Heterogeneous cell types in the corpus luteum of sheep, goats and cattle

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Summary. Data on the structure, quantitation, origins and functions of the large luteal (LL) and small luteal (SL) cells of sheep, goats and cattle are reviewed. Both LL and SL cells show ultrastructural features consistent with a steroidogenic function. However, in addition to differences in size and shape, LL cells differ from SL cells primarily in possessing large numbers of secretory granules, suggesting an additional protein/ polypeptide synthetic and secretory function. In sheep, morphometric estimates show that the corpus luteum (CL) contains $\simeq 10 \times 10^6$ LL cells and $\simeq 50$–60×10^6 SL cells: individual LL cells are $\simeq \times 6$ greater in volume than SL cells. During formation of the CL, granulosa and theca cells are incorporated, and evidence suggests that granulosa cells give rise to LL cells and theca cells to SL cells. However, SL cells, or cells of thecal origin, may also give rise to some LL cells. Both LL and SL cells produce progesterone *in vitro*. On a per cell basis, LL cells produce more progesterone than do SL cells, but SL cells show a much greater progesterone-secretory response to LH. Oxytocin is synthesized, and secreted in granule form, only by the LL cells, and relaxin, whose presence has been demonstrated convincingly only in cattle, also appears to be produced only by LL cells. The two types of luteal cell in ruminants therefore show major differences in function: the occurrence of any significant functional interaction remains to be established.

Introduction

The corpus luteum (CL) in ruminants, as in other mammals, contains specific hormone-producing (luteal) cells and other cells of several types. The latter include endothelial cells and pericytes, smooth muscle cells, fibrocytes, macrophages, leucocytes and occasional plasma cells (O'Shea *et al.* 1979; Rodgers *et al.*, 1984). Although there is some evidence that macrophages may serve a specific stimulatory function in relation to progesterone synthesis (Kirsch *et al.*, 1983), the non-luteal cell populations are presumed to be primarily concerned with their conventional ancillary roles.

In several groups of mammals, including perissodactyls (e.g. horse), cetaceans (e.g. whales, dolphins) and artiodactyls (e.g. pig, ruminants), cellular heterogeneity is also seen within the population of steroidogenic luteal cells. In all of these groups, two distinct populations of luteal cells have been recognized histologically (Mossman & Duke, 1973), and variously termed large and small luteal cells or granulosa and theca luteal (or lutein) cells on account of their putative origins. This review will deal only with heterogeneity within the luteal cell population, and consider data relevant to qualitative and quantitative aspects of structure, to the origins, and to the functions of the large and small luteal cells of domestic ruminants. Reasons why it may be advantageous for the CL to possess two types of luteal cell, in terms of distinctive functions and possible functional interactions, are also considered.

Structural features

Sheep

Large luteal cells. The large luteal (LL) cells of cyclic ewes attain their mature size and structure (Figs 1–3) about 6 or 7 days after ovulation, and show little change thereafter until the onset of structural luteal regression. They are large, polyhedral cells with a single, round or oval, pale vesicular nucleus situated close to the centre of the cell and containing one or more prominent nucleoli. Nuclear:cytoplasmic ratio, at $\simeq 1{:}27$, is very low (Rodgers *et al., 1984*).

Details of the ultrastructure of these cells have been reported by many workers, including Deane *et al.* (1966), Gemmell *et al.* (1974), O'Shea *et al.* (1979) and Paavola & Christensen (1981), and the description below draws heavily on these reports. The cytoplasm of the LL cells shows specializations consistent with both a steroidogenic and a protein synthetic and secretory function. Features characteristic of steroidogenic cells include large quantities of smooth endoplasmic reticulum and high numbers of mitochondria whose cristae are frequently tubular in form. Lipid droplets are not abundant until the onset of luteal regression. Smooth endoplasmic reticulum occurs in these cells in the form of anastomosing networks of branching tubules, and does not form whorl-like arrays. As in many other cells which secrete proteins or polypeptides, LL cells contain multiple parallel arrays of flattened cisternae of rough endoplasmic reticulum, prominent Golgi complexes and numerous membrane-bound granules ('secretory granules'), $\simeq 0{\cdot}15{-}0{\cdot}3\,\mu m$ in diameter, whose electron-dense contents can frequently be seen to be released by exocytosis (Corteel, 1973; Gemmell *et al.,* 1974). Not all granules in the LL cells are related to protein secretion, as lysosomes, multivesicular bodies and peroxisomes are also present (Paavola & Christensen, 1981).

The surfaces of these cells, which are covered by a moderately well-defined basal lamina except at sites of close contact with neighbouring cells, are extensively folded to form microvillous or flattened projections which usually extend parallel to the cell surface. These projections commonly interdigitate with others from the same or neighbouring cells, including both large and small luteal cells. At sites of interdigitation the membranes of adjacent processes are frequently separated by a rather constant space of $\simeq 20\,nm$, and resemble 'septate-like' junctions (Friend & Gilula, 1972). Other forms of specialized junction are rarely seen, and although occasional gap junctions ('abutment nexuses') have been observed (O'Shea *et al.,* 1979), Higuchi *et al.* (1976) were unable to demonstrate electrical coupling between luteal cells of sheep.

Characteristic LL cells persist throughout pregnancy (Gemmell *et al.,* 1977; O'Shea *et al.,* 1979) and into the early post-partum period (O'Shea & Wright, 1985), but show progressive structural modifications. As pregnancy advances these cells become more rounded, and the extent of surface contacts is reduced. Cytoplasmic lipid droplets accumulate in large numbers, and many mitochondria acquire large, dense, rounded matrix granules which may be $\geqslant 1\,\mu m$ in diameter. Secretory granules become reduced in number, with less evidence of exocytosis, and both smooth and rough endoplasmic reticulum become less prominent.

Small luteal cells. These cells (SL; Figs 4–7), described in detail by O'Shea *et al.* (1979), are smaller, more angular and often elongated or spindle-shaped. Their nuclei are less regular in outline

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Fig. 1. Large luteal cell from a cyclic sheep, showing abundant mitochondria and many dense granules (arrows). $\times 7850$ (bar $= 1\,\mu m$).

Fig. 2. Detail from Fig. 1, showing mitochondria with tubular cristae, smooth endoplasmic reticulum, and the release of secretory granules (arrow). $\times 23\,200$ (bar $= 0{\cdot}5\,\mu m$).

Fig. 3. Detail from Fig. 1. Membrane-bound secretory granules, showing the dense core and lighter periphery (arrow) which characterize many of these granules. $\times 23\,200$ (bar $= 0{\cdot}5\,\mu m$).
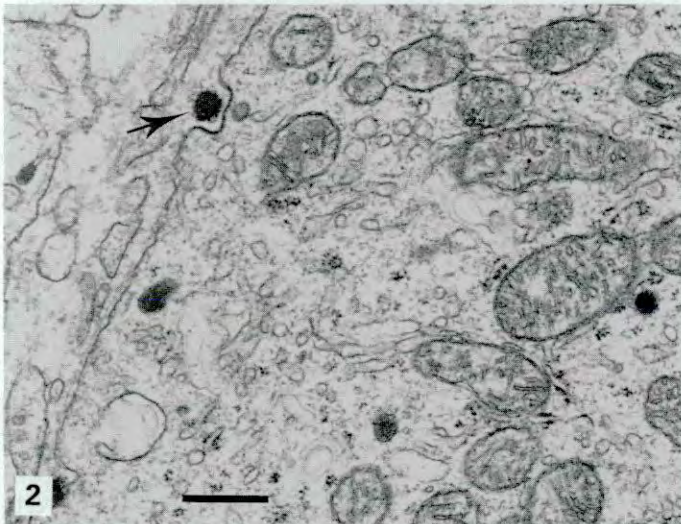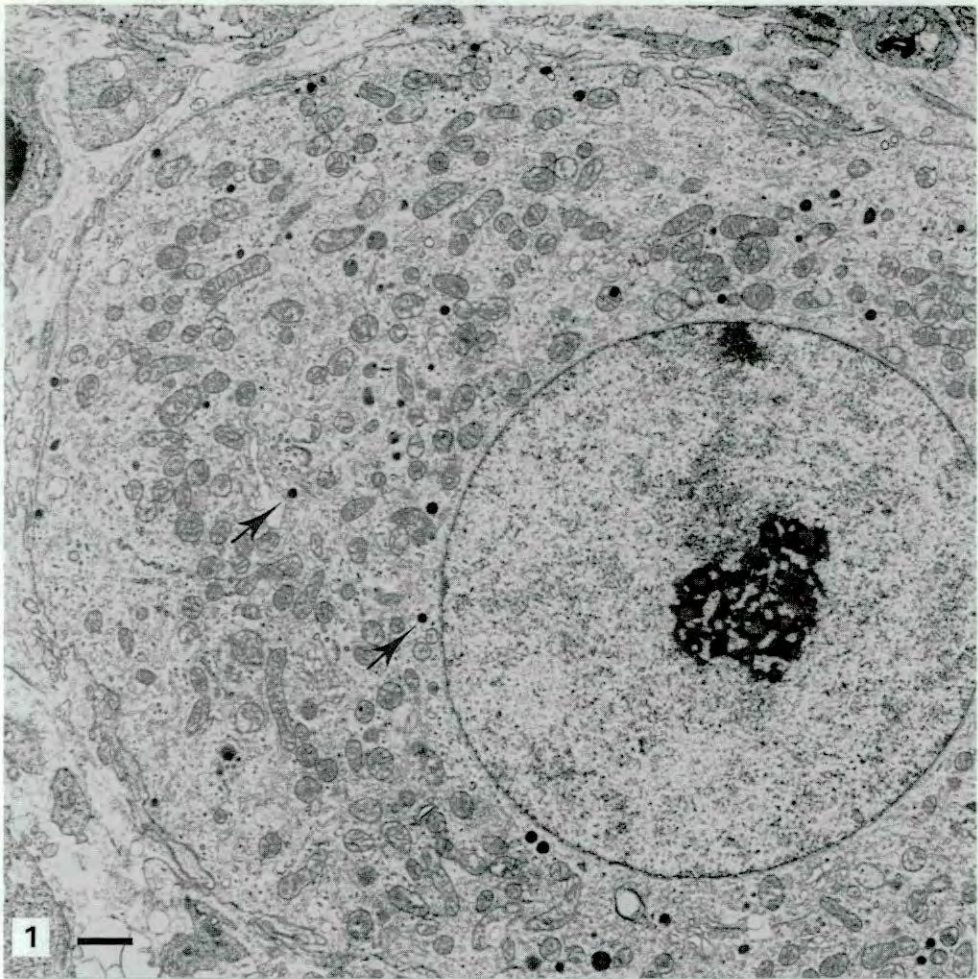
and more densely staining due to a coarser pattern of heterochromatin. One or more small nucleoli are commonly seen. Complex infoldings of the nuclear envelope are frequently present, and large nuclear inclusions containing modified cytoplasmic organelles, often apparently wholly detached from the remaining cell cytoplasm, are a striking feature of a small proportion of these cells. Nuclear:cytoplasmic ratio, at $\simeq 1{:}8$, is higher than in the LL cells (Rodgers *et al.*, 1984).

The cytoplasm shows features consistent with a steroidogenic function, including large amounts of tubular endoplasmic reticulum on which small groups of ribosomes are sparsely distributed, many mitochondria which contain both tubular and lamellar cristae, and scattered lipid droplets. Mitochondria are, however, less densely packed, and more irregular in size, than in the large luteal cells. Free ribosomes, and one or more small Golgi complexes, are also present, but these cells lack focal aggregates of rough endoplasmic reticulum and secretory granules. Small numbers of dense, membrane-bound granules, possibly lysosomes, are present, but there is no evidence of exocytosis.

Surface folding and basal lamina formation are less marked than in LL cells, and surface projections are more often microvillous in character. Changes associated with the advancement of pregnancy are also less marked, some of these cells appearing essentially unchanged even into the immediate post-partum period (O'Shea & Wright, 1985).

Goat

The limited ultrastructural data available for this species indicate a close similarity between sheep and goats in LL and SL cell structure (Gemmell *et al.*, 1977; Azmi & Bongso, 1985). The only significant difference so far reported is in relation to secretory granules in LL cells during the last part of pregnancy. In goats, granules remain abundant at least until Day 140, with continuing release by exocytosis (Gemmell *et al.*, 1977).

Cow

It is clear from the ultrastructural studies of Priedkalns & Weber (1968) and Fields *et al.* (1985), of cyclic and pregnant cows respectively, that LL and SL cells broadly comparable to those in sheep occur also in the cow. However, some apparent contradictions are found between the reports of different workers studying the bovine CL, which suggest that clearcut distinction between LL and SL cells is less easy in cattle than in sheep or goats. In particular, Singh (1975) described LL ("granulosa lutein") cells in the pregnant cow but incorporated several ultrastructural features (notably whorls of smooth endoplasmic reticulum and stacks of rough endoplasmic reticulum) which, based on the subsequent report of Fields *et al.* (1985), may in fact have been present in adjoining SL cells. Parry *et al.* (1980), describing LL cells in cyclic cows, were unable to distinguish

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Fig. 4. Small luteal cell from a cyclic sheep, showing irregularity of nuclear outline and absence of secretory granules. A capillary (C) is seen at top, and part of a macrophage (M) at upper left. × 7850 (bar = 1 μm).

Fig. 5. Detail from Fig. 4, showing cytoplasm rich in smooth endoplasmic reticulum but containing relatively few mitochondria. × 23 200 (bar = 0·5 μm).

Fig. 6. Nucleus of a small luteal cell showing an inclusion containing cytoplasmic material, surrounded by an inverted nuclear envelope. × 23 200 (bar = 0·5 μm).

Fig. 7. Detail from Fig. 6, showing a narrow neck of continuity (arrow) between the cell cytoplasm and the contents of the inclusion. × 46 400 (bar = 0·2 μm).
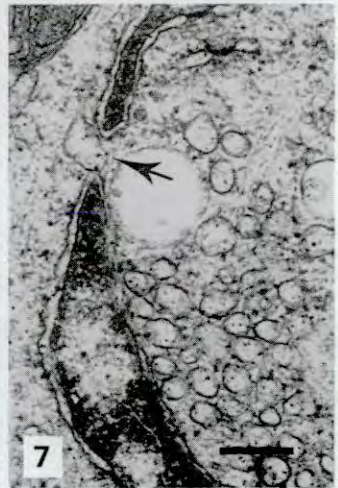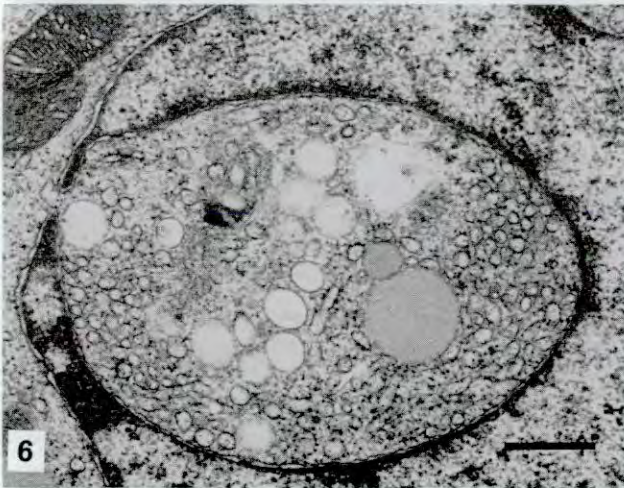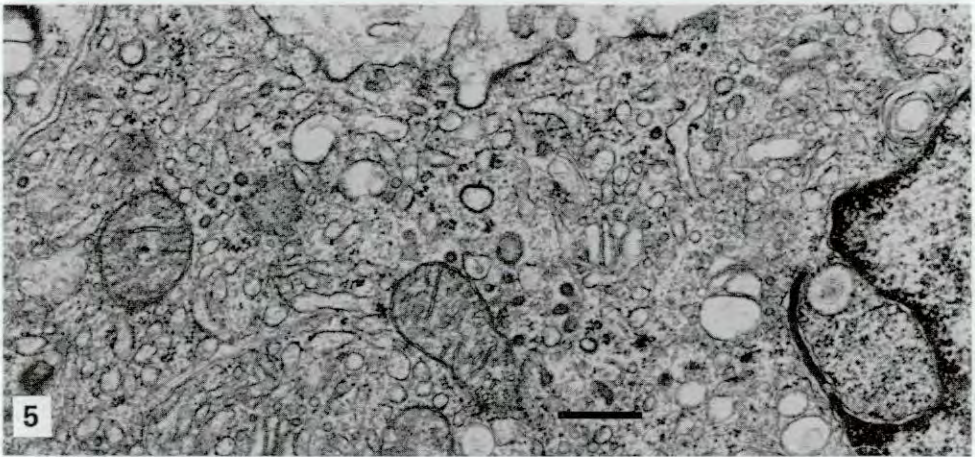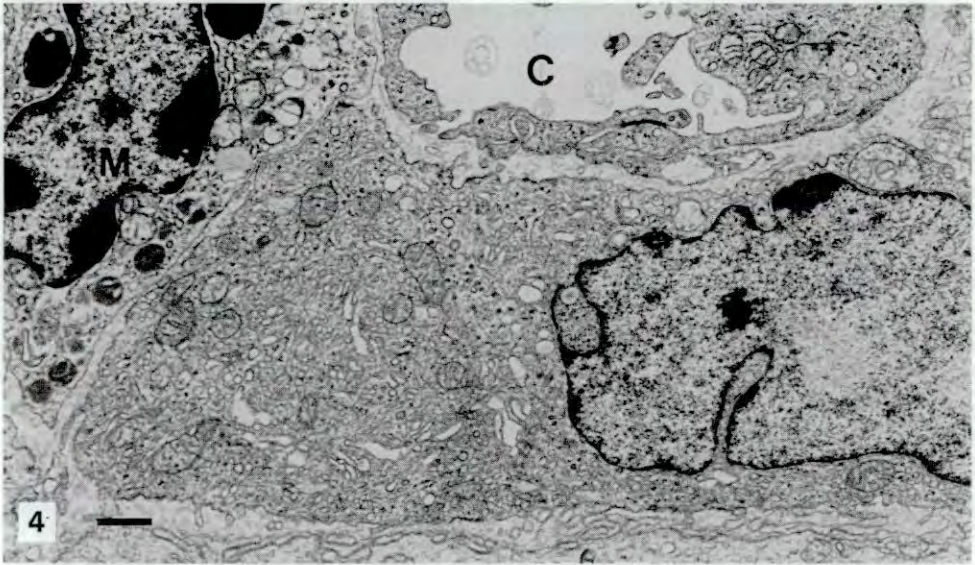
between these and SL cells, and regarded the two types as "part of the same population". Contrasts with the sheep, as outlined below, are therefore based mainly on the recent report of Fields *et al.* (1985).

The LL cells of the cow appear to differ from those of sheep in that they lack parallel stacks of cisternae of rough endoplasmic reticulum, and in that their secretory granules commonly aggregate in the central regions of the cell and subsequently migrate *en masse* towards the cell periphery where they are released by exocytosis. Secretory granules reach their maximum numbers per cell early in the last third of pregnancy, and then decline towards term (Fields *et al.*, 1985). The SL cells, on the other hand, possess stacks of cisternae of rough endoplasmic reticulum, and also whorl-like arrays of smooth endoplasmic reticulum, neither of which are characteristic of sheep SL cells. Crystalline-like inclusions in mitochondria have also been observed in SL cells of cows (Fields *et al.*, 1985), and lipid droplets are particularly abundant during both the oestrous cycle and pregnancy (Priedkalns & Weber, 1968; Fields *et al.*, 1985). Although small numbers of 'secretory granules' have been described in bovine SL cells by Fields *et al.* (1985), no evidence of their release by exocytosis has been presented.

Quantitative aspects of structure

Volume density

Estimates of the percentage composition (volume density) of mature, functional CL from naturally cyclic ewes, based on standard point- (hit-) counting methods, have been made using both light (Niswender *et al.*, 1976) and electron (Rodgers *et al.*, 1984; O'Shea *et al.*, 1986) microscopy. Findings in relation to LL and SL cells are summarized in Table 1, the remaining proportions of the tissue being composed of cells of other types, connective tissue fibres and intercellular spaces, and vascular lumina. The combined populations of LL and SL cells thus constitute up to $\simeq 50\%$ of the total luteal tissue, with LL cells occupying the greater volume. A higher ultrastructural estimate ($> 70\%$) of the volume density of combined luteal cells in cyclic CL of cows was obtained by Parry *et al.* (1980).

Cell size

Three types of estimate of the sizes of LL and SL cells in the sheep are available. These are based on measurement of diameters of cells in tissue sections and in enzymically-dissociated cell populations, and on tissue morphometry. The first method is clearly of limited value, being based on random two-dimensional images of cells whose shape is irregular, and will not be considered further.

Since dissociated luteal cells in suspension adopt a more-or-less spherical form, measured diameters of free-floating cells should be meaningful. Data derived using this approach, coupled in

Table 1. Volume density (% $\pm$ s.e.m.) of the corpus luteum of the cyclic ewe occupied by large and small luteal cells

Source of data	Day of cycle	Large luteal (granulosa lutein) cells	Small luteal (theca lutein) cells	Total
Niswender *et al.* (1976)	10	$33\cdot8 \pm 2\cdot5$	$16\cdot5 \pm 1\cdot6$	$50\cdot3$
Rodgers *et al.* (1984)	Not known	$25\cdot4 \pm 2\cdot7$	$17\cdot5 \pm 0\cdot6$	$42\cdot9$
O'Shea *et al.* (1986)	9	$28\cdot3 \pm 2\cdot1$	$22\cdot2 \pm 1\cdot4$	$50\cdot5$

Table 2. Measured diameters of large and small luteal cells after enzymic dissociation

Source of data	Species	Reproductive status	Large luteal cells (μm)	Small luteal cells (μm)
Rodgers & O'Shea (1982)	Sheep	Cycle, days unknown	>19	13·5–19
Fitz *et al.* (1982)	Sheep	Cycle, Days 8–12, after superovulation	23–25	12–22
Ursely & Leymarie (1979)	Cow	Pregnant, days 60–100	30–50 (mean = 37)	15–20 (mean = 18)
Koos & Hansel (1981)	Cow	Cycle, Days 11–13	>25	10–20
Chegini *et al.* (1984)	Cow	Pregnant, days unknown	18–45	15–18

Table 3. Cell volume (mean ± s.e.m.) and derived* cell diameters of large and small luteal cells from cyclic ewes

Source of data	Day of cycle	Cell volume ($\mu m^3 \times 10^{-3}$)		Cell diameter (μm)	
		Large luteal cells	Small luteal cells	Large luteal cells	Small luteal cells
Rodgers *et al.* (1984)	Not known	13·1 ± 2·1	2·1 ± 0·2	29·2	15·8
O'Shea *et al.* (1986)	9	15·6 ± 1·3	2·1 ± 0·3	31·0	16·0

*Diameters derived from measured cell volume (volume = $4/3\pi r^3$) assuming cells were to take on a spherical form.

some cases with ultrastructural (Koos & Hansel, 1981; Rodgers & O'Shea, 1982) or enzyme histochemical (Fitz *et al.*, 1982) methods to aid identification of the cell populations in question, are summarized in Table 2. This method, as a means of estimating the sizes of LL and SL cells in the original tissue does, however, have some disadvantages. The actual cells measured cannot be identified as to type by any objective criteria other than size, and there is no certainty that the populations obtained by tissue dissociation are accurately representative of those in the original tissue. Nor, in view of the somewhat arbitrary size limits used to categorize cells as LL or SL types, is it possible to be certain that the populations defined as LL and SL cells by different workers are necessarily identical.

The third type of estimate, at present available only for cyclic sheep, is based on the morphometric measurement of mean cell volume for each type of luteal cell in whole luteal tissue. Volume thus measured can also be converted to cell diameter if it be assumed that the cells were to take on a spherical form (Table 3). These estimates indicate that individual LL cells are at least 6 times the volume of SL cells, an important factor when comparing the functional capacity of the two cell types on a per cell basis.

Cell numbers

Estimates of the total number of cells of all types per CL in the sheep have been obtained using three independent methodologies. In the first, based on counts of free cells in suspension following enzymic dissociation of functional luteal tissue from cyclic ewes, totals of $\simeq 40 \times 10^6$ (Rodgers & O'Shea, 1982) and $\simeq 60 \times 10^6$ (Niswender *et al.*, 1985) have been reported. This approach has the disadvantage that some cells are almost certainly lost during dissociation, and the extent of this loss cannot be quantitated. Hence some degree of underestimate appears inevitable.

The second method, based on measurement of cell and tissue DNA, gives a much higher estimate of 238×10^6 cells (Rodgers *et al.*, 1984). This estimate is, however, reasonably close to estimates obtained by the third method, ultrastructural morphometry, giving values of 258×10^6 (Rodgers *et al.*, 1984) and 201×10^6 (O'Shea *et al.*, 1986). The relatively close correspondence between the findings from these last two methods, in which questions of non-measurable losses do not arise, suggests that the cyclic CL of the sheep probably contains $\geqslant 200 \times 10^6$ cells, indicating the loss of very significant numbers of cells during tissue dissociation.

Morphometric estimates further place the mean numbers of LL and SL cells per CL at $10 \cdot 7 \times 10^6$ and $49 \cdot 2 \times 10^6$ (Rodgers *et al.*, 1984) and $9 \cdot 6 \times 10^6$ and $57 \cdot 5 \times 10^6$ (O'Shea *et al.*, 1986), respectively. Thus the combined total of both types of luteal cells, at $60-70 \times 10^6$, represents a minority of the total cells per CL, and the data indicate a ratio of 5 or 6 SL cells for each LL cell.

Origins of luteal cells

There is now clear evidence that both the granulosa and theca layers contribute cells to the developing CL in ruminants. Many studies in sheep (McClellan *et al.*, 1975; O'Shea *et al.*, 1980) and cattle (Priedkalns *et al.*, 1968; Alila & Hansel, 1984) provide support for this conclusion. However, the precise nature of the contributions of granulosa and theca cells to developing and established CL remains more controversial.

Genesis of the CL

Fate of the granulosa cells. All of the studies cited above are consistent in reaching the conclusion that following ovulation the granulosa cells differentiate into LL cells. This conclusion is based on sequential observations of light and electron microscopic structure, together with the use of 'markers' in the form of glycogen bodies and monoclonal antibodies. Glycogen bodies (Le Beux, 1969), involving whorled arrangements of smooth membranes with or without the presence of associated glycogen particles, are a characteristic feature of granulosa cells in sheep preovulatory follicles (Corteel, 1973) and persist for 24–48 h after ovulation. Since these structures are not seen in thecal cells they form a convenient ultrastructural marker of granulosa-derived cells in the early postovulatory period (O'Shea *et al.*, 1980). Monoclonal antibodies raised against granulosa cell surface antigens have been used as markers of granulosa-derived cells in the cow CL (Alila & Hansel, 1984). Finally, numbers of granulosa cells in preovulatory sheep follicles (O'Shea *et al.*, 1985) are similar to numbers of LL cells per CL (Rodgers *et al.*, 1984; O'Shea *et al.*, 1986), which would be expected in view of evidence that granulosa-derived cells undergo little if any post-ovulatory mitosis (McClellan *et al.*, 1975).

Fate of the theca cells. Many morphological studies have indicated that SL cells may be of thecal origin. This possibility has been supported in cattle by Donaldson & Hansel (1965) and Priedkalns *et al.* (1968), and in sheep by Deane *et al.* (1966) and McClellan *et al.* (1975). Markers, including the enzyme alkaline phosphatase and monoclonal antibodies, have also been used in tracing the fate of theca-derived cells after ovulation. In sheep, alkaline phosphatase is confined to the theca, and particularly the theca interna, in preovulatory follicles. Use of this marker has clearly demonstrated the incorporation of theca cells into the developing CL, and supported the concept of a thecal origin for the SL cells (O'Shea *et al.*, 1980). However, the value of alkaline phosphatase as a thecal marker is limited to the first 48 h after ovulation, as this enzyme subsequently becomes widely distributed throughout the CL. Monoclonal antibodies against bovine theca cell surface antigens, used to identify cells of thecal origin after ovulation, have similarly pointed to the theca as a source of at least a large proportion of the SL cells in the cow CL (Alila & Hansel, 1984).

Cellular transformation in the CL

The question as to whether LL and SL cells persist as discrete and closed populations throughout the lifespan of the CL is more controversial. Several lines of evidence suggest that SL cells may be able to differentiate into LL cells, and it has further been suggested that there may be a population of stem cells in the CL which differentiate into small steroidogenic cells which in turn differentiate into LL cells (Niswender *et al.*, 1985).

Morphological evidence. If SL cells do differentiate into LL cells then it would be expected that some cells of intermediate structure would be demonstrable by electron microscopy. In an early study of the ultrastructure of luteinization in the cow Priedkalns *et al.* (1968) did appear to recognize the presence of such cells, and Parry *et al.* (1980) were subsequently unable to distinguish bovine LL and SL cells as separate cell types. On the other hand, O'Shea *et al.* (1979) and Fields *et al.* (1985) were able to identify two distinctive cell populations, in sheep and cattle, respectively, and failed to observe cells of intermediate structure. However, the subjective nature of all of these observations needs to be recognized, and the question cannot be regarded as definitively resolved.

Evidence based on cell numbers. In a study of numbers of large and small steroidogenic cells obtained by enzymic tissue dissociation, Fitz *et al.* (1981) observed a rise in numbers of large cells and a fall in numbers of small cells during the later stages of the oestrous cycle in the sheep. While these findings are suggestive of the possibility of transformation of some SL cells to LL cells, cells were categorized solely on the basis of diameter in suspension. Hence simple enlargement of some or all of the SL cells, without true cytodifferentiation to LL cells, could equally validly explain these observations. Furthermore there is a risk that cell populations obtained by tissue dissociation may not be truly representative of those in the original tissue. A subsequent study based on morphometry failed to show any rise in numbers of LL cells, as identified by electron microscopy, during the later part of the luteal phase of the sheep oestrous cycle (O'Shea *et al.*, 1986).

An increase in the numbers of LL cells and a decrease in SL cells in dissociated populations from cyclic sheep CL have also been demonstrated in response to luteotrophic stimulation by human chorionic gonadotrophin (hCG) (Niswender *et al.*, 1985). While these observations are also consistent with the idea of transformation of SL cells to LL cells, they remain subject to the general problems associated with quantitation based on dissociated cell populations.

Evidence based on luteinization of follicular cysts. The strongest evidence in sheep that theca-derived cells may be able to differentiate into LL cells is found in a study by Cran (1983): luteinization of follicular cysts, formed in response to PMSG, in which granulosa cells had largely degenerated, was accompanied by the formation of cells whose ultrastructure closely resembled that of LL cells.

Evidence based on monoclonal antibodies. The studies of Alila & Hansel (1984), referred to above, also provide evidence related to the possible transformation of SL cells to LL cells: the numbers of LL cells binding a granulosa-specific antibody declined progressively throughout the oestrous cycle, while the number binding a theca-specific antibody increased. The majority of the SL cells bound the theca-specific antibody, and no SL cells bound the granulosa-specific antibody after Day 6 of the cycle. The findings of this study, in which numbers of cells binding both of these antibodies were reduced progressively throughout pregnancy, obviously add considerable strength to the case for transformation of theca-derived cells, and presumably of SL cells, to LL cells in the normal cow CL. While the possibility that granulosa-derived cells might at a later stage in their life cycle express antigens originally limited to the theca cannot be wholly eliminated, Alila & Hansel (1984) argue strongly against this on the grounds that no cells were ever detected which expressed both granulosa-specific and theca-specific antigens at the same time. The concept that theca-derived LL cells replace the original granulosa-derived cells would be strengthened if direct evidence of the death and disposal of the original LL cells could be provided.

Implications of cellular transformation. Although granulosa and theca cells may share a common origin during ovarian histogenesis (Byskov, 1986), they are structurally and functionally distinct cell types at least from the time of folliculogenesis, and are separated by a complete basal lamina until close to the time of ovulation. Hence, subsequent convergent differentiation to a common, new type of specialized cell, the LL cell, would appear intrinsically surprising. Nonetheless, if the dual origin theory of LL cell formation can be confirmed to be correct, it remains an open question as to why LL cells of granulosa origin should need to be replaced by theca-derived cells. One possibility might be that granulosa-derived LL cells have a limited lifespan, perhaps sufficient for the oestrous cycle but inadequate for pregnancy. Disappearance of the original granulosa-derived cells might then serve as a stimulus for further differentiation of theca-derived cells, as appears to have happened in the luteinized follicles studied by Cran (1983). An additional possibility could be that, although superficially similar, theca-derived LL cells are functionally different from those of granulosa origin. For example, replacement might be necessary to provide a relaxin-synthetic LL cell type in place of an oxytocin-synthetic type, leading to the testable hypothesis that individual LL cells may be able to synthesize either oxytocin or relaxin, but not both.

Function

Knowledge as to the distinctive functions of LL and SL cells has been obtained primarily from in-vitro studies of enriched populations derived from enzymically dissociated luteal tissue. Emphasis here will be placed on data related to the individual functions of LL and SL cells, and to the possibility of functional interactions. While other functions of the CL as a whole have been identified, the major products which have been linked with one or other luteal cell type are progesterone, relaxin and oxytocin. Further discussion will therefore be limited to these hormones.

Progesterone

Control of synthesis. While luteinizing hormone (LH) appears to be the major luteotrophic hormone in domestic ruminants (Niswender *et al.*, 1985), evidence from several studies of sheep (Fitz *et al.*, 1982; Rodgers & O'Shea, 1982; Rodgers *et al.*, 1983a) and cattle (Ursely & Leymarie, 1979; Koos & Hansel, 1981) indicates a marked difference in response to LH by LL and SL cells *in vitro*. This evidence, which is consistent with the much higher numbers of LH receptors on SL cells (Fitz *et al.*, 1982), shows that the progesterone secretory response to LH is confined largely, if not wholly, to the SL cells. The response by SL cells is mediated by cAMP, intracellular concentrations of which are elevated in response to LH (Hoyer *et al.*, 1984). Dibutyryl cAMP (Fitz *et al.*, 1982; Rodgers *et al.*, 1983a) or activators of adenylate cyclase (Hoyer *et al.*, 1984) also stimulate pro-gesterone production by these cells. Progesterone synthesis by SL cells is also stimulated by hCG, although quantitative aspects of the response differ from those to LH (Bourdage *et al.*, 1984). The only documented exception to the response of SL cells to LH is found in cows during the later stages of pregnancy (Chegini *et al.*, 1984; Weber *et al.*, 1984), and may represent a regressionary change.

The response to LH by LL cells is consistently less than that of SL cells, and in studies of sheep can be explained in terms of contamination of the LL cell populations by small numbers of LH-responsive SL cells (Fitz *et al.*, 1982; Rodgers *et al.*, 1983a). LL cells also fail to show any progesterone-secretory response to dibutyryl cAMP (Fitz *et al.*, 1982) or to activators of adenylate cyclase, although these activators do increase the synthesis of cAMP by LL cells (Hoyer *et al.*, 1984).

Studies *in vitro* also suggest the possibility that other hormones, including prostaglandins, oestradiol, catecholamines and oxytocin, may have physiological actions on the progesterone-secretory function of cells of ruminant CL. In relation to prostaglandins (PG), Fitz *et al.* (1984a) demonstrated a stimulatory effect of PGE-1 and PGE-2 on basal (i.e. without LH) progesterone production by ovine LL cells, in the absence of any changes in cAMP levels in the cells or media,

while SL cells did not respond to these PGs. PGF-2α, known to be a luteolytic hormone in sheep, caused a decrease in progesterone production by the LL cells (Fitz *et al.*, 1984b). These findings are again consistent with the demonstrated distribution of receptors for PGE and PGF, which are confined largely to the LL cells (Fitz *et al.*, 1982). In contrast to the findings of Fitz *et al.* (1984a, b), Rodgers *et al.* (1985) found no effect of PGE-2 on basal progesterone production by either type of sheep luteal cell, and Koos & Hansel (1981) were unable to detect any effect of PGF-2α on progesterone production by LL or SL cells from cow CL, with or without LH stimulation.

The presence of a cytosolic receptor for oestradiol has been demonstrated in ovine luteal cells, primarily LL cells, by Glass *et al.* (1984). However, the physiological significance of this receptor is not yet clear. Rodgers *et al.* (1985) observed no effect of oestradiol on basal progesterone production by either type of ovine luteal cell *in vitro*, while Ursely & Leymarie (1979) reported a dose-dependent inhibitory effect on LH-stimulated progesterone secretion by bovine LL and SL cells, suggesting a possible physiological role in luteolysis.

β-Adrenergic stimulation of progesterone secretion by isoprenaline, and its blockage by the β-adrenergic antagonist propranolol, have been demonstrated in mixed populations of luteal cells from sheep (Jordan *et al.*, 1978) and cattle (Battista & Condon, 1986). Increased synthesis of cAMP was also observed in sheep (Jordan *et al.*, 1978), suggesting that SL cells rather than LL cells may have been responsible for the increased progesterone production. However, Rodgers *et al.* (1985), using separated populations of ovine LL and SL cells, observed that isoprenaline produced a significant stimulation of progesterone production only by LL cells. The reason for this apparent discrepancy is not clear, and the cellular location of β-adrenergic receptors in ruminant CL has not been investigated.

Evidence from mixed populations of bovine luteal cells (Tan *et al.*, 1982) suggests that oxytocin may produce a minor stimulatory action on progesterone production at low doses, and a marked suppression of hCG-stimulated progesterone production at higher doses. In sheep, oxytocin has been reported to inhibit LH-stimulated progesterone production by SL cells (Niswender *et al.*, 1985), although Rodgers *et al.* (1985) were unable to detect any effect on LL or SL cell progesterone synthesis. Various degrees of damage to receptors arising from variations in method of tissue dissociation could perhaps explain these findings.

In addition to hormonal influences, there is also evidence of the importance of intracellular levels of calcium in progesterone secretion by sheep luteal tissue (Higuchi *et al.*, 1976). The importance of calcium fluxes is also suggested by the observation that the Ca^{2+} channel-blocking agent verapamil substantially suppresses basal progesterone production by both LL and SL cells from sheep (Rodgers *et al.*, 1985).

Quantitative aspects of secretion. With the exception of the work of Chegini *et al.* (1984) on pregnant cows, all of the published studies comparing basal progesterone production by ruminant LL and SL cells have indicated that LL cells, on a per cell basis, produce substantially more progesterone than do SL cells. Quantitative comparisons become more complicated, however, when considering contributions to total luteal progesterone production. Two attempts (Rodgers *et al.*, 1983a; Niswender *et al.*, 1985) have been made to assess the relative contributions of LL and SL cells to total progesterone production by the sheep CL. To do this one needs quantitative data on basal secretion, on responses to LH, on absolute or relative numbers of the two cell types in luteal tissue, and on luteal (or blood) levels of LH *in vivo*. It is also necessary to extrapolate findings on the functional behaviour of LL and SL cells from an in-vitro to an in-vivo situation, clearly a hazardous undertaking. However, on these bases, and using different sets of data, Rodgers *et al.* (1983a) concluded that progesterone production by the SL cell type could exceed that from the LL cells, while Niswender *et al.* (1985) estimated an SL cell contribution of less than 22%. It seems unlikely that either of these estimates is very accurate, but they do suggest that the contribution of SL cells to luteal progesterone production may well be quantitatively significant and should never be ignored.

Morphological correlates of secretion. As indicated above, LL and SL cells possess features consistent with a steroidogenic function. However, discussion of the structural correlates of luteal progesterone secretion in ruminants has been dominated for over a decade by the suggestion that progesterone (presumably bound to a carrier protein) may be released in association with the exocytosis of the secretory granules of the LL cells. This suggestion, originally advanced by Corteel (1973) and Gemmell *et al.* (1974), has received wide support based primarily on a large amount of circumstantial evidence linking the timing and extent of granule release to that of progesterone secretion (Gemmell *et al.*, 1977; Sawyer *et al.*, 1979; Parry *et al.*, 1980; Heath *et al.*, 1983). Additional evidence derives from the demonstration that progesterone is concentrated in association with secretory granules after differential centrifugation of bovine luteal tissue homogenates (Quirk *et al.*, 1979). However, the granule theory of progesterone secretion has become less tenable since it has been established that SL cells also secrete progesterone, but without the exocytosis of granules. Furthermore, a recent attempt to demonstrate the presence of a progesterone-binding protein in the secretory granules of ovine LL cells was unsuccessful (Sernia *et al.*, 1982), and granule release can be induced in sheep by PGF-2α *in vivo* without any accompanying increase in ovarian venous blood levels of progesterone (Fairclough *et al.*, 1985).

LL–SL cell interaction. Using incubation of LL and SL cells separately and after recombination, and a superfusion system in which SL cells were incubated in series with, and upstream from, LL cells, Lemon & Mauléon (1982) demonstrated an interaction between pig LL and SL cells in the synthesis of progesterone. The data suggest that some product(s) of the SL cells was able to enhance progesterone production by the LL cells. Attempts to demonstrate a similar interaction in cow (Ursely & Leymarie, 1979) and sheep (Rodgers *et al.*, 1985) CL have so far been unsuccessful.

The possibility of an inhibitory interaction between ruminant LL and SL cells, in which oxytocin might be released from LL cells under the influence of PGF-2α and suppress the response of SL cells to LH, has also been postulated (Rodgers *et al.*, 1985; Niswender *et al.*, 1985). As discussed above, however, evidence of any inhibitory action of oxytocin on progesterone production *in vitro* is equivocal, and oxytocin receptors have not yet been demonstrated on SL cells.

Relaxin

Cattle. Fields *et al.* (1980) were able to demonstrate relaxin in bovine LL cells from the middle third of pregnancy by using the immunoperoxidase method with light microscopy. Although the polyclonal antibodies used were produced against pig relaxin, strong evidence from biochemical, immunological and bioassay studies supported the conclusion that the antigen localized was in fact relaxin. Reaction product was confined to the cytoplasm of the LL cells, but it was not possible to establish its subcellular localization. However, both by extrapolation from data from other species and in terms of known mechanisms of secretion of polypeptides by other cell types, storage and release of relaxin in granule form would be predicted.

Sheep. Evidence of the occurrence of relaxin in sheep CL is less convincing than that in the cow. Kruip *et al.* (1976) reported a positive identification of relaxin in CL from ewes in the last half of pregnancy, using immunofluorescence with an antibody produced against pig relaxin (NIH-R-P1), but also noted that sections treated without the antirelaxin serum were "not completely negative". No confirmatory evidence of the occurrence of relaxin in sheep CL was produced. Subsequent immunological localization studies in CL from pregnant ewes, using polyclonal (Renegar & Larkin, 1985) and monoclonal (J. Patterson, J. D. O'Shea, C. S. Lee & M. R. Brandon, unpublished data) antibodies to pig relaxin, have given wholly negative results.

Oxytocin

Sheep. In this species, many lines of evidence now indicate that oxytocin is synthesized, stored and secreted by LL cells. Localization of oxytocin and neurophysin I in LL cells, but not SL cells,

was demonstrated with light microscopic immunocytochemistry by Watkins (1983), and has since been confirmed by electron microscopy (Theodosis *et al.*, 1986). Synthesis of oxytocin *in vitro* has also been shown to be restricted to LL cells (Rodgers *et al.*, 1983b). There is close correlation between the content and release of luteal oxytocin and the content and exocytosis of LL cell secretory granules. Granule loss of LL cells during luteal tissue dissociation (Rodgers & O'Shea, 1982) is accompanied by a substantial fall in the oxytocin content of the LL cells (Rodgers *et al.*, 1983b), while PGF-2α-induced release of oxytocin *in vivo* is associated with the exocytosis of large numbers of secretory granules (Fairclough *et al.*, 1985). Immune electron microscopy (Theodosis *et al.*, 1986) has now provided definitive proof of the granular localization of oxytocin and neurophysin I in ovine LL cells.

Cattle. Although data are less complete than for sheep, it is clear that the LL cells, but not the SL cells, of cyclic cows contain immunoreactive oxytocin and neurophysin (Guldenaar *et al.*, 1984; Kruip *et al.*, 1985). Secretory granule release in response to PGF-2α also occurs in cow CL (Heath *et al.*, 1983), but this has not yet been linked directly to oxytocin secretion.

Goats. Although morphological studies have shown that secretory granules are plentiful in LL cells through the cycle and into late pregnancy, and goat CL do contain oxytocin (Freeman & Currie, 1985), it has not yet been established whether the LL-cell granules contain oxytocin, relaxin, or some other product.

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