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Cellular composition of the cyclic corpus luteum of the cow

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Summary. The cellular composition of CL from 6 cows on ~Day 12 of the oestrous cycle, after synchronization with cloprostenol, was studied by ultrastructural morphometry. Point-count measurements of volume density (mean ± s.d.) showed that large luteal cells occupied 40·2 ± 7·0% of the luteal tissue, and small luteal cells 27·7 ± 6·3%. Of the total of 393·4 ± 52·0 × 10^3 cells per mm^3 of luteal tissue, large luteal cells made up only 3·5% and small luteal cells 26·7%, a ratio of 1:7·6. Endothelial cells/pericytes, at 52·3%, were the most numerous cell type. The mean volume per large luteal cell was 29·6 ± 6·3 × 10^3 μm^3, while that of small luteal cells was 2·7 ± 0·4 × 10^3 μm^3. In spherical form, these volumes would represent mean diameters of 38·4 μm and 17·2 μm respectively, and are consistent with published measurements on dispersed luteal cells. However, the values for cell numbers are much higher than published values based on luteal tissue dispersion, suggesting that dispersion may result in substantial and possibly selective losses of luteal cells.

Keywords: corpus luteum; oestrous cycle; cow; morphometry; luteal cells

Introduction

Several studies of the function *in vitro* of enzymically dispersed cells from the corpus luteum (CL) of the cow have identified two functionally distinct cell populations, large and small luteal cells (Ursely & Leymarie, 1979; Koos & Hansel, 1981; Chegini *et al.*, 1984; Rodgers *et al.*, 1986; Weber *et al.*, 1987). Criteria for separation of these populations have been based on cell diameter after tissue dispersion, but the cut-off points for the two classes of cell have varied substantially from study to study, making direct comparisons difficult. Chegini *et al.* (1984) regarded cells > 18 μm in diameter as large luteal cells, whereas Weber *et al.* (1987) used 23 μm, Koos & Hansel (1981) 25 μm and Rodgers *et al.* (1986) 26 μm as the smallest diameters for these cells.

On the basis of counts of dispersed cell populations from cyclic bovine CL, Hansel *et al.* (1987) concluded that small luteal cells outnumbered large luteal cells by a ratio of 20:1–40:1, whereas Weber *et al.* (1987) reported a ratio of only 10·2:1. The relationships of these ratios to the actual numbers of the different cell types in intact luteal tissue are important if realistic estimates of the contributions of the two cell populations to overall luteal function are to be made. In sheep, it has been found that dispersion of luteal tissue may result in selective losses of certain cell types, such that counts of dispersed populations may not accurately reflect the tissue populations (Rodgers *et al.*, 1984). Hence other forms of estimation of cell numbers are desirable, and morphometry has now provided a clearer picture of cell numbers in sheep (Rodgers *et al.*, 1984; O'Shea *et al.*, 1986; Farin *et al.*, 1986; O'Shea & McCoy, 1988).

The numbers of cells of different types in luteal tissue are also important in the evaluation of evidence relating to the stability, or interchangeability, of the populations of large and small luteal cells. Alila & Hansel (1984) have provided evidence, based on the use of monoclonal antibody

'markers', that small luteal cells of theca origin may enlarge and progressively replace the original, granulosa-derived, large luteal cells in the CL of the cow. The numbers used in support of this argument were based on counts of dispersed cells, showing shifts in the percentages of cells stained by theca-specific and granulosa-specific antibodies as a function of CL age. The interpretation of these findings is critically dependent on the numerical relationships between the dispersed cell populations studied and the populations in the original tissue.

The major purpose of the present study, using ultrastructural morphometry of CL from ~Day 12 of the oestrous cycle, was to obtain estimates of the numbers and sizes of the large and small luteal cells in intact bovine luteal tissue. Such estimates, providing a basis for comparison with estimates made following tissue dispersion, and with previous studies in sheep, could contribute to the evaluation of existing data on luteal cell function *in vitro* and on the histogenesis of the CL.

Materials and Methods

Animals and tissues. Cyclic Shorthorn heifers were maintained under field conditions. Ovulation was synchronized by 2 intramuscular injections of 500 μg cloprostenol (Estrumate: Coopers Ltd, North Ryde, New South Wales) 11 days apart (Cooper, 1974). Ovaries were removed following slaughter 14 days after the second injection (assumed to be ~Day 12 of the oestrous cycle), and CL were carefully dissected out, sliced through their centres to remove any fluid in central cavities, blotted and weighed. A complete thin (1 mm) slice of luteal tissue was cut from the central surface of each CL, and placed in fixative for electron microscopy. Additional tissue was fixed for light microscopy.

Processing for light and electron microscopy. Tissues for paraffin-wax embedding were fixed in Bouin's fluid, embedded, sectioned at 10 μm, and stained with haematoxylin and eosin. Tissues for plastic embedding were fixed for 24 h in a modified FGP fixative (Ito & Karnovsky, 1968) containing 2·5% paraformaldehyde, 5% glutaraldehyde, 0·5% picric acid and 1·6 mM-calcium chloride in 0·1 M-cacodylate buffer, pH 7·2, at room temperature. Blocks of tissue were taken from inner and outer regions of the luteal tissue as described by O'Shea & McCoy (1988), rinsed in buffer, and post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 h. Blocks were rinsed, dehydrated in acetone, and embedded in Spurr's embedding medium. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM300 electron microscope. From each of 5 blocks per CL, 5 randomly-selected grid squares of a 200-mesh grid were photographed at a magnification of × 1000, and prints prepared at × 2500.

Morphometric methods. Methods used for measurement of volume density, cell number and cell volume have been reported in detail elsewhere (Rodgers *et al.*, 1984) and are summarized only briefly here. Volume density (P_p) was determined by the point-counting method (Weibel, 1979). Forty-two points were counted per micrograph, a total of 1050 points per CL and 6300 points for the 6 CL examined.

Cytoplasmic:nuclear ratios were calculated from the numbers of points on cytoplasm and nuclei for each cell type. Cell identification was based on features reported by Rodgers *et al.* (1986). Cell numbers per unit volume of tissue (N_v) were calculated using the formula

$$N_v = \frac{K}{\beta}\sqrt{\left(\frac{N_A^3}{P_p}\right)} \quad \text{(Formula 2·85, Weibel, 1979),}$$

where N_A is the number of nuclei per unit area of micrograph as measured by image analysis. Size distribution coefficient K was given a value of 1, indicating uniform size for nuclei of all types. Shape coefficient β was given values of 1·382 for nuclei of large and small luteal cells and "other cell types or unidentified", of 1·85 for fibrocytes, and of 2·12 for endothelial cells and pericytes, calculated as described by Rodgers *et al.* (1984). A linear shrinkage factor of 0·85 was applied in calculating values for cell numbers per unit volume. Tissue specific gravity, and shrinkage during fixation, were calculated by measuring weight, and volume by fluid displacement, of slices of luteal tissue from 4 additional cyclic CL, before and after fixation. Cell volume was calculated by dividing volume density (%) by the number of cells per unit volume of tissue, and cell diameter was calculated from mean cell volume assuming a spherical shape.

Results

Luteal weight of the 6 CL studied was 3·8 ± 0·8 g (mean ± s.d.), and volume 3·7 ± 0·8 cm^3. In terms of volume density (Table 1), the combined large and small luteal cells occupied 67·9% of the luteal tissue, with large luteal cells occupying more of the luteal volume than small luteal cells. The cytoplasmic:nuclear ratio of large luteal cells was ≃4·5-fold greater than that of the small luteal cells (Table 1), with lesser ratios observed for endothelial cells/pericytes and fibrocytes.

Table 1. Volume density and cytoplasmic:nuclear ratios in the corpus luteum of the cow on Day 12 of the oestrous cycle

Component of luteal tissue	Volume density (%)	Cytoplasmic:nuclear ratio
Endothelial cells and pericytes	13·3 ± 1·7	4·6 ± 0·9
Large luteal cells	40·2 ± 7·0	44·5 ± 8·9
Small luteal cells	27·7 ± 6·3	10·1 ± 2·8
Fibrocytes	6·2 ± 5·3	4·1 ± 1·8
Other cell types or unidentified	1·9 ± 0·6	
Vessel lumen	1·2 ± 0·4	
Intercellular space	9·5 ± 3·2	

Values are mean ± s.d. for 6 CL.

Endothelial cells and pericytes represented slightly more than 50% of all cells within the luteal tissue, while large luteal cells provided 3·5% and small luteal cells 26·7%, a ratio of large to small luteal cells of 1:7·6 (Table 2). Total numbers of cells of all types were estimated at $\simeq 1{\cdot}5 \times 10^9$ per CL.

Table 2. Quantitative data on individual cell types in the corpus luteum of the cow on Day 12 of the oestrous cycle

Cell type	No. of cells per $mm^3 \times 10^{-3}$ (%)	No. of cells per CL $\times 10^{-6}$	Cell vol. ($\mu m^3 \times 10^{-3}$)	Cell diam.* (μm)
Endothelial cells and pericytes	205·4 ± 42·5 (52·3)	779·8 ± 331·5	0·67 ± 0·15	10·8
Large luteal cells	13·8 ± 1·8 (3·5)	51·5 ± 15·4	29·55 ± 6·25	38·4
Small luteal cells	105·2 ± 22·1 (26·7)	392·4 ± 135·1	2·65 ± 0·35	17·2
Fibrocytes	39·4 ± 32·3 (10·0)	147·5 ± 128·9	1·70 ± 1·06	14·8
Other cell types or unidentified	29·6 ± 25·5 (7·5)	106·4 ± 103·7		
Total	393·4 ± 52·0 (100)	1477·6 ± 513·1		

Values are mean ± s.d. for 6 CL.
*Diameter (mean) calculated from cell volume if cells assumed to be spherical.

The mean volume per individual large luteal cell, at $30 \times 10^3\ \mu m^3$, was 11 times that per individual small luteal cell (Table 2). These volumes, converted to a spherical shape, provided estimates of mean diameter of 38·4 μm and 17·2 μm for the large and small luteal cells (Table 2).

Discussion

The present point-count data on volume density, showing a combined value of almost 70% for bovine large and small luteal cells at mid-cycle, accord closely with values obtained previously by Parry *et al.* (1980). They are, however, higher than estimates obtained for sheep in which values were closer to 50% (O'Shea *et al.*, 1986). While some real inter-species variation may exist it is likely that a major reason for this difference lies in the method of tissue fixation. Perfusion fixation, as used in sheep, resulted in a combined value of $\simeq 28\%$ for vascular lumina and intercellular

spaces. The equivalent value of $\simeq 11\%$ in the present study, using immersion fixation, could largely account for the apparent between-species difference in luteal cell volume density.

Cell numbers have not previously been estimated by morphometry in the cow, but the values obtained here on a per-unit-volume basis are similar to those obtained previously for mid-cycle sheep (O'Shea *et al.*, 1986). While the present estimate for large luteal cells is a little lower than that for sheep, both in absolute and percentage terms, the individual large luteal cells appeared to be substantially larger in the cow. However, the present morphometric estimates of cell diameter accord closely with those of Ursely & Leymarie (1979), who reported mean diameters of 37 μm and 18 μm for dispersed large and small luteal cells of cows.

While there is some evidence that the CL formed after ovulation synchronized by prostaglandin analogues in cows may not be identical to CL formed during natural cycles, at least in *Bos indicus* cattle (Hardin & Randell, 1982; Hansen *et al.*, 1987), comparisons with data from Parry *et al.* (1980) and Ursely & Leymarie (1979) for naturally cyclic cows show close similarities with the present observations. Therefore, although caution is necessary, some comparisons between the present morphometric data and findings from studies using dissociated cell populations may be of value.

With regard to total cell numbers obtained following tissue dispersion, Hansel *et al.* (1987) obtained estimates of $1{\cdot}13 \times 10^6$ large luteal cells and $19{\cdot}3 \times 10^6$ small luteal cells per gram of tissue on Days 10–12 of the oestrous cycle (compared with $13{\cdot}6 \times 10^6$ and $103{\cdot}3 \times 10^6$ respectively in the present study). If mean volumes for cells in these two populations are calculated using the mean diameters (37 μm for large luteal cells, 18 μm for small luteal cells) provided by Ursely & Leymarie (1979), the combined population of large and small luteal cells obtained by Hansel *et al.* (1987) would account for less than 10% of the volume in 1 g of luteal tissue. This compares with point-count estimates of 70% by Parry *et al.* (1980), and 67·9% in the present study, suggesting heavy losses of cells during dispersion. Even lower values of cell dispersion recovery ($0{\cdot}017 \times 10^6$ large and $0{\cdot}173 \times 10^6$ small luteal cells/g) were obtained by Weber *et al.* (1987) from bovine CL on Day 14 of the oestrous cycle.

In terms of the proportions of small to large luteal cells, the present estimate of 7·6:1 is a little lower than that of 10·2:1 obtained by Weber *et al.* (1987) from dispersed luteal tissue on Day 14, and substantially lower than that of 20:1–40:1 reported by Hansel *et al.* (1987) for cyclic cows. The extent of these discrepancies suggests that the losses occurring during luteal tissue dispersion may be selective, with a preferential loss of large luteal cells.

If selective losses of large luteal cells did occur in the studies of Alila & Hansel (1984) and Hansel *et al.* (1987), the percentages of cells staining with the two antibodies used may not have accurately reflected the percentages in the original tissues. Hence granulosa-derived large luteal cells could have been systematically underestimated. These considerations, together with the observation of Alila & Hansel (1984) that a progressively increasing number of large luteal cells bound neither the theca-specific nor the granulosa-specific antibody, suggest that it may be premature to conclude that the granulosa-derived large luteal cells "disappear during early pregnancy" (Alila & Hansel, 1984). In fact, none of the available data are really inconsistent with the idea that some, or even all, of the granulosa-derived cells persist until the onset of luteal regression.

In conclusion, the present morphometric results indicate that the cellular composition of the cyclic CL of the cow is similar to that of the ewe. They further suggest that tissue dispersion can result in substantial and possibly selective losses of cells, so that cell populations obtained by dispersion may not accurately represent the populations in intact tissues. These findings have important implications in interpretation of the cell kinetics of the bovine CL.

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