Weight, composition, mitosis, cell death and content of progesterone and DNA in the corpus luteum of pregnancy in the ewe

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Summary. Changes in luteal weight from about Day 20 to near term, and in quantitative histology as assessed by ultrastructural morphometry and light microscopic counts of mitosis and cell death on Days 30, 60, 100 and 142, were studied in 168 pregnant ewes.

Luteal weight (mean ± s.d.) remained constant at 0.56 ± 0.11 g until Day 120, and fell thereafter to reach 0.31 ± 0.11 g after Day 140 (P < 0.01). Up to Day 100, quantitative aspects of the composition of the luteal tissue showed no significant change, and values for volume density, cytoplasmic:nuclear ratio, cell number/mm³ and cell volume were comparable to values previously obtained for corpora lutea (CL) of the cycle. By Day 142 structural evidence of luteal regression was present, but regressive changes were much more marked in some CL than others. Mitosis was seen in a few cells (0.02–0.04%) on all of the days studied, but never in large luteal cells. Cell death was rarely seen up to Day 100, but had increased in incidence by Day 142 (P < 0.01). Luteal progesterone content, 55.2 ± 15.9 nmol/g on Day 30, was not significantly changed on Days 60, 100 or 142.

It is concluded that (1) structural regression of the CL of pregnancy does not begin until much later than the time (about Day 50) when pregnancy ceases to depend on the CL; (2) structural luteal regression begins before parturition, but its time of onset and/or rate of progression vary widely between animals; and (3) large and small luteal cells remain as distinctive populations throughout pregnancy, and their numbers at all stages can be accounted for by survival of the cells which differentiate during the genesis of the CL.

Keywords: corpus luteum; pregnancy; sheep; morphometry; luteal weight

Introduction

Ovariectomy in the ewe leads to the termination of pregnancy (duration about 150 days) until about Day 50 (Casida & Warwick, 1945; Denamur & Martinet, 1955). However, after Day 50 the placenta becomes the major source of progesterone (Linzell & Heap, 1968), and the corpus luteum (CL) is no longer essential for the support of pregnancy.

Luteal weight up to Day 40 of pregnancy appears to be similar to that attained during the oestrous cycle (Thwaites & Edey, 1970), and has declined substantially by the time of parturition (O’Shea & Wright, 1985). However, no quantitative data are available on the pattern of changes, if any, which occur during the period in which the CL ceases to be the major source of progesterone, or in the subsequent stages of pregnancy.

In addition to their interest in relation to the changing functional status of the CL during pregnancy, aspects of the cell dynamics of the CL of pregnancy are also relevant to questions on the
origins and interrelationships of the two types of luteal cell in sheep. There is now persuasive evidence that these two cell types, large and small, are derived from follicular granulosa and theca cells respectively (O’Shea, 1987), although Cran (1983) has provided experimental evidence suggesting that small luteal cells, or more specifically cells of thecal origin, may in some circumstances be able to differentiate into large luteal cells. However, ultrastructural morphometric studies of cell numbers at different stages of the oestrous cycle (O’Shea et al., 1985, 1986; Farin et al., 1986) provide no support for the idea that conversion of small luteal to large luteal cells is a normal event during the oestrous cycle.

In cattle, the findings of Alila & Hansel (1984), using specific monoclonal antibody markers, appear to indicate that while large luteal cells are initially granulosa-derived, theca-derived cells may subsequently replace the original large luteal cells. This replacement seemed to occur primarily during the oestrous cycle, but to continue into pregnancy such that by Day 150 no large luteal cells expressed the granulosa-specific antigen. No evidence exists as to whether parallel events occur in sheep, but the conclusions of Alila & Hansel (1984) for cattle imply the disappearance of the original large luteal cell population, which comprises a substantial part of the total mass of luteal tissue. They also imply quantitative changes in the population of theca-derived cells, and by inference in the small luteal cell population.

Against this background it seemed important to examine quantitative aspects of the cell populations in the CL during pregnancy in the ewe. Particular emphasis was placed on the numbers of cells of the various types, and on cell division and cell death. Data are also presented on luteal weight, tissue composition, cell size and progesterone content, as indicators of the functional status of the CL.

**Materials and Methods**

**Animals and tissues.** For the measurement of luteal weight, complete reproductive tracts from 153 pregnant ewes of various breeds were collected from an abattoir and transported to the laboratory. All corpora lutea were carefully dissected from both ovaries, bisected to remove any fluid in central cavities, blotted, and weighed to 0.1 mg. All fetuses were removed and straight crown–rump length (Cloete, 1939) was measured to 1 mm. When more than one CL or more than one fetus was present, a single mean value was calculated per animal and used for subsequent calculations. Approximate stage of pregnancy in days was calculated from fetal crown–rump length using data from Cloete (1939): cut-off points for the 7 groups used were as follows: 20–40 days, 0–6–4–2 cm; 41–60 days, 4–3–11–1 cm; 61–80 days, 11–2–19–2 cm; 81–100 days, 19–3–28–2 cm; 101–120 days, 28–3–37–1 cm; 121–140 days, 37–2–45–7 cm; 141 days–term, >45–7 cm.

For the remaining studies a single set of tissues was obtained from 4 groups each of 5 Merino ewes slaughtered at Days 30 (30–31), 60 (59–61), 100 (100–103) and 142 (141–142) of pregnancy. These ewes were housed in pens and given a pelleted feed and pasture hay ad libitum. Mating was synchronized by removal of intravaginal sponges impregnated with 40 mg fluorogesterone acetate and 60 mg medroxyprogesterone acetate 12 days after insertion, and rams were introduced 24–48 h later. Mating dates with rams fitted with a harness and crayon were recorded (day of mating = Day 0). Precise mating dates for 2 of the ewes in the Day 30 group were not known, and stage of pregnancy was estimated from fetal crown–rump length. Immediately after slaughter, reproductive tracts were removed intact, ovaries separated from the tracts, and CL counted. Mean numbers of CL per ewe in the 4 groups were: Day 30, 1–6; Day 60, 1–4; Day 100, 2–4; Day 150, 1–4. One CL from each ewe was removed and sliced into almost equal halves. The slightly larger portion was immersed in fixative for electron microscopy, and samples from the remainder were weighed to 0.1 mg, snap-frozen in sealed vials in solid CO₂ and subsequently stored at −20°C for assay of tissue progesterone and DNA content.

**Processing for light and electron microscopy.** Tissues were fixed 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 for 2–4 h. A thin slice (~1 mm) was then cut from the exposed surface of the CL and divided into 4 equal quadrants. From each quadrant, two blocks of tissue ~1 mm³ were taken, one from the outer part of the quadrant and the other from the inner. Outer blocks were taken about one-third of the way from the capsule of the CL to its centre, and inner blocks from about two-thirds of this distance. For morphometry and counts of mitosis and cell death, all 4 outer blocks but only one randomly-selected inner block were used to represent inner and outer regions of the luteal tissue in their proper proportions. These blocks were placed in fresh fixative for up to 16 h at 4°C, rinsed in buffer, and post-fixed in 1% osmium tetroxide in cacodylate buffer for 2 h at room temperature. Blocks were then rinsed in buffer, dehydrated in acetone, and embedded in Spurr’s embedding medium. Ultrathin sections were stained with a saturated solution of uranyl acetate in 70% ethanol, and with lead
citrate, and examined in a Philips EM300 electron microscope. From each block, 5 randomly-selected complete grid squares of a 200-mesh grid were photographed at a magnification of ×1000. Prints for morphometry were produced at a final magnification of ×2500. Semithin sections (1 µm) from each of these blocks were stained with 1% toluidine blue in 1% borax for light microscopic histology and counts of mitosis and cell death.

**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 (Pv) was determined by the point-counting method (Weibel, 1979). Forty-two points were counted for each micrograph and a total of 5250 points per group. Cytoplasmic:nuclear ratio was calculated from the ratio of numbers of hits on cytoplasm and nuclei for each cell type. Cell numbers per unit volume of tissue (Nv) were calculated using the formula

\[ Nv = \frac{K \sqrt{N_n^3}}{P_v} \]

(Formula 2.85: Weibel, 1979).

where Nn is the number of nuclei per unit area of micrograph. Size distribution coefficient K was given a value of 1, indicating uniform size, for nuclei of all cell 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 1.7 for fibrocytes, and of 1.9 for endothelial cells and pericytes (Rogers et al., 1984). The areas of electron micrographs were measured by image analysis. A linear shrinkage factor of 0.88 was applied in calculating values for cell numbers per unit volume. The mean volume of cells of each type was calculated by dividing volume density (%) by the number of cells per unit volume of tissue.

**Mitosis and cell death.** Using a microscope fitted with an eyepiece graticule marked with a square area containing 10 × 10 1-mm squares, counts of numbers of cells in mitosis or showing unequivocal signs of cell death (Kerr et al., 1972) were made on sections from all blocks used for morphometry. A total of 500 cells was counted per section, giving 2500 cells per CL and 12500 cells per group. Because large luteal cells were represented at only ~5% of total cells, and their incidence of mitosis and death was of particular interest, counts of large luteal cells only were continued on each section until 100 cells had been counted (i.e. 500 large luteal cells per CL, and 2500 per group).

**Assays for progesterone and DNA.** Progesterone was measured by radioimmunoassay (Carson et al., 1986) using reagents supplied under the World Health Organization Matched Reagents Program and in accordance with WHO recommendations. Luteal tissues were homogenized and extracted with diethyl ether before assay, and all samples were handled in a single assay. The within-assay coefficient of variation was <15% between 36 and 2750 fmol/tube, calculated by the methods and computer programme of Burger et al. (1972), and the limit of detection was 9.1 ± 1.9 fmol/tube.

DNA was measured by the method of Burton (1956), and all samples were measured in a single assay. A standard curve was constructed to range from 0 to 80 µg DNA/tube, and all samples diluted to fall within a range of 10–50 µg/tube.

**Statistical analysis.** Data were analysed by Student's t test or by analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) method (Sokal & Rohlf, 1969). Arcsine transformation was applied to all percentage values for statistical analysis. Data are reported as mean ± standard deviation (s.d.).

**Results**

**Luteal weight**

Of 153 pregnant tracts examined, 81 contained single CL, 67 twin CL and 5 triple CL. Data from triple-ovulating ewes were not used for further analyses. Of the 67 ewes with twin CL, 40 had twin fetuses and 27 single fetuses. Overall fetal crown–rump length varied from 0·9 to 47·6 cm in the 148 ewes with single or twin CL. For the purpose of subdivision of these 148 pregnancies into groups based on 20-day intervals (Fig. 1), data from ewes with single and twin CL were pooled after it was shown that neither luteal weight (0·54 ± 0·15 and 0·50 ± 0·11 g) nor fetal crown–rump length (26·7 ± 14·1 and 24·8 ± 13·8 cm) differed significantly between ewes with 1 or 2 CL (P > 0·05, t tests). Nor were there any significant differences between single and twin-ovulating ewes within any individual 20-day group.

As shown in Fig. 1, luteal weight remained constant until Day 120 (overall mean 0·56 ± 0·11 g) and fell progressively thereafter. Mean luteal weights in the groups from Day 121–140 (0·42 ± 0·13 g) and after Day 140 (0·31 ± 0·11 g) were significantly lower than in any other group (P < 0·01, ANOVA/SNK), while those after Day 140 were lower than those from ewes at Day 121–140 (P < 0·05). The ranges of luteal weight in these last two groups (0·11–0·68 and 0·11–0·44 g respectively) were particularly wide.
Stage of pregnancy (days)

Fig. 1. Weight of the CL (g) against estimated stage of pregnancy (days) in 148 ewes, arranged into 20-day groups (mean ± s.d.) based on crown–rump length. Numbers shown within bars are numbers of ewes per group. Luteal weight after Day 120 was lower ($P < 0.01$) than at any earlier stage.

Histology and ultrastructure

General features of the luteal tissue at Days 30, 60 and 142 are shown in Figs 2–5. The tissue showed little change between Days 30 and 100. By Day 142 there was greater variability between individual CL. In one animal, regressive changes were marked, with considerable numbers of dead cells scattered throughout the tissue and within the lumina of capillaries. This and one other CL were heavily infiltrated with leucocytes, primarily lymphocytes, although eosinophils in one of these CL, and macrophages in both, were also present in substantial numbers. Small, thick-walled arterial blood vessels were also prominent in these two CL. The remaining three CL in this group were more similar in appearance to those at the earlier stages of pregnancy. Binucleate large luteal cells, which were rarely observed up to Day 100, increased in incidence at Day 142, especially in the CL showing marked regressive changes, possibly indicating cell fusion in late pregnancy. Nuclear shrinkage, associated with a sometimes pronounced irregularity of nuclear outline (Fig. 5), was also observed in many large luteal cells at Day 142. Cytoplasmic inclusions within the nuclei of small luteal cells (Fig. 4), as described previously (O'Shea et al., 1979), were present at all stages of pregnancy examined here. Their incidence (% of nuclei containing one or more inclusions) in the nuclei used for the morphometric study was 8.9% overall, and there were no significant differences between days.

Morphometry

Volume density (Table 1) of the various constituents of the luteal tissue remained relatively constant throughout the period studied, and no significant differences between days were observed. The combined large and small luteal cells occupied ~60% of the luteal tissue on all days. The mean number of hits on ‘other cell types or unidentified’ was considerably higher on Day 142 than on any
**Fig. 2.** Luteal tissue at Day 60, showing large (L) and small (S) luteal cells. An endothelial cell in mitosis (arrow) is present in the wall of a capillary. Toluidine blue, × 1000.

**Fig. 3.** Electron micrograph of luteal tissue at Day 30. Large luteal cells (L) contain abundant small, dense 'secretory granules' (arrows). Small luteal cells (S), fibrocytes (F) and endothelial cells (E) are also present. × 2500.
other day: however, this non-significant increase was primarily due to a high number of hits on leucocytes, macrophages and smooth muscle cells of the walls of blood vessels in 2 CL within this group.

Cytoplasmic:nuclear ratios (Table 1), which were extremely high in large luteal cells, also showed no significant differences between days. However, the trend towards increased cytoplasmic: nuclear ratio in large luteal cells in late pregnancy was consistent with the observed nuclear shrinkage and crenation in these cells at Day 142.

Mean cell volume for the four major cell types did not change significantly over the four stages of pregnancy examined (Table 1). Total numbers of cells per unit volume, and numbers of each individual cell type, remained stable between Days 30 and 100 (Table 1). The mean number of total cells/mm³ was, however, greater on Day 142. This increase, which did not achieve significance, resulted largely from an almost 3-fold increase in the number of ‘other cell types or unidentified’, again associated with marked changes in 2 of the 5 CL in the Day 142 group. Means for endothelial cells and pericytes, large luteal cells and fibrocytes were also slightly, but not significantly, elevated at Day 142.

Up to Day 100 the category of ‘other cell types or unidentified’ was composed primarily of smooth muscle cells of blood vessel walls (39-0%), macrophages (30-0%) and lymphocytes (25-8%). Only 4% of cells in this category were unidentified up to Day 100, whereas 11-5% were unidentified in the Day 142 group, reflecting a greater difficulty in cell identification in late pregnancy. Eosinophils, at 9-2% overall on Day 142, were numerous in only one CL in this group.

Mitosis and cell death

Small numbers of mitotic figures were observed in CL on each of the 4 days studied (Table 1), with no significant differences between days. Dividing cells included endothelial cells (Fig. 2), smooth muscle cells of the walls of blood vessels, fibrocytes, and occasional small, unidentified cells in the interstitium. No mitotic figures were observed in 10 000 luteal cells examined. It was not possible to be certain that mitosis did not occur in small luteal cells, due to difficulties in cell identification by light microscopy; however, no clear evidence of small luteal cell division was seen, and most of the small number of mitotic figures observed were clearly not in cells of this type.

Dead cells (Fig. 5) were very rarely observed up to and including Day 100 (Table 1), and no evidence of cell death was seen in the 7500 large luteal cells examined up to this stage. Dead cells had increased substantially in numbers by Day 142, but were unevenly distributed among the 5 CL in this group. Degenerating cellular debris within the lumina of small blood vessels was not counted for the purposes of this study. In view of the marked cytoplasmic and nuclear condensation accompanying cell death it was frequently difficult or impossible to identify the cells involved other than by their location (e.g. within the walls of blood vessels). A total of 3 large luteal cells was identified as dead among the 2500 cells of this type counted on Day 142, all within the single CL showing the most marked regressive changes.

Progesterone

Luteal tissue progesterone content showed no significant change over the four stages of pregnancy examined (Table 1). However, variability between CL was very great at Day 142 (range 9-5-136-7 nmol/g).

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Fig. 4. Luteal tissue, Day 60. The same cell types as in Fig. 4 are identified, and a small luteal cell contains an intranuclear inclusion (arrow). × 2500.

Fig. 5. Luteal tissue, Day 142. Cell identification as above. At this stage large luteal cells contain fewer, larger, dense granules and abundant lipid droplets. The nucleus (arrow) of one large luteal cell is very irregular in outline, and an unidentified dead cell (DC) is present. × 2500.

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Table 1. Measures of corpus luteum characteristics at four stages of pregnancy in the ewe

<table>
<thead>
<tr>
<th>Stage of pregnancy (days)</th>
<th>30</th>
<th>60</th>
<th>100</th>
<th>142</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume density (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells and pericytes</td>
<td>14.2 ± 1.5</td>
<td>11.5 ± 1.6</td>
<td>11.9 ± 2.7</td>
<td>14.4 ± 2.3</td>
</tr>
<tr>
<td>Large luteal cells</td>
<td>41.1 ± 5.4</td>
<td>40.3 ± 5.7</td>
<td>40.4 ± 3.9</td>
<td>40.8 ± 4.4</td>
</tr>
<tr>
<td>Small luteal cells</td>
<td>18.2 ± 2.0</td>
<td>21.6 ± 3.2</td>
<td>22.3 ± 2.8</td>
<td>18.0 ± 6.8</td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>8.7 ± 2.5</td>
<td>7.9 ± 2.1</td>
<td>8.2 ± 2.5</td>
<td>8.6 ± 4.2</td>
</tr>
<tr>
<td>Other cell types or unidentified</td>
<td>2.8 ± 1.6</td>
<td>2.2 ± 1.1</td>
<td>1.3 ± 0.2</td>
<td>6.6 ± 6.0</td>
</tr>
<tr>
<td>Vessel lumen</td>
<td>3.2 ± 1.2</td>
<td>2.6 ± 1.3</td>
<td>3.9 ± 1.5</td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td>Intercellular space</td>
<td>11.8 ± 2.9</td>
<td>13.9 ± 3.5</td>
<td>12.0 ± 2.8</td>
<td>9.1 ± 1.8</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

| **Cytoplasmic:nuclear ratio** |      |      |      |      |
| Endothelial cells and pericytes | 3.5 ± 0.5 | 3.6 ± 0.9 | 3.4 ± 0.6 | 2.8 ± 0.6 |
| Large luteal cells             | 35.5 ± 6.8 | 54.3 ± 29.8 | 49.6 ± 11.1 | 61.0 ± 24.2 |
| Small luteal cells             | 8.6 ± 0.8  | 9.1 ± 1.4  | 10.2 ± 1.3 | 7.6 ± 1.5  |
| Fibrocytes                     | 5.8 ± 1.5  | 6.6 ± 1.6  | 5.0 ± 1.5  | 4.9 ± 0.9  |

| **Cell Volume (μm³ × 10⁻³)** |      |      |      |      |
| Endothelial cells and pericytes | 0.64 ± 0.05 | 0.49 ± 0.05 | 0.49 ± 0.12 | 0.54 ± 0.09 |
| Large luteal cells             | 27.3 ± 10.8 | 25.8 ± 3.2 | 28.3 ± 4.6 | 23.2 ± 9.8 |
| Small luteal cells             | 2.6 ± 0.6  | 3.0 ± 0.5  | 3.3 ± 1.0  | 2.9 ± 0.9  |
| Fibrocytes                     | 1.5 ± 0.4  | 1.6 ± 0.6  | 1.6 ± 0.6  | 1.6 ± 0.5  |

| **No. of cells/mm³ × 10⁻³** |      |      |      |      |
| Endothelial cells and pericytes | 220.1 ± 12.0 | 234.8 ± 33.6 | 253.6 ± 64.4 | 269.5 ± 56.0 |
| Large luteal cells             | 17.1 ± 5.7  | 15.7 ± 2.1  | 14.7 ± 2.6  | 23.2 ± 15.0 |
| Small luteal cells             | 73.4 ± 15.7 | 73.0 ± 6.0  | 73.9 ± 26.7 | 64.3 ± 19.9 |
| Fibrocytes                     | 61.0 ± 17.4 | 51.8 ± 7.9  | 52.6 ± 15.8 | 81.7 ± 67.1 |
| Other cell types or unidentified | 49.6 ± 33.6 | 42.2 ± 12.6 | 51.3 ± 35.2 | 142.4 ± 146.1 |
| Total                          | 421.2 ± 46.1 | 417.5 ± 40.8 | 446.1 ± 136.0 | 581.1 ± 195.1 |

| **Mitosis and cell death** |      |      |      |      |
| No. of cells counted/CL (%)  | 2500 | 2500 | 2500 | 2500 |
| No. of mitotic figures/CL (%) | 1.0 ± 1.2 | 0.8 ± 0.8 | 0.6 ± 0.9 | 0.6 ± 0.9 |
| No. of dead cells/CL (%)     | 0   | 0.2 ± 0.4 | 0   | 5.8 ± 5.4*** |

| **Tissue content of progesterone and DNA** |      |      |      |      |
| Progesterone (nmol/g)         | 55.2 ± 15.9 | 84.9 ± 39.2 | 92.3 ± 19.2 | 58.0 ± 53.0 |
| DNA (mg/g)                    | 2.6 ± 0.5  | 2.5 ± 0.8  | 2.4 ± 0.3  | 6.9 ± 6.3  |
| Estimated† no. of cells/mg × 10⁻³ | 317.9 ± 66.2 | 307.9 ± 94.9 | 312.0 ± 67.7 | 848.9 ± 777.8 |

Values are mean ± s.d. for 5 CL per day.

**P < 0.01 (ANOVA/SMK) compared with all other days.
†Estimated from DNA content and a value of 8.14 pg/cell DNA (Rodgers et al., 1984).

**DNA**

DNA content remained stable up to Day 100 (Table 1). The mean value at Day 142 (actual values 2.9, 3.6, 4.2, 5.7 and 18.1 mg/g) was higher, but not significantly higher. However, when the single highest value was excluded from consideration, DNA content on Day 142 (4.1 ± 1.2 mg/g, n = 4) was higher than on any other day (P < 0.05, ANOVA/SMK).

When total numbers of cells/mg luteal tissue were calculated from these DNA measurements (Table 1), values up to Day 100 were consistently ~25% lower than the morphometric estimates of
cells/mm$^3$ (Table 1). At Day 142 a higher (not significant) mean estimate of total cells/mg was obtained when all 5 CL in this group were included. However, if the CL showing the highest value for DNA was excluded, the estimate in this group would have been $505.8 \pm 148.3 \times 10^3$ cells/mg ($n = 4$), a significant increase compared with any other day ($P < 0.05$) and again lower than the morphometric estimate of cells/mm$^3$ on Day 142.

**Discussion**

Taken together, the observations reported here show a remarkable consistency in the size and cellular composition of the CL of the pregnant ewe until at least two-thirds of the way through pregnancy. Furthermore, there was no reduction in luteal progesterone content over this period. Thus in none of the measures examined here was there any evidence of luteal decline around Day 50, when the essential role of the CL in the maintenance of pregnancy comes to an end. Obviously this does not exclude the possibility that biochemical changes are occurring before structural signs of regression are apparent.

Clear evidence of luteal decline was obtained during the final month of pregnancy. Luteal weight fell significantly, and structural evidence was also consistent with the onset of luteal regression before parturition. However, regressive changes appeared to begin and/or proceed at a very uneven rate in different animals, as seen in terms of both luteal weight and histological structure. Evidence of an actual net loss of cells from the CL in late pregnancy can be drawn from the pattern of substantial weight loss accompanied by little if any increase in the numbers of cells of the major types per unit volume. This loss was apparently balanced in part by an influx of leucocytes into the luteal tissue, associated with elevated amounts of DNA per unit weight of tissue. As shown previously (O'Shea & Wright, 1985), structural luteal regression continues at an accelerated rate after parturition.

The presence of high levels of tissue progesterone until Day 100, and even until Day 142 in some CL, is consistent with earlier evidence that progesterone synthetic function is sustained into late pregnancy (Edgar & Ronaldson, 1958; Lindner et al., 1964; Linzell & Heap, 1968). However, it is not necessarily true that tissue levels of progesterone are an accurate indicator of the extent of progesterone-synthetic function, as Bjersing et al. (1970) have shown that luteal tissue progesterone levels remain high in hysterectomized animals in which blood concentrations of progesterone are low.

At least until Day 100, the composition of the luteal tissue in both qualitative and quantitative terms appeared to be similar to that which has been observed in sheep CL of the cycle (Rodgers et al., 1984; O'Shea et al., 1986; Farin et al., 1986), although the individual large luteal cells of pregnancy may be slightly larger than those of the cycle. Each of the major cell types of cyclic luteal tissue (O'Shea et al., 1979) was represented, and distinctive populations of large and small luteal cells were identifiable in numbers similar to those seen in the cycle. Large luteal cells showed neither mitosis nor death up to Day 100, and the numbers of these cells present could be accounted for in full by persistence of cells formed during the genesis of the CL shortly after ovulation. These numbers are in turn similar to those of granulosa cells in preovulatory follicles (O'Shea et al., 1987). Hence in quantitative terms there is no need to seek additional sources of large luteal cells. Small luteal cells also remained in numbers similar to those found in the cycle, showed little if any mitosis or death up to Day 100, and were ultrastructurally quite distinct from large luteal cells at all stages. Even the proportion of small luteal cells whose nuclei contained cytoplasmic inclusions remained unchanged throughout the period studied. While these observations cannot disprove the idea that some cellular conversions may occur during pregnancy, they are certainly entirely consistent with the simpler view that large and small luteal cells, once formed, are discrete populations which persist more or less unchanged until the onset of regressive changes during late pregnancy. The possibility that sheep differ from cattle in this regard warrants serious consideration.
The idea that there may be stem cells present in the CL of the ewe which give rise progressively to new small and thence large luteal cells (Niswender et al., 1985) also received no support from this study. Neither here nor in other ultrastructural studies of the CL (O'Shea et al., 1979; Rodgers et al., 1984) has any population been observed which might fit this role. However, since stem cells could be present in small numbers their existence must remain a possibility.

In an earlier study (Rodgers et al., 1984), estimates of the total number of cells per CL by morphometry and by DNA measurement were closely similar. However, in the present study a lower estimate was obtained by DNA measurement than by morphometry. This would in part have been due to the different basis of measurement, per unit weight for DNA and per unit volume for morphometry. Although not measured here, luteal specific gravity exceeds 1 and hence the comparison is not equal. There is also likely to have been some inbuilt bias towards an overestimate in the morphometric data in as far as nuclear shape varied more than the shape factors used would allow. This would be true particularly for the category of 'other cell types or unidentified', whose nuclei are pleomorphic and for which no published shape factor could adequately be applied. Furthermore, irregularity of nuclear shape, particularly in the large luteal cell population, seemed to increase in late pregnancy.

As reported earlier (O'Shea et al., 1979), and shown here in Fig. 3, 'secretory granules' are still present in substantial numbers in the large luteal cells of CL of pregnancy in the ewe. Theodosis et al. (1986) have shown that at least a high proportion of granules of this type contain oxytocin and its neurophysin in cyclic ewes. However, the oxytocin content of luteal tissue in pregnant ewes is low even by Day 20 (Flint & Sheldrick, 1986), and luteal cells from Day 25 of pregnancy fail to produce measurable amounts of oxytocin in vitro (Harrison et al., 1987), raising the possibility that secretory granules in pregnant ewes might contain some other protein or polypeptide. Relaxin is clearly a possible candidate, but as yet the specific localization of relaxin in sheep has not been achieved.

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