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Morphometric Analysis of Cell Types in the Ovine Corpus Luteum throughout the Estrous Cycle[1]

C. E. FARIN, C. L. MOELLER, H. R. SAWYER, F. GAMBONI, and G. D. NISWENDER[2]

Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

ABSTRACT

The cellular composition of ovine corpora lutea obtained during the early (Day 4), mid (Days 8 and 12), and late (Day 16) stages of the estrous cycle was determined by morphometric analysis. Individual corpora lutea were collected via midventral laparotomy from a total of 19 ewes. A center slice from each corpus luteum was processed for electron microscopy and subsequent morphometric analysis of the numbers and sizes of steroidogenic and nonsteroidogenic cells.

Luteal weight progressively increased throughout the estrous cycle ($p<0.05$). Corpora lutea collected on Day 16 were assigned to one of two subgroups on the basis of gross appearance and weight: nonregressed (NR, 542 ± 25 mg) or regressed (R, 260 ± 2 mg). There were no significant changes in the proportion of the corpus luteum occupied by small luteal cells (19 ± 2%) or large luteal cells (36 ± 1%) throughout the estrous cycle. The total number of steroidogenic cells per corpus luteum increased from 21.8 ± 3.7 (× 10^6) on Day 4 to 61.7 ± 5.4 (× 10^6) on Day 8 ($p<0.05$) and remained elevated thereafter. The number of small luteal cells was 10.0 ± 2.7 (× 10^6), 39.7 ± 1.4 (× 10^6), 46.1 ± 5.8 (× 10^6), 49.0 ± 13.7 (× 10^6), and 29.9 ± 8.6 (× 10^6) on Days 4, 8, 12, 16 (NR), and 16 (R), respectively ($p<0.05$, Day 4 vs. Days 8, 12, 16 NR). In contrast, the number of large luteal cells was 11.8 ± 1.5 (× 10^6) on Day 4 and did not vary significantly during the remainder of the estrous cycle. The numbers of nonsteroidogenic cell types increased ($p<0.05$) from Day 4 to Day 16 (NR) but were decreased in regressed corpora lutea (Day 16 R). Regression was characterized by a 50% decrease ($p<0.05$) in the total number of cells per corpus luteum from 243 ± 57 (× 10^6) on Day 16 (NR) to 125 ± 14 (× 10^6) on Day 16 (R) ($p<0.05$). Small luteal cells remained constant in volume throughout the entire estrous cycle (2520 ± 270 μm^3), whereas large luteal cells increased in size from 5300 ± 800 μm^3 on Day 4 to 16,900 ± 3300 μm^3 on Day 16 (NR) ($p<0.05$). In summary, small luteal cells increased in number but not size throughout the estrous cycle, whereas large luteal cells increased in size but not number.

INTRODUCTION

The corpus luteum of the ewe is composed of two types of steroidogenic cells, commonly referred to as "small" and "large" luteal cells (Warbritton, 1934; Deane et al., 1966; O'Shea et al., 1979). These cell types differ morphologically (O'Shea et al., 1979, 1980) and biochemically (Fitz et al., 1982; Glass et al., 1985; Hoyer and Niswender, 1985). Briefly, small luteal cells are spindle-shaped cells, approximately 12–18 μm in diameter. These cells contain irregularly shaped nuclei, numerous mitochondria, lipid droplets, and an abundance of smooth endoplasmic reticulum characteristic of steroid-secreting cells (O'Shea et al., 1979). They also contain the majority of the luteal receptors for luteinizing hormone (LH) (Fitz et al., 1982) coupled with a highly responsive adenylate cyclase system (Hoyer and Niswender, 1985). In contrast, large luteal cells are predominantly spherical in shape with a diameter ranging from 25 to 40 μm. These cells, characterized by a highly folded plasma membrane and a pronounced basal lamina, also contain spherical nuclei, numerous mitochondria, lipid droplets, and an abundance of smooth endoplasmic reticulum. In contrast to small luteal cells, large luteal cells contain isolated stacks of rough endoplasmic

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reticulum and a unique class of electron-dense secretory granules that are known to be released by exocytosis (Gemmell et al., 1974; Sawyer et al., 1979; Paavola and Christensen, 1981). Compared to small luteal cells, large luteal cells have few receptors for LH but contain the majority of receptors for estradiol (Glass et al., 1985) and prostaglandins $F_{2\alpha}$ and E_2 (Fitz et al., 1982). Although large luteal cells contain an active adenylate cyclase system, this system does not appear to be involved in regulating the synthesis or secretion of progesterone in this cell type (Hoyer and Niswender, 1985).

Three types of nonsteriodogenic cells can be readily identified in the ovine corpus luteum: fibroblasts, capillary endothelial cells and pericytes (Warbritton, 1934; O'Shea et al., 1979, 1980; Rodgers et al., 1984). Both small and large luteal cells are found closely associated with these nonsteroidogenic cells.

On the basis of an analysis of the size-distribution of luteal cells after enzymatic dissociation, Niswender et al. (1985) and Schwall et al. (1986) concluded that the corpus luteum undergoes dynamic changes in cellular composition throughout the luteal phase of the estrous cycle. These authors have reported that the number of both small and large luteal cells increases from early to midcycle, peaking at approximately Day 8 to Day 12 and declining thereafter. These data are based on cell counts obtained after enzymatic dissociation of luteal tissue; therefore, differential losses of either cell type could occur during the dissociation procedure. Such phenomena have been reported to occur (Rodgers and O'Shea, 1982). To overcome this problem, we have examined the changes in the cellular composition of the ovine corpus luteum throughout the estrous cycle by morphometric analysis of fixed luteal tissue.

MATERIALS AND METHODS

Animals and Tissue Preparation

Nineteen western range ewes of mixed breeding that exhibited normal estrous cycles were used in this study. Corpora lutea were collected by midventral laparotomy under pentobarbital anesthesia on Days 4 (n=4 ewes), 8 (n=4 ewes), 12 (n=5 ewes), and 16 (n=6 ewes) of the estrous cycle. All tissue collections were conducted during the breeding season (December 1984). Individual corpora lutea were decapsulated, weighed, and their volume determined by water displacement in a graduated cylinder (Wiebel, 1979). A Stadie-Riggs hand microtome was used to cut each corpus luteum into a series of 0.5-mm slices on a plane parallel to the surface of the ovary. A single slice from the center of each corpus luteum was then divided into 6 tissue blocks, approximately equal in size, which were fixed by immersion in a solution of 4% glutaraldehyde-0.1 M cacodylate buffer (pH 7.3) containing 6% sucrose. After primary fixation, the tissue blocks were washed in cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Polybed 812 (Polysciences, Warrington, PA). Thin sections were prepared from randomly selected areas from each of the 6 tissue blocks per ewe. Sections were mounted on 300 mesh hexagonal grids, stained with uranyl acetate and lead citrate, and examined with a Philips 400T electron microscope. A sequential pattern of 5 negatives was taken at a magnification of 2800X from a randomly chosen portion of a single, thin section representing one tissue block. This procedure was repeated for each tissue block from each ewe. Negatives were printed at a final magnification of 5300X.

Morphometric Analysis

A Ziess Videoplan Image Analyzer (Carl Zeiss Inc., New York, NY), equipped with a digitizing pad and stereology software, was used to collect primary data required for areal profile analysis (Wiebel, 1979). All data from each tissue block were corrected for shrinkage to 92% of original size due to fixation. This figure was based on a comparison of the linear measurements of 2-mm-square fresh tissue blocks taken before and after fixation and embedding procedures.

The total number of each cell type present in whole corpora lutea (including small and large luteal cells, fibroblasts, capillary endothelial cells and pericytes and eosinophils) was determined by analysis of the profiles of nuclei of the particular cell type of interest using the method of Wiebel and Gomez (1962). This procedure has been described in detail by Rodgers et al. (1984). Briefly, an areal profile analysis was used to determine volume density and number of nuclei per unit area. Calculations per corpus luteum were based on the sum of areas pooled over all the tissue blocks from that corpus luteum. A total of 26,101 ± 403 μm^2 of tissue from each ewe was used in the analysis. The number of cells per unit volume (N_v) was calculated using the following formula:

$$N_v = \frac{1}{\beta} \frac{N_A^{1.5}}{V_v^{0.5}}$$

(Wiebel, 1979), where N_v is the number of cell nuclei per unit volume, N_A is the number of nuclei per unit area of the micrograph, and V_v is the volume density of nuclei (equal to the sum of the areas of the nuclei of interest from the electron micrographs divided by the total area of the electron micrographs). The correction factor for nuclear shape (β) was given a value of 1.382 for small luteal cells and large luteal cells, 1.7 for fibroblasts, and 1.9 for capillary endothelial cells and pericytes (Rodgers et al., 1984). In addition, β was given a value of 1.382 for eosinophils that had lobulated nuclei with a spherical shape. The total number of cells per corpus luteum was the product of the number of cells per unit volume and the volume of the corpus luteum. The mean cell volume was equal to the volume density of each cell type divided by the number of cells of that type per unit volume. The mean cell diameter was determined based on the calculated mean cell volume assuming a spherical shape.

Statistical Analysis

All data were evaluated using a one-way analysis of variance. When a significant F-statistic was obtained, a Duncan's procedure was used for separation of means. When heterogeneous variance was present, a log transformation of the data was used prior to final analysis.

RESULTS

Portions of the data regarding weight of corpora lutea used in this study have been previously reported (Schwall et al., 1986). The weight of corpora lutea progressively increased from 158 ± 10 mg on Day 4 to a maximum of 649 ± 35 mg on Day 12 ($p<0.05$; Table 1). Six corpora lutea were collected on Day 16 of the cycle; however, it was evident that there were 2 distinct subgroups with significantly different mean weights (542 ± 25 mg and 260 ± 2 mg; $p<0.05$). The heavier corpora lutea appeared normal and well-vascularized, whereas the lighter corpora lutea were whitish, as is typical of regressing corpora lutea. Accordingly, the subgroup of Day 16 corpora lutea weighing >500 mg will be referred to as "Day 16 nonregressed" corpora lutea, whereas the subgroup with mean weight <300 mg will be referred to as "Day 16 regressed" corpora lutea.

Figure 1 illustrates the various steroidogenic and nonsteroidogenic cell types present in Day 12 corpora lutea as they appear at the level of magnification used for morphometric analysis (5300×). Structural characteristics used to identify the respective cell types were as follows: 1) large luteal cells—large spherical nucleus, presence of membrane-bound secretory granules which are released via exocytosis,

TABLE 1. Summary of luteal weight, number of nuclei, and total tissue area used for morphometric analysis.

Day of cycle	No. of ewes	Mean (± SE) weight of corpora lutea (mg)	No. nuclei counted				Total area analyzed (10^3 μm²)
			SLC*	LLC†	FB‡	CE/P§	
4	4	158 ± 10[a]	45	65	96	92	101.6
8	4	510 ± 31[b]	61	43	37	106	100.5
12	5	649 ± 35[c]	74	33	62	147	135.6
16 NR‖	3	542 ± 25[b]	47	16	54	119	78.9
16 R#	3	260 ± 2[d]	49	22	82	84	79.4

*Small luteal cells.

†Large luteal cells.

‡Fibroblasts.

§Capillary endothelial cells and pericytes.

‖Nonregressed (see text for details).

#Regressed (see text for details).

[a–d]Means differ, $p<0.05$.

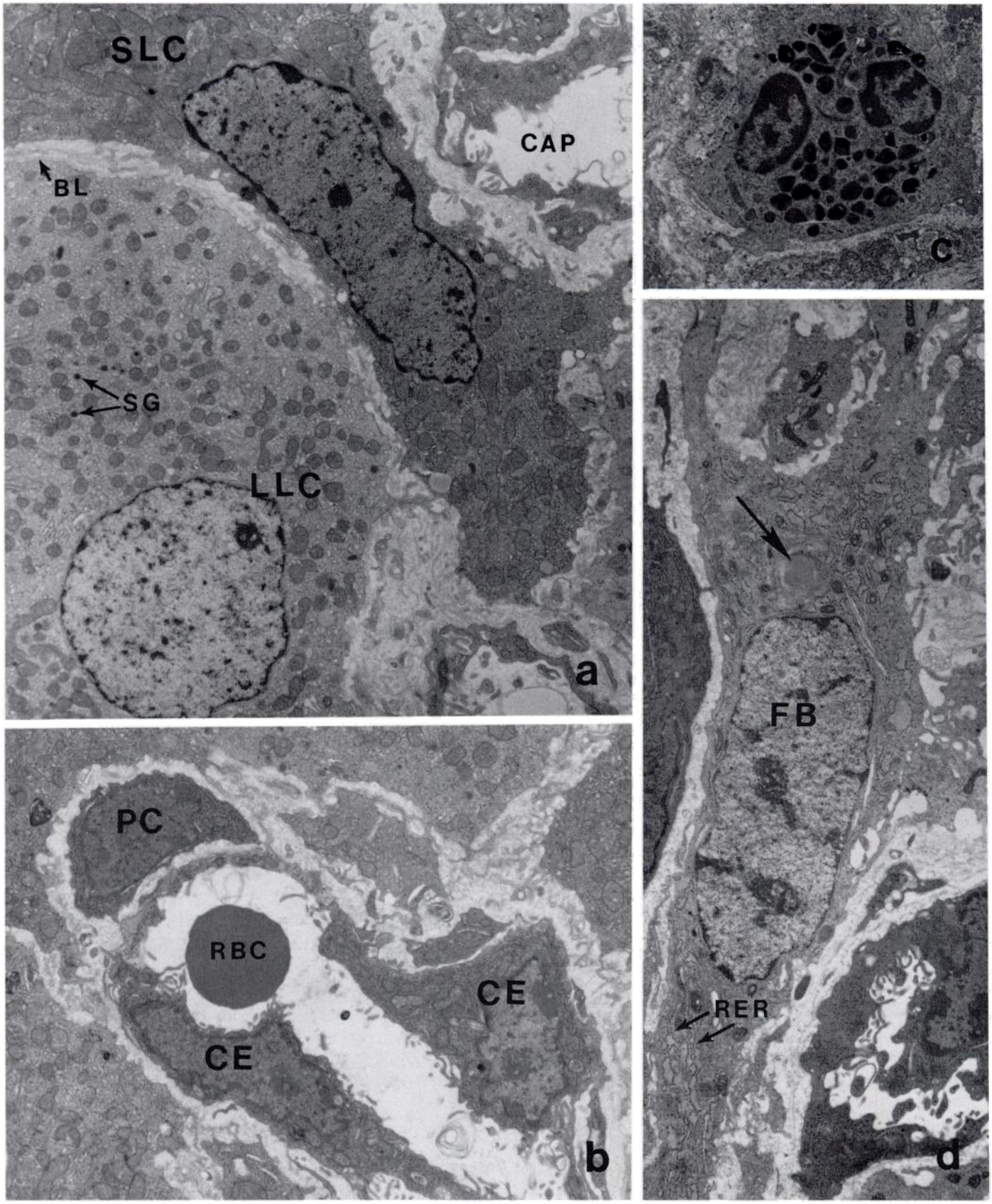

FIG. 1. Cell types found in the ovine corpus luteum. (*a*) Steroidogenic cells: small luteal cell (*SLC*), large luteal cell (*LLC*) (×5300); (*b*) Vascular cells: capillary endothelial cell (*CE*), pericyte (*PC*) (×5300); (*c*) eosinophil (×5300); (*d*) fibroblast (*FB*), note lipid droplet within cytoplasm (*arrow*) (×5300). Capillary space (*CAP*), basal lamina (*BL*), secretory granule (*SG*), red blood cell within capillary space (*RBC*), and rough endoplasmic reticulum (*RER*).

isolated stacks of rough endoplasmic reticulum, numerous mitochondria, highly folded plasma membrane associated with a pronounced basal lamina, and presence of tubular smooth endoplasmic reticulum and lipid droplets; 2) small luteal cells—convoluted nucleus, large amounts of tubular smooth endoplasmic reticulum and lipid droplets, lack of secretory granules and/or rough endoplasmic reticulum, plasma membrane with fewer microvillus-like projections, and the absence of a distinct basal lamina; 3) fibroblasts—presence of continuous rough endoplasmic reticulum with dialated cisternae, lack of tubular smooth endoplasmic reticulum, and elongated nucleus with large amounts of heterochromatin; 4) capillary endothelial cells and pericytes—proximity to capillary vessels, cytoplasm with few organelles, prominent aggregates of heterochromatin in nuclei, a distinct basal lamina, and a large nuclear to cytoplasmic ratio. A total of 1334 cell nuclei were used in the morphometric analysis, with a mean of 267 ± 17 cell nuclei counted on each day of the cycle. A breakdown of the number of nuclei of the four major cell types by day of cycle is shown in Table 1.

Volume Density of Luteal Cell Types

There were no significant changes in the volume density of small luteal cells or large luteal cells over the course of the estrous cycle. Collectively, steroidogenic cells comprised 55 ± 1% of luteal tissue on any day of the cycle with 19 ± 2% composed of small luteal cells and 36 ± 1% composed of large luteal cells (Table 2). In contrast, there were significant changes in the volume densities of nonsteroidogenic cell types. The volume density of fibroblasts decreased from Day 4 to Day 8 ($p<0.05$; Table 2) and remained constant throughout the remainder of the cycle. The proportion of the corpus luteum occupied by capillary beds (i.e., capillary endothelial cells, pericytes and capillary luminal space) remained constant at 10 ± 0.4% from Days 4 to 16 nonregressed and then decreased to 6 ± 1% in Day 16 regressed corpora lutea ($p<0.05$; Table 2). There was a nonsignificant increase in the volume density of extracellular matrix as the corpus luteum developed from Day 4 to Day 12. There was a significant increase ($p<0.05$) in the amount of nonidentifiable tissue components ("other"; Table 2) associated with regressed corpora lutea.

TABLE 2. Volume density (%) of luteal tissue components throughout the estrous cycle.

Component	Day of cycle*				
	4	8	12	16NR†	16 R‡
Total steroidogenic§	53 ± 3	56 ± 2	56 ± 2	56 ± 2	54 ± 4
Small luteal	15 ± 1	18 ± 1	23 ± 2	22 ± 3	18 ± 4
Large luteal	38 ± 3	38 ± 2	33 ± 3	34 ± 3	36 ± 1
Fibroblasts	15 ± 3^{a}	7 ± 1^{b}	6 ± 0.4^{b}	9 ± 1^{b}	8 ± 1^{b}
Capillaries	9 ± 1^{a}	11 ± 0.3^{b}	10 ± 1^{a,b}	10 ± 1^{a,b}	6 ± 1^{c}
CE/P	7 ± 1^{a}	9 ± 3^{a}	9 ± 6^{a}	8 ± 1^{a}	5 ± 1^{b}
Luminal space	1 ± 3^{a,b}	2 ± 1^{b,c}	1.5 ± 1^{b,c}	1.5 ± 2^{c}	0.7 ± 2^{a}
Eosinophils	0.1 ± 0.05	0 ± 0	0.1 ± 0.05	0.2 ± 0.2	0 ± 0
Matrix	23 ± 1	26 ± 1	27 ± 1	25 ± 3	26 ± 2
Other	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}	0.5 ± 0.2^{a}	0.2 ± 0.1^{a}	6.3 ± 0.1^{b}

*Mean ± SE.

†Nonregressed (see text for details).

‡Regressed (see text for details).

§Steroidogenic cells classified on a morphological basis.

$^{a-d}$Means within rows with different superscripts differ, $p<0.05$.

TABLE 3. Number of cells per corpus luteum throughout the estrous cycle (X 10^6).

Type of cell	Day of cycle* 4	8	12	16 NR†	16 R‡
Total steroidogenic§	21.8 ± 3.7[a]	61.7 ± 5.4[b]	61.3 ± 6.0[b]	61.9 ± 17.4[b]	38.5 ± 10.3[a,b]
Small luteal	10.0 ± 2.7[a]	39.7 ± 1.4[b]	46.1 ± 5.8[b]	49.0 ± 13.7[b]	29.9 ± 8.6[a,b]
Large luteal	11.8 ± 1.5	22.0 ± 5.1	15.2 ± 3.1	13.0 ± 5.0	8.6 ± 1.9
Fibroblasts	21.6 ± 6.5[a]	21.5 ± 6.6[a]	43.3 ± 5.8[a,b]	53.8 ± 18.7[b]	45.4 ± 4.1[a,b]
Endothelial cells and pericytes	18.7 ± 5.1[a]	71.2 ± 14.6[b,c]	94.6 ± 7.6[c,d]	119.0 ± 18.4[d]	41.3 ± 10.3[a,b]
Eosinophils	5.7 ± 4.0	0 ± 0	2.8 ± 2.8	8.7 ± 4.5	0 ± 0
Total	63.3 ± 12.6[a]	154.4 ± 21.4[b,c]	202.0 ± 9.9[b,c]	243.5 ± 57.3[c]	125.2 ± 13.9[b]

*Mean ± SE.

†Nonregressed (see text for details).

‡Regressed (see text for details).

§Steroidogenic cells classified on a morphological basis.

[a–d]Means within rows with different superscripts differ, $p<0.05$.

Numbers of Luteal Cells

The number of steroidogenic cells per corpus luteum increased from Day 4 to Day 16 in nonregressed corpora lutea ($p<0.05$; Table 3). Concurrent with luteal regression, however, was a 38% decrease in the number of steroidogenic cells. Changes in the numbers of steroidogenic cells throughout the cycle were accounted for primarily by alterations in the number of small luteal cells. Small luteal cell number increased fourfold from Day 4 to Day 8 ($p<0.05$) and remained at this level until Day 16 in nonregressed corpora lutea. With luteal regression, the number of small luteal cells decreased 39%; however, this decrease was not significant. There were no significant changes in the number of large luteal cells throughout the estrous cycle.

The number of nonsteroidogenic cells fluctuated throughout the estrous cycle. From Day 4 to Day 8, the number of fibroblasts remained constant (Table 3); however, they increased twofold by Day 12 and continued increasing until Day 16 in nonregressed corpora lutea. The most dramatic change in cell number was associated with the vascular compartment. The number of capillary endothelial cells and their associated pericytes increased fourfold from Days 4 to 8 of the cycle ($p<0.05$; Table 3). In the absence of luteal regression, these increases continued ($p<0.05$) throughout Day 16 of the cycle. Luteal regression was characterized by a threefold decrease in the number of cells in the vascular compartment ($p<0.05$; Table 3).

Although eosinophils were present in several of the corpora lutea, they were few in number and their presence was not associated with any particular stage of the estrous cycle.

There was a significant increase in total number of cells per corpus luteum from Day 4 to Day 8 ($p<0.05$; Table 3) that was accounted for by increases in the numbers of both small luteal cells and cells of the vascular compartment. The continued increase in total cell numbers in Day 12 and Day 16 nonregressed corpora lutea was due to increases in small luteal cells, vascular cell types, and fibroblasts. Although the 50% decrease ($p<0.05$) in total number of cells in regressed corpora lutea was associated with a decrease in the number of all cell types, this was pri-

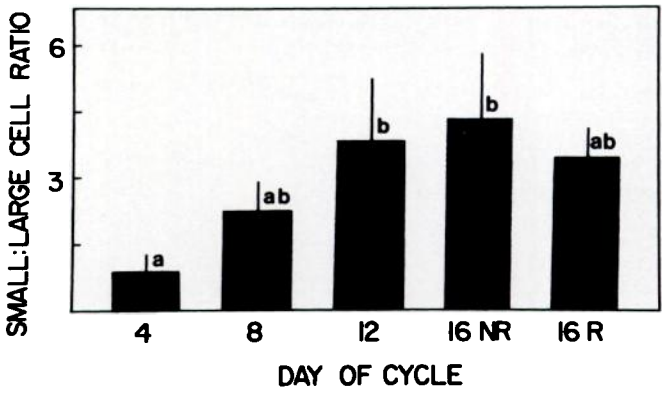

FIG. 2. Ratio of the number of small luteal cells to large luteal cells throughout the estrous cycle. Each *bar* represents the mean ± SE. Different superscripts (a,b) denote differences at the $p<0.10$ level of significance.

TABLE 4. Cell volume and diameter of cell types throughout the estrous cycle.

	Day of cycle*				
	4	8	12	16 NR†	16 R‡
Steroidogenic cells§					
Small luteal					
Cell volume (10^3 μm^3)	2.6 ± 0.5	2.2 ± 0.1	3.3 ± 0.4	2.8 ± 0.9	1.7 ± 0.3
Cell diameter (μm)	16.9 ± 1.0	16.2 ± 0.3	18.2 ± 0.8	17.2 ± 1.8	14.8 ± 0.8
Large luteal					
Cell volume	5.3 ± 0.8[a]	9.9 ± 2.0[a,b]	15.2 ± 2.0[b,c]	16.9 ± 3.3[c]	11.7 ± 1.4[a,b,c]
Cell diameter	21.5 ± 1.0[a]	26.3 ± 1.7[b]	30.5 ± 1.2[b,c]	31.5 ± 1.2[c]	28.0 ± 1.1[b,c]
Nonsteroidogenic cells					
Fibroblasts					
Cell volume (10^3 μm^3)	1.2 ± 0.1[a,b]	1.8 ± 0.3[a]	0.9 ± 0.2[b,c]	1.0 ± 0.2[b,c]	0.5 ± 0.03[c]
Cell diameter (μm)	13.2 ± 0.3[a,b]	14.9 ± 0.9[a]	12.0 ± 0.7[b]	12.2 ± 0.8[b]	9.6 ± 0.2[c]
Capillary endothelial/pericyte					
Cell volume	0.8 ± 0.03[a]	0.7 ± 0.08[a,b]	0.6 ± 0.03[b,c]	0.4 ± 0.03[d]	0.3 ± 0.05[d]
Cell diameter	10.9 ± 0.4[a]	10.9 ± 0.4[a]	10.4 ± 0.2[a]	9.0 ± 0.2[b]	8.7 ± 0.4[b]

*Mean ± SE.

†Nonregressed (see text for details).

‡Regressed (see text for details).

§Steroidogenic cells classified on a morphological basis.

[a–d]Means within rows with different superscripts differ, $p<0.05$.

marily due to a decrease in the number of cells in the vascular compartment.

Small Luteal Cell to Large Luteal Cell Ratio

Changes in the ratio of small luteal cells to large luteal cells, based on the number of each cell type per corpus luteum, are depicted in Figure 2. As the estrous cycle progressed, there was a fivefold increase ($p<0.1$) in the small to large cell ratio from Day 4 to Day 16 in nonregressed corpora lutea.

Mean Cell Volume and Diameter

Small luteal cells remained constant in size throughout the estrous cycle, averaging 2520 ± 270 μm^3 in volume and 16.7 ± 0.6 μm in diameter (Table 4). In contrast, large luteal cells progressively increased ($p<0.05$) in size from 5300 ± 800 μm^3 in volume and 21.5 ± 1 μm in diameter on Day 4 to 16,900 ± 3300 μm^3 in volume and 31.5 ± 1.2 μm in diameter on Day 16 in nonregressed corpora lutea. Luteal regression was not associated with any significant changes in size of large luteal cells. The size of nonsteroidogenic cell types decreased as the estrous cycle progressed ($p<0.05$; Table 4).

DISCUSSION

The data presented in this study support the concept that in terms of cellular composition, the corpus luteum is a dynamic structure throughout the estrous cycle. Luteal development occurs continuously with increases in cell number from Day 4 through Days 8, 12 and, in the absence of luteal regression, even to Day 16.

The final tissue magnification chosen for the present study was 2 to 5 times greater than that used in previously published reports (Nett et al., 1976; O'Shea et al., 1984; Rodgers et al., 1984). The increased magnification was chosen to allow a better identification of nuclear and cytoplasmic characteristics of the various cell types in an attempt to increase accuracy in classifying cytoplasmic cross sections. The advantage of the more precise cell identification afforded by higher magnification is coupled, however, with the disadvantage that less tissue can be examined per photomicrograph. Although the total number of nuclei counted and area of tissue examined in this study were smaller than those used in previously published studies (Nett et al., 1976; O'Shea et al., 1984; Rodgers et al., 1984), good agreement on various morphometric parameters was obtained. Based on morphological characteristics, the volume density of cells classified as steroidogenic remained constant throughout the estrous cycle at approximately 55%. This agrees well with reports of 51%, 50%, 52%, and 57% by Nett et al. (1976), O'Shea et al. (1984),

and Niswender et al. (1976, 1986), respectively, but is slightly greater than the 43% figure reported by Rodgers et al. (1984). The volume density of the large and small luteal cells on various days of the cycle is also in good agreement with the reports of Nett et al. (1976), Niswender et al. (1976, 1986), and O'Shea et al. (1984).

In contrast to the constant proportion of the corpus luteum taken up by small and large luteal cells, the volume densities of the various nonsteroidogenic cell types varied with stage of the estrous cycle. Fibroblasts decreased from a volume density of 15% on Day 4 to 7% on Day 8 and remained constant thereafter. The latter value agrees well with previous reports for fibroblast volume density on comparable days of the estrous cycle (Days 9–12) (Nett et al., 1976; O'Shea et al., 1984; Rodgers et al., 1984; Niswender et al., 1986). In addition, the pattern of change in the volume density of fibroblasts is similar to that reported for corpora lutea collected at various stages of gestation in the rat (Meyer and Bruce, 1979). Differences in the volume occupied by vascular elements between this report and those of O'Shea et al. (1984) and Rodgers et al. (1984) are likely due to differences in initial procedures used for fixing recovered corpora lutea (immersion vs. perfusion, respectively; Dharmarajan et al., 1983). However, the decrease in volume of corpora lutea occupied by capillaries after luteal regression is consistent with previous reports of diminished blood flow and morphologic degeneration of capillary beds during normal (Niswender et al., 1975, 1976; Gemmell et al., 1976; O'Shea et al., 1977) and prostaglandin-induced luteal regression in the ewe (Niswender et al., 1975, 1976; Chamley and O'Shea, 1976; Nett et al., 1976; Stacy et al., 1976).

Data from the present study indicate that the number of large luteal cells present on Day 4 (10–12 million cells) does not change during the remainder of the cycle, even in regressed corpora lutea on Day 16 post-estrus. These estimates for large luteal cell numbers are consistent with previous morphological studies of various days during the estrous cycle including Days 9 and 12 (O'Shea et al., 1984; Niswender et al., 1986), as well as for mature (luteal phase) corpora lutea (Rodgers et al., 1984). The number of small luteal cells increased dramatically from Day 4 to Day 8 and did not appear to change until the occurrence of luteal regression, when a slight decrease was noted. These changes in the number of small luteal cells throughout the cycle appear to account for changes seen in the small to large cell ratio throughout luteal development. It may seem difficult to reconcile the present data with the hypothesis of luteal cell conversion supported by the observations of Donaldson and Hansel (1965), Alila and Hansel (1984), and Farin et al. (1985). However, until we understand the control of luteal cell development and turnover, we cannot conclude that conversion of small luteal cells to large luteal cells does not occur during the estrous cycle.

The numbers of nonsteroidogenic cells continually increased as the estrous cycle progressed. The number of fibroblasts on Days 8 and 12 was the same or slightly higher than that reported previously (O'Shea et al., 1984; Rodgers et al., 1984), whereas the number of capillary endothelial cells and pericytes was similar to or slightly lower than previously reported. The occurrence of continued increases in the number of capillary endothelial cells and pericytes in corpora lutea has also been reported for the pregnant rat throughout gestation (Meyer and Bruce, 1979). It is apparent from the present study that the principal nonsteroidogenic cell types continually increase in number throughout the estrous cycle and are solely responsible for the increases in total cell number seen between Days 8 and 16 of the estrous cycle. In this study, there were few cells that could not be positively identified as to type (estimated to be between 2 and 5 million cells per corpus luteum for Days 4 to 16 nonregressed, data not shown). This is in contrast to approximately 23 million cells classified as 'other' cell types (macrophages, leucocytes, smooth muscle cells, or unidentified cell types) reported by O'Shea et al. (1984) and Rodgers et al. (1984). This difference in classification may be a result of differences in tissue magnification used for the analysis. It is likely that cytoplasmic details could be more clearly identified at the higher magnification used in the present study, which would result in a greater number of positive identifications.

Although the numbers of small luteal cells increased during the estrous cycle, their size remained relatively constant. In contrast, large luteal cells, while remaining constant in number, continually increased in size as the estrous cycle progressed. These observations for large luteal cells are consistent with reports of increasing luteal cell volume throughout gestation in the rat (Meyer and Bruce, 1979). In contrast to reports by Deane et al. (1966), Gemmell et al.

(1974, 1976), and Stacy et al. (1976), we found no significant decrease in the size of luteal cells with the occurrence of luteal regression. The previous authors did not use quantitative morphometric methods to substantiate their conclusions. The fact that small and large luteal cells are similar in size early in the cycle may explain why it is not possible to obtain pure small and large cell preparations from corpora lutea between Days 1 and 6 of the estrous cycle. Thus, references to 'large' and 'small' luteal cells early in the estrus cycle are inaccurate. It may be simpler to refer to these cell types according to the terminology suggested by Foley and Greenstein (1958) as Type I (analogous to 'small luteal cells') and Type II (analogous to 'large luteal cells'). The actual size distribution profiles of both Type I and II cells have yet to be established, and it may be that the actual size ranges for these cells will overlap. Schwall et al. (1986) have shown that the size distribution of steroidogenic luteal cells form a continuum rather than two separate populations. Thus, it is possible that early in the cycle many Type II ('large' luteal) cells may, in fact, have smaller diameters than Type I ('small' luteal) cells.

The observation that Type II cells increase in size as the estrous cycle progresses may indicate that these cells undergo further growth and development after luteinization. It is possible that these cells are not functionally mature early in the cycle when they are smaller in size. It would be of interest to know whether Type II cells contain their full complement of prostaglandin receptors during the early period of luteal development. If they did not, this may explain why corpora lutea early in the estrous cycle are insensitive to injections of prostaglandin $F_{2\alpha}$ although the same number of Type II cells are present as is found later in the cycle when corpora lutea are $PGF_{2\alpha}$-sensitive.

A comparison of the changes in the populations of luteal cells in the present study with that of Schwall et al. (1986), who drew conclusions based on dissociated luteal tissue, shows that although the numbers of small and large luteal cells in these two studies differ, there is good agreement on the relative changes observed throughout most of the estrous cycle. Schwall et al. (1986) reported that the numbers of steroidogenic luteal cells increased dramatically between Days 4 and 8 of the cycle. They also noted an increase in the average size of dissociated luteal cells as the estrous cycle progressed. The increase in the number of Type I luteal cells as well as the progressive increase in the size of Type II luteal cells seen in the present study are consistent with Schwall's observations. In addition, the progressive decline in the mean cell size of nonsteroidogenic cells is consistent with previous reports based on dissociated luteal tissue (Niswender et al., 1985). Two trends observed by Schwall et al. (1986) were not observed in the present study. First, Schwall and coworkers reported an increase in the number of large luteal cells between Days 4 and 8 of the estrous cycle. In their study, classification of luteal cell types was based on measurements of cell diameter. In the present study, however, it was demonstrated that Type II (large luteal) cells increase in size between Days 4 and 8 of the cycle. Thus, it is possible that some Type II cells were classified by Schwall et al. (1986) as 'small' luteal cells on Day 4 but as 'large' luteal cells on Day 8. The second trend observed by Schwall et al. (1986), which was not observed in the present study, was a decrease in the number of small-sized steroidogenic cells (presumably Type I cells) in Day 16 nonregressed corpora lutea. It is possible that the small-sized steroidogenic cells are more fragile later in the cycle and do not survive during the dissociation process.

In summary, corpora lutea undergo continual development throughout the entire estrous cycle. The number of steroidogenic cells increases early in the cycle while the number of nonsteroidogenic cells tends to increase later in the cycle. Type I, or small luteal cells, were found to increase in number but not size as the estrous cycle progressed. In contrast, Type II, or large luteal cells, were found to increase in size but not number throughout luteal development.

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