal of the corpus luteum before parturition improves reproductive performance, the conclusion drawn by O'Shea and Wright (1985) "... it seems unlikely that the CL of pregnancy serves any important function in the postpartum period" remains valid.

Conclusion

Structural and functional properties of the corpus luteum of pregnancy are similar to those of the corpus luteum of the oestrous cycle. Small and large luteal cells persist as distinct populations throughout pregnancy and collectively occupy approximately 60% of the corpus luteum. Although there is a reduction in steroidogenic capabilities as pregnancy advances, the ability of small and large luteal cells to synthesize and secrete progesterone is maintained. There is no doubt that the primary function of the corpus luteum of pregnancy is to produce progesterone; however, the potential roles of other luteal factors in modulating endocrine and intraovarian functions during pregnancy and post partum warrant attention.

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