

The Ultrastructure of the Corpus Luteum of the Goat

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ABSTRAK

Ultrastruktur sel-sel lutea dan peratus sel-sel berlainan jenis dalam korpus luteum yang aktif dalam kambing telah dikaji menggunakan mikroskop elektron. Terdapat dua jenis sel lutea, sel lutea besar dan sel lutea kecil, dalam korpus luteum spesies ini. Sel lutea besar berbentuk bulat dan mempunyai banyak mitokondria, granul tumpat elektron yang diliputi membran, retikulum endoplasma tanpa granul yang ekstensif dan retikulum endoplasma bergranul di mana organel ini kadangkala kelihatan seperti sisterna tersusun rapi dan bertindih-tindih. Hanya sedikit titik lipid terdapat dalam sitoplasma sel jenis ini sementara retikulum endoplasma tanpa granul berbentuk pusar tidak kelihatan. Antara sel-sel lutea besar terdapat sel-sel lutea kecil dengan cuaran-cuaran sitoplasmanya yang meruncing. Sel ini berbeza daripada sel lutea besar dari segi ianya mempunyai hanya sedikit mitokondria dan granul tumpat elektron yang diliputi membran. Kadangkala nukleus sel lutea ini mempunyai badan-badan rangkuman.

ABSTRACT

The ultrastructure of luteal cells and the proportion of the different cell types in the functional corpus luteum of the goat were studied using the electron microscope. Two luteal cell types, large and small, were present in the corpus luteum of this species. The large more rounded luteal cell possessed numerous mitochondria and electron dense membrane-bound granules, extensive agranular endoplasmic reticulum and granular endoplasmic reticulum which at times appeared as stacks of closely packed cisternae. Few lipid droplets were present in the luteal cell cytoplasm while whorled agranular endoplasmic reticulum was absent. Interspersed amongst the large luteal cells were smaller luteal cells with tapering cytoplasmic processes. These cells differed from the large luteal cells in that they possessed fewer mitochondria and electron dense membrane-bound granules. Occasional nuclei of the small luteal cells contained cytoplasmic inclusion bodies.

INTRODUCTION

Reproduction is a major contributing factor to efficiency of meat and milk production in the goat. Beyond improving the kid crop, a knowledge of the reproductive phenomena of the goat is essential to good herd management. The diversity of genotypes of the goat is such that it is difficult to have specific parameters for reproductive characteristics. The goat shows a

seasonal cycle in reproductive activity in temperate latitudes which is usually related to the length of the photoperiod. However, near the equator and in tropical environments like Malaysia the cycle is year round. As such, there have been numerous reports on the length of the oestrous cycle in the goat. These reports suggest that most goats in the tropics show an oestrous cycle length of 19 to 21 days (Carrera and Butterworth, 1968; Devendra and Burns, 1970).

In temperate environments, proestrus is known to last 24 hours, while oestrus or standing heat varies from 12 to 36 hours and ovulation is reported to occur 12 to 36 hours after the onset of standing heat (Smith, 1980). Diestrus or the period of corpus luteum function is the longest portion of the cycle and the exact duration of diestrus, together with the morphological structure and hormonal function of the corpus luteum (CL) during this period is not known for goats in tropical environments. Further, the goat depends on the CL for maintenance of pregnancy. The goat also appears to be more susceptible to abortion than other species of livestock (Shelton, 1978). The exact cause for this is unknown but the fact that the goat is CL dependent may predispose the animal to abort when there is an interference with or the absence of a functional corpus luteum. Prior to conducting some experiments on the use of the prostaglandins to regulate the oestrous cycle of the goat for oestrus synchronisation, a preliminary study was undertaken to examine the normal ultrastructural features of the goat CL, so as to have a better understanding of the mechanism of luteolytic action of prostaglandins at the cellular level.

This report presents some electron microscopic features of the CL of the goat which apparently seems to be quite vital for the reproductive function of this species.

MATERIALS AND METHODS

Functional CL from cross bred goats (*Capra hircus*) were obtained within 10 minutes of death

from a slaughter house. Functional CL were selected on the basis of size, colour and no evidence of regressionary changes as reported in other species (Crombie *et al.*, 1971; O'Shea *et al.*, 1977). Each CL was immediately quartered with a sharp razor blade and small blocks (1 mm³) were taken from each quarter and fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C. The tissues were post-fixed in cacodylate-buffered 1% osmium tetroxide, dehydrated in a graded series of dilutions of acetone in distilled water and embedded in araldite. Thin sections were stained with a saturated solution of uranyl acetate in 70% filtered methanol, and lead citrate (Reynolds, 1963) and examined with a Philips HMG 400 electron microscope.

For determining the proportion of different cell types in the CL, low magnification ($\times 1,000$ plate magnification) electron micrographs were obtained randomly by photographing the bottom left corner of grid squares. All cells in which nuclei were observed were counted from 36 electron micrographs from 9 CL of four goats.

RESULTS

General Features

A prominent feature in the CL of the goat was a discreet population of large polyhedral luteal cells 19–25 μ m in diameter with centrally located nuclei, approximately 8 μ m in diameter, and prominent nucleoli (Fig. 1). Interspersed amongst the large luteal cells was a population of

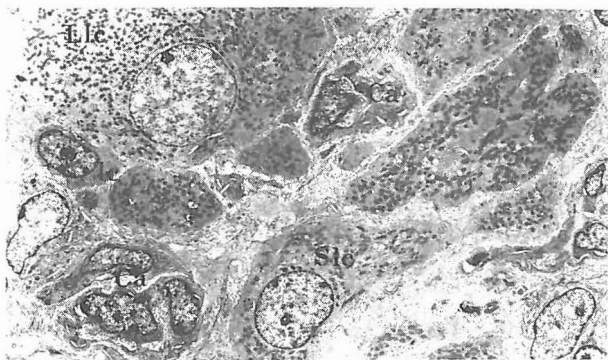


Fig. 1. Electron micrograph of luteal tissue from a functional corpus luteum showing large (Llc) and small (Slc) luteal cells and capillaries (Ca). The large luteal cell at the top left hand corner shows close surface relationship with other luteal cells (lc) but not with a neighbouring capillary. $\times 3,300$.

small cells which were of two types. One type consisted of cells approximately 10 μm in diameter with rounded nuclei, approximately 7 μm in diameter, and distinct nucleoli. These cells are hereafter referred to as small luteal cells. The cytoplasm of these cells at times extended for a considerable distance from the main cell body. Another population of small cells consisted of cells with spindle shaped nuclei and little cytoplasm around their nuclei. These cells were tentatively identified as endothelial cells or pericytes or fibroblasts: The ultrastructure of the CL in this study focuses mainly on the large and small luteal cells.

Large Luteal Cells

The plasmalemma of large luteal cells possessed numerous cytoplasmic projections which appeared either as circular profiles or elongated projections which interdigitated with cytoplasmic projections from neighbouring large or small luteal cells. There was, however, no close surface relationship between large luteal cells and endothelial cells or fibroblasts.

A prominent feature of the large luteal cells was the abundance of mitochondria dispersed throughout its cytoplasmic matrix with profiles which were rounded, oval or elongated (Fig. 2). Branched mitochondria were rarely seen and mitochondrial cristae were predominantly tubular.

There was an extensive distribution of agranular endoplasmic reticulum (ER) in the cytoplasm of large luteal cells. Whorl arrangement of agranular ER was not observed. Granular ER occurred as discrete patches but in some luteal cells this organelle appeared as stacks of closely packed cisternae (Fig. 3). Moderate amounts of polyribosomes were also present in the cytoplasm of the luteal cells.

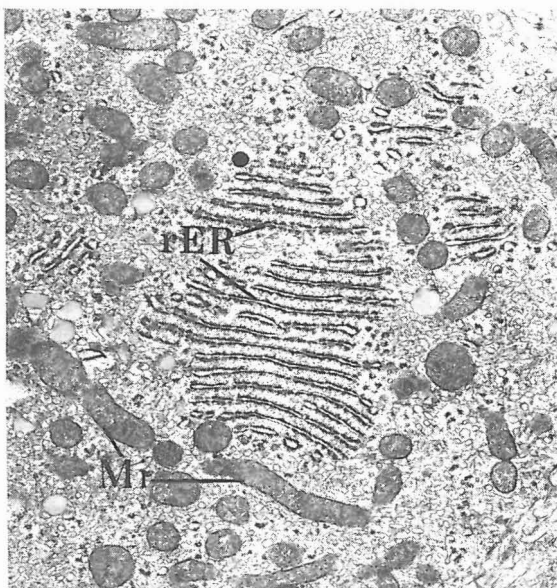


Fig. 3. Granular endoplasmic reticulum (rER) of a large luteal cell arranged as stacks of closely packed cisternae. The cristae of mitochondria (Mi) are predominantly tubular. $\times 15,400$.

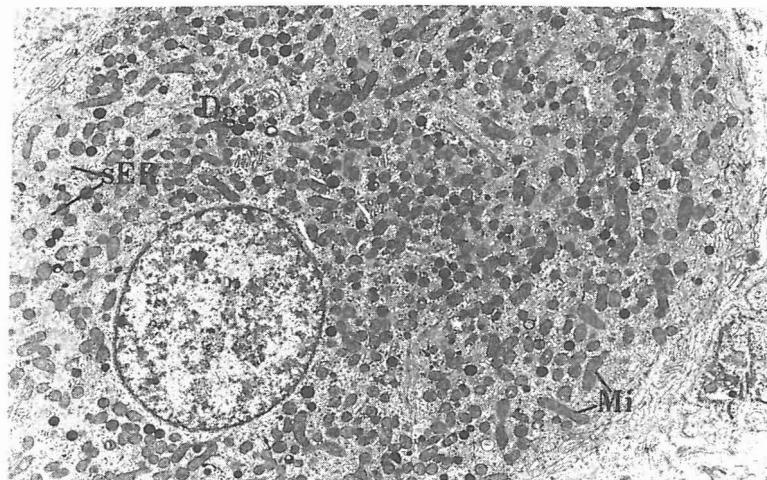


Fig. 2. A large luteal cell containing numerous mitochondria (Mi), dense granules (Dg) and an extensive agranular endoplasmic reticulum (sER). $\times 7,000$.

A number of Golgi complexes in the cytoplasm of the luteal cell were associated with coated and uncoated vesicles and membrane-bound dense granules (Fig. 4). These granules were usually spherical although some pleomorphism such as C-shaped configurations were observed. No continuities between the cisternae of Golgi apparatus and dense granules were observed suggesting that these granules have been "budded off" from the Golgi complex.

Morphologically, membrane-bound dense granules scattered throughout the cytoplasm of the large luteal cell could be classified into two different types. The first type approximately 0.2 μm in diameter were more numerous and displayed a dense core surrounded by a lighter periphery (Fig. 5) with occasional granules containing more than one dense core. Granules of this type were at times present in the extracellular spaces (Fig. 6). The second type, also approximately 0.2 μm in diameter, were uniformly electron dense with their membranes closely apposed to their contents (Fig. 5).

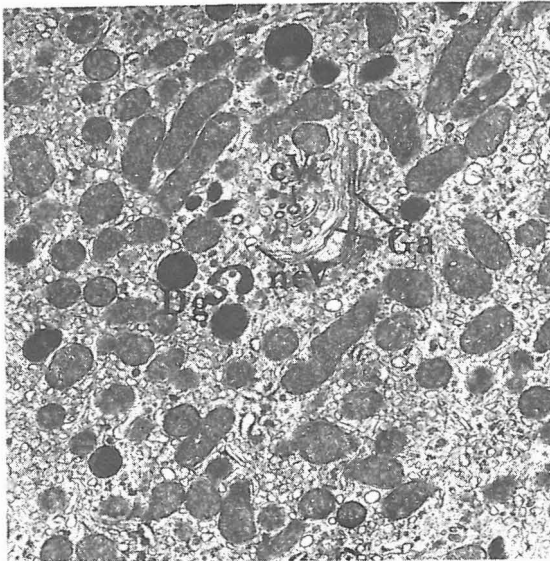


Fig. 4. Golgi apparatus (Ga) of a large luteal cell is closely associated with coated (cV) and uncoated (ncV) vesicles. Some of the dense granules (Dg) in the vicinity of the Golgi apparatus are C-shaped. $\times 20,000$.

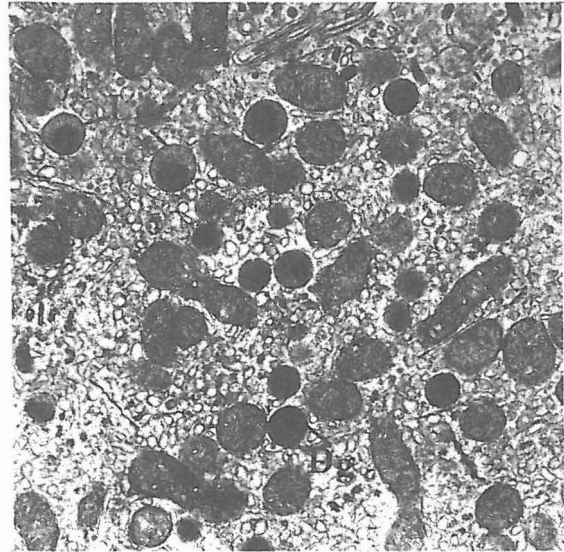


Fig. 5. Membrane-bound granules in the cytoplasmic matrix of a large luteal cell. The granules either contain a dense core with a lighter periphery (Lg) or their content is uniformly electron dense (Dg) $\times 25,000$.

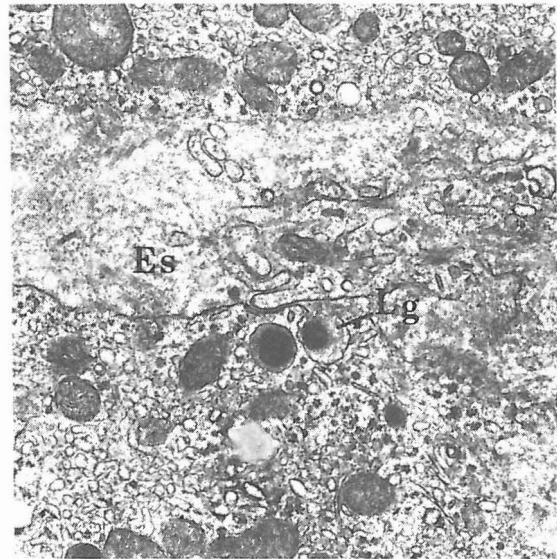


Fig. 6. Membrane-bound granules containing a dense core and lighter periphery appear to be expelled into the extracellular space (Es). $\times 25,000$.

Small Luteal Cells

The ultrastructure of a small luteal cell is shown in Fig. 7. Small luteal cells differed from large luteal cells in several aspects. a) The nuclei of some small luteal cells contained cytoplasmic inclusion bodies consisting of granular ER and ribosomes. No continuity between cytoplasmic nuclear inclusion and the surrounding cytoplasm was observed but serial sections would be necessary to establish this. b) The cytoplasm of small luteal cells contained moderate numbers of mitochondria with profiles which were rounded or elongated. In spindle shaped small luteal cells, mitochondria appeared to be localized at the poles of the cell. c) An extensive agranular ER in the cytoplasm of the small luteal cell was predominantly tubular and fewer cisternae of granular ER were present. d) Fewer membrane-bound dense granules were present and these consisted of the types which was uniformly electron dense. e) Multivesicular bodies were more numerous in the cytoplasm of the small luteal cell.

Quantitative Aspects of Luteal Histology

The percentages of each of the major cell types in a total count of 506 cells are shown in Table 1.

TABLE 1
Percentages of different cell types in the corpus luteum of the goat. Numbers of cells types in parentheses

Cell type	Proportion (%)
Large luteal cell	10.5 (53)
Small luteal cell	17.0 (86)
Endothelial cell/pericyte	61.6 (312)
Fibroblast	9.7 (49)
Miscellaneous or unidentified cell	1.2 (6)

Ninety nine percent of all cells counted could be classified under four main headings. Approximately 60% of all nucleated cells counted were either endothelial cells and/or pericytes. Luteal cells accounted for approximately 27% of the cells counted in which about two thirds were small luteal cells. Fibroblasts accounted for approximately 10%.

DISCUSSION

Previous studies on the ultrastructure of the goat CL were confined to the relationship between the presence of membrane-bound electron dense granules in the cytoplasm of luteal cells

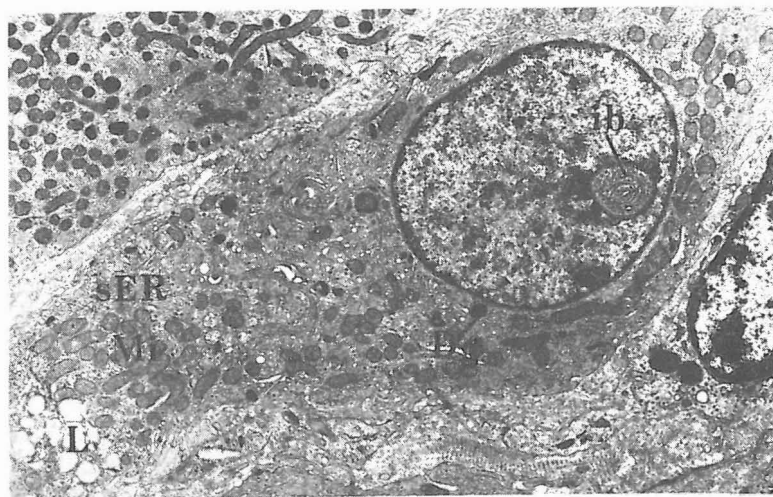


Fig. 7. A small luteal cell containing within its cytoplasmic matrix numerous mitochondria (Mi) and an extensive agranular endoplasmic reticulum (sER). Few dense granules (Dg) are present and lipid droplets (L) are present at one pole of the cell. The nucleus contains cytoplasmic inclusion body (lb). $\times 9,200$.

and progesterone secretion during the oestrous cycle (Gemmell *et al.*, 1977; Gemmell and Stacy, 1979).

Endothelial cells and pericytes constitute the most numerous cell types in the functional CL of the goat. Large and small luteal cells are intermediate in proportion. The high proportion of endothelial cells or pericytes indicate the high vascularity of luteal tissues which has also been observed in the rat (Meyer and Bruce, 1979; 1980); sheep (O'Shea *et al.*, 1979; Rodgers *et al.*, 1984) and guinea pig (Azmi *et al.*, 1984).

The morphological features of the large luteal cell in the goat were comparable to those described for steroid secreting cells generally, and for luteal cells of other mammals (Fawcett *et al.*, 1969; Christensen and Gillim, 1969; Enders, 1973). Many features in the luteal cell of the goat were however similar to those reported in the sheep which include presence of a few lipid droplets and absence of concentric whorls of agranular ER in the cytoplasm of the large luteal cell (McClellan *et al.*, 1975). Large concentric whorls of agranular ER on the other hand have been observed during maximal steroid synthesis in the sow (Bjersing, 1967; Goodman *et al.*, 1968); rabbit (Blanchette, 1966b); bat and ferret (Enders, 1973); hamster (Leavitt *et al.*, 1973) and dog (Abel *et al.*, 1975). Other features of the goat CL which are similar to that of the sheep are the proportion of the two luteal cell populations, and the presence of cytoplasmic inclusion bodies in the nuclei of small luteal cell (O'Shea *et al.*, 1979).

Electron-dense membrane-bound granules were reported present in the cytoplasm of large luteal cells and in extracellular spaces of the goat, cow and rabbit (Gemmell and Stacy, 1979), and in sheep (Gemmell *et al.*, 1974, O'Shea *et al.*, 1979; Paavola and Christensen, 1981). In the sheep, these granules were abundant between Days 9 and 11 of the oestrous cycle (Gemmell *et al.*, 1974) which coincides with the time of maximum progesterone secretion (Thorburn *et al.*, 1969). However evidence on the secretion of progesterone by the large luteal cells released via these dense granules is still limited. In the present study, the dense granules

observed in the extracellular spaces and the type with a dense core and lighter periphery which are similar to those observed in the sheep (Paavola and Christensen, 1981).

The present study confirms the existence of a second population of luteal cell, viz. small luteal cells, in the CL of the goat. Small luteal cells were also reported in a number of other species (Mossman and Duke, 1973) but little is known as to their functional significance. However, certain morphological features of the small luteal cells of the goat observed in the present study suggest that this cell type could be steroid secreting, since it conforms to the criteria of steroid secreting cells described by Christensen and Gillim (1969). The features of a steroid secreting cell described by these authors include abundant tubular agranular ER, lipid droplets and dispersed Golgi elements. These features were present in the cytoplasm of the small luteal cells of the goat.

Dahl (1970) reported the presence of nuclear inclusion bodies in a number of organs and cell types of normal chickens, rats and monkeys. The author considered these nuclear inclusions as normal organelles with no pathological or etiological significance and had arbitrarily classified them into three different types. The nuclear inclusions observed in the small luteal cells in the present study, as well as in the sheep (O'Shea *et al.*, 1979, Rodgers and O'Shea, 1982) do not however conform to any of the nuclear inclusions described by Dahl (1970). Further work is required to elucidate the significance of the cytoplasmic nuclear inclusions in the small luteal cells of the sheep and goat.

It can be concluded that many ultrastructural features in the large luteal cell are similar to that observed in the sheep. The goat also possesses a second luteal cell type whose intrastructural features demonstrate that it may be the steroid secreting cell type.

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