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Quantitative Cell Composition of Human and Bovine Corpora Lutea from Various Reproductive States[1]

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ABSTRACT

The cell composition of human and bovine corpora lutea (CL) from various reproductive states was investigated by computerized video-based interactive Bioquant image analysis system IV and by light microscope immunocytochemistry. Human and bovine CL contained more nonluteal cells than luteal cells. Human CL contained a lower number of luteal and a greater number of nonluteal cells than bovine CL. Regardless of the reproductive state, human CL contained more small luteal cells than large luteal cells. In all reproductive states except in the late luteal phase, the bovine CL also contained more small luteal cells than large luteal cells. The average sizes of all the cells in human CL were smaller than in bovine CL. Human CL contained more vascular space than bovine CL during mid and late luteal phases.

The number of luteal cells increased and nonluteal cells decreased from early to mid luteal phase, and then luteal cells decreased and nonluteal cells increased in late luteal phase and in degenerating human and bovine CL. While the change of number of small and large luteal cells first occurred from early to mid luteal phase in human CL, it did not take place until the late luteal phase in bovine CL. The average size of large luteal cells in humans and of small luteal cells in cattle did not change, whereas size of the other cells changed in different reproductive states in both human and bovine CL. The cell composition of term pregnancy human CL was similar to mid or late luteal phase, whereas the cell composition of early pregnancy bovine CL was similar to mid luteal phase.

The number of macrophages in human CL increased from early to mid luteal phase and from mid to late luteal phase. The number of fibroblasts, on the other hand, decreased from early to mid luteal phase and then nearly doubled in late luteal phase CL. The vascular space increased from early to mid luteal phase, followed by a considerable decline in late luteal phase human and bovine CL.

INTRODUCTION

Corpus luteum (CL) is a transient endocrine gland formed from the Grafiaan follicle following ovulation [1]. It grows, develops, and reaches structural and functional maturity by mid luteal phase and then begins to regress in a nonfertile cycle. The regressive changes continue until CL is completely degraded and eventually absorbed into the ovarian stroma [1]. Corpus albicans is the terminal stage of CL before incorporation into ovarian stroma. In a fertile cycle, CL is rescued from regression and it continues to function during pregnancy. In women, CL is functional throughout pregnancy, but is not needed to maintain pregnancy after the luteoplacental shift, which occurs at about 9–12 wk of pregnancy [1–10]. In cattle, CL function is required until the last 70 days of pregnancy [11].

CL of humans and cattle, as those of other animal species, contain small and large luteal cells as well as nonluteal cells [12–16]. In cattle, small luteal cells appear to arise from theca interna cells and large luteal cells from granulosa cells as well as from small luteal cells by cellular differentiation [17]. The nonluteal cells, which represent many different cell types, probably originate from similar cells in the Graafian follicle and from the neovascularization processes and cell migration during the formation and development of the CL. Small and large luteal cells are steroidogenic and they differ not only in size, but also in morphology, function, and responses to hormones [12–22]. Although not steroidogenic, the nonluteal cells may play a role in structure, function, and regression of CL.

Small and large luteal cells from human and bovine CL have been separated for morphological and functional studies [18–22]. However, there are no studies on in situ quantitative analysis of cell composition of human CL, and there is only one study on bovine CL determining cell composition only during the mid luteal phase [23]. Cell separation studies do not give this information because nonluteal cells are often not recovered, selective losses of luteal cells can occur, and some luteal components such as blood vessels are not represented. This paper presents for the first time data on in situ quantitative analysis of cell composition of human and bovine CL from various reproductive states.

MATERIALS AND METHODS

All the materials for light and electron microscopy were purchased from Polysciences, Inc. (Warrington, PA), Vectastain ABC Kits from Vector Laboratories, Inc. (Burlingame,

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CA), monoclonal mouse antibodies to human macrophages (macrophage CD68, lot #099) and fibroblasts (vimentin, lot #059) from Dako Corp. (Carpinteria, CA); all other reagents were purchased from regular commercial sources.

Human CL were obtained from sixteen normally cycling women undergoing abdominal hysterectomy for a variety of benign gynecological conditions at the University of Louisville and Emory University affiliated hospitals. Most of the CL obtained were biopsy specimens. According to the menstrual history of the patients and/or morphology of the CL [12], five were from early (Days 14–19), three from mid (Days 22–25) and three from late (Days 26 and 27) luteal phase of the cycle and two were corpora albicantia. Serum progesterone levels were measured in some of the patients prior to surgery to confirm appropriate luteal phase. Three CL were obtained from pregnant women undergoing cesarian section at term. The use of tissues was approved by institutional Human Experimentation Committees.

Bovine CL were collected from three Holstein heifers each at Days 6 (early luteal phase), and 12 (mid luteal phase), and 18 (late luteal phase) of the first estrous cycle following cycle synchronization at Cornell University, Ithaca, New York. The CL were collected through an incision in the anterior vagina after epidural anesthesia with 2% procaine [24]. Estrous cycle synchronization was accomplished by the combined PRID (progesterone-releasing intravaginal device)-prostaglandin ($PGF_{2\alpha}$) method described by Smith et al. [25]. In this method, the PRIDs remain in place for 7 days and the $PGF_{2\alpha}$ (25 mg) is administered subcutaneously on Day 6. Fertility of animals inseminated 80 h after the $PGF_{2\alpha}$ injection was shown to be equal to that obtained after insemination of normally cycling control heifers during estrus. The peripheral plasma progesterone levels at the time of CL removal and luteal weights were consistent with appropriate luteal phases. Three bovine CL of early pregnancy (~90 days, determined from crown-to-rump length of the fetus) were collected from a local slaughterhouse.

Immediately following removal, CL were washed with chilled physiological saline to remove blood, cut into 5-mm-thick pieces, fixed overnight in Bouin's solution. In a totally randomized fashion, several tissue blocks were prepared from each luteal specimen, 30–50 sections of 5-μm thickness were cut from different tissue blocks, and 5–10 sections and 3–5 areas per section were used for determination of cell composition.

The quantitative composition of small and large luteal cells, nonluteal cells and vascular space was determined by computerized video-based interactive Bioquant image analysis system IV [26]. This is a state-of-the-art software system that can interface with the most powerful microcomputers. It comes as a three-disk set, which includes statistics and graphic components. The images of luteal tissue components were displayed on a video screen from a microscope slide and then the number of cells were counted by touch counting. The sizes of luteal and nonluteal cells were determined by individual distance measurements. Luteal vascular space was quantified by tracing the outline of vascular lumen. We found this system to be efficient and accurate for obtaining these cell composition data.

The combination of size, shape, and morphology at the light microscope level was used in distinguishing luteal cells from nonluteal cells and small luteal cells from large luteal cells [20]. Nonluteal cells are not round, are smaller in size, and contain highly condensed nuclei compared to luteal cells. The cell size and morphological differences seen at the light microscope level were used in distinguishing small from large luteal cells. The size ranges were 7–16 μm and 17–30 μm, 15–19 μm, and 20–50 μm for human and bovine small and large luteal cells, respectively. Arbitrary size cutoffs of 16.5 μm and 19.5 μm were used for counting small and large luteal cells in human and bovine CL, respectively. At the light microscope level, large luteal cells were polyhedral in shape and usually contained lipid droplets, and small luteal cells stained darker than large luteal cells.

Vascular space was identified by the presence of smooth muscle and endothelial cells. The macrophages and fibroblasts were identified by light microscope immunocytochemistry in human CL. The antibodies used did not cross-react with the corresponding cells in bovine CL and the antibodies specific for bovine cells are not commercially available.

The light microscope immunocytochemistry was performed by an avidin-biotin immunoperoxidase method as previously described [27]. The sections were counterstained with hematoxylin after immunostaining. The human macrophage and vimentin antibodies were used at dilutions of 1:100 and 1:500, respectively. The immunostained cells were counted as before, using the Bioquant image analysis system IV. Some large luteal cells were also immunostained with vimentin antibody, but they were not counted. For controls, primary antibodies were omitted and nonspecific IgGs were substituted. Immunostaining was completely absent in the controls.

The number of cells or area used in counting the cells per luteal specimen are given in table legends. These numbers multiplied by the number of CL used for each reproductive state gave the total numbers required for valid statistical analysis. The significant differences between reproductive states for each cell type were determined by analysis of variance and Duncan's multiple range test and indicated by superscript letters in Tables 1–5 [28]. The significant differences between corresponding cells in human and bovine CL were similarly tested and are indicated by asterisks in Tables 1–3 and 5 [28].

RESULTS

Figures 1 and 2 show phase-contrast photographs of predominantly small (A), large (B), and nonluteal cells (C) in

TABLE 1. Cell composition of human and bovine CL from various reproductive states.

Reproductive state	Percent of total cells ($\bar{x}$ ± SEM)*			
	Human		Bovine	
	Luteal cells	Nonluteal cells	Luteal cells	Nonluteal cells
Early luteal phase	16.9 ± 0.8[a]†	83.1 ± 0.8[a]†	25.8 ± 0.3[a]	74.2 ± 0.1[a]
Mid luteal phase	31.0 ± 0.7[b]†	69.0 ± 0.7[b]†	40.0 ± 0.2[b]	60.0 ± 0.3[b]
Late luteal phase	22.4 ± 0.5[c]	77.6 ± 0.8[c]	25.4 ± 0.4[a]	74.6 ± 0.5[a]
Corpora albicantia	14.0 ± 0.3[d]	86.1 ± 0.3[d]	—	—
Pregnancy	29.1 ± 1.6[b]†	70.9 ± 1.6[b]†	39.5 ± 0.4[b]	60.5 ± 0.3[b]

*Three hundred to 450 cells were counted in a total area of 43 000 μm^2 from each CL.
†The values in horizontal rows with daggers symbol (†) indicate differences ($p < 0.05$) compared to corresponding cell type from bovine CL.
[a–d]The values in vertical columns with different superscript letters are different, $p < 0.05$.

TABLE 2. Small and large luteal cell distribution in human and bovine CL from various reproductive states.

Reproductive state	Percent of total luteal cells ($\bar{x}$ ± SEM)*			
	Human		Bovine	
	Small	Large	Small	Large
Early luteal phase	89.8 ± 1.4[a]†	10.2 ± 1.4[a]†	54.7 ± 0.5[a]	45.3 ± 0.2[a]
Mid luteal phase	72.0 ± 1.7[b]†	28.0 ± 1.7[b]†	55.9 ± 0.4[a]	44.1 ± 0.4[a]
Late luteal phase	76.2 ± 1.2[c]†	24.3 ± 1.1[c]†	39.3 ± 0.3[b]	60.7 ± 0.3[b]
Corpora albicantia	83.5 ± 0.5[d]	17.5 ± 0.5[d]	—	—
Pregnancy	76.5 ± 0.3[c]†	23.5 ± 0.3[c]†	59.7 ± 0.4[c]	40.3 ± 0.3[c]

*One hundred luteal cells in each corpus luteum were counted.
†The values in horizontal rows with dagger symbol (†) indicate differences ($p < 0.05$) compared to corresponding cell type from bovine CL.
[a–d]The values in vertical columns with different superscript letters are different, $p < 0.05$.

mid luteal phase human (Fig. 1) and bovine (Fig. 2) CL. The size and shape of these cells differ. Luteal cells are bigger and rounder than nonluteal cells. Nonluteal cells include different cell types that cannot easily be distinguished from each other by light microscopy. Blood vessels were seen traversing nonluteal (Figs. 1C and 2C) and luteal cells (Fig. 1A).

Table 1 shows that human and bovine CL contained more nonluteal cells than luteal cells in all reproductive states examined. Human CL contained a lower number of luteal cells and a greater number of nonluteal cells than bovine CL. As also shown in this table for both species, the number of luteal cells increased and nonluteal cells decreased from early to mid luteal phase. Then the number of luteal cells decreased and nonluteal cells increased in late luteal phase and degenerating CL. The composition of luteal and nonluteal cells of term pregnancy human and early pregnancy bovine CL was similar to that of the corresponding mid luteal phase CL.

The vast majority of luteal cells were the small cells in human CL in all reproductive states (Table 2). The number of small luteal cells decreased and large luteal cells increased from early to mid luteal phase, followed by an increase of small luteal cells and decrease of large luteal cells in late luteal phase and degenerating human CL (Table 2). The composition of small and large luteal cells of term pregnancy human CL was similar to that of late luteal phase CL.

Table 2 also shows that there were more small luteal cells than large luteal cells in early and mid luteal phase and early pregnancy bovine CL. In late luteal phase, however, there were more large luteal cells than small luteal cells. The number of small and large bovine luteal cells did not change from early to mid luteal phase. In late luteal phase, however, small luteal cells decreased and large luteal cells increased (Table 2). The composition of small and large luteal cells in early pregnancy CL was similar to mid luteal phase bovine CL.

The average size of nonluteal cells was less than that of small luteal cells, which in turn was less than the size of large luteal cells in human and bovine CL (Table 3). Compared to the sizes of bovine cells, the average size of all the cells was less in human CL. The average size of large luteal cells in humans and that of small luteal cells in cattle

TABLE 3. Size distribution of small and large luteal and nonluteal cells in human and bovine CL from various reproductive states.

Reproductive state	Average cell size, μm ($\bar{x}$ ± SEM)*					
	Human			Bovine		
	Small	Large	Nonluteal	Small	Large	Nonluteal
Early luteal phase	11.6 ± 0.3[a]†	22.5 ± 1.4[a]	6.3 ± 0.1[a]†	14.2 ± 0.2[a]	23.8 ± 0.4[a]	9.4 ± 0.2[a]
Mid luteal phase	13.2 ± 0.3[b]†	21.6 ± 0.4[a]†	7.6 ± 0.1[b]†	14.7 ± 0.1[a]	24.1 ± 0.3[a]	9.6 ± 0.1[a]
Late luteal phase	11.4 ± 0.3[a]†	22.5 ± 0.8[a]†	6.7 ± 0.1[c]†	15.0 ± 0.3[a]	31.4 ± 0.6[b]	9.3 ± 0.2[a]
Corpora albicantia	11.7 ± 0.3[a]	23.5 ± 1.1[a]	6.4 ± 0.2[a]	—	—	—
Pregnancy	11.8 ± 0.3[a]†	22.0 ± 0.9[a]	6.0 ± 0.1[a]†	14.8 ± 0.2[a]	23.2 ± 0.5[a]	8.9 ± 0.1[b]

*The size was determined on 100 cells from each CL.
†The values in horizontal rows with dagger symbol (†) indicate differences ($p < 0.05$) compared to corresponding cell type from bovine CL.
[a–c]The values in vertical columns with different superscript letters are different, $p < 0.05$.

TABLE 4. Distribution of macrophages and fibroblasts in human CL from various reproductive states.

Reproductive state	Cells/10 000 μm² (x̄ ± SEM)*	
	Macrophages	Fibroblasts
Early luteal phase	16.1 ± 2.1[a]	38.6 ± 1.7[a]
Mid luteal phase	31.4 ± 1.8[b]	33.4 ± 1.0[b]
Late luteal phase	97.7 ± 1.5[c]	65.8 ± 1.5[c]

*Twenty 10 000-μm² areas of each CL were used in counting these cells.
[a-c]The values in vertical columns with different superscript letters are different, $p < 0.05$.

did not change in any of the reproductive states examined (Table 3).

In humans, the average size of small luteal cells and nonluteal cells increased from early to mid luteal phase followed by a decrease in late luteal phase, degenerating, and term pregnancy CL (Table 3). The average size of large bovine luteal cells was greater in late luteal phase compared to early and mid luteal phase and pregnancy CL. The average size of nonluteal cells did not change during the cycle, but it did decrease in early pregnancy bovine CL (Table 3).

Multiple cell types make up the nonluteal cells in CL. Among these, macrophages and fibroblasts were identified in human CL. Figure 3 shows that macrophages (A–C) and fibroblasts (D–F) are present in CL of all three luteal phases. Their quantities and distribution patterns, however, varied with luteal phase. In early and mid luteal phases, the macrophages were scattered in increasing numbers among luteal cells (3A and B). In late luteal phase, however, macrophages greatly increased in number and were seen as if they were invading the CL from the periphery (C). The fibroblasts were scattered among the luteal cells and their numbers dramatically increased in late luteal phase CL (D–F). Some large (but not small) luteal cells in all three luteal phases were also immunostained with vimentin antibody (D–F).

Table 4 and Figure 3 show that the quantity of human luteal macrophages progressively increased from early to mid luteal phase and mid to late luteal phase. The fibroblasts, on the other hand, decreased in number from early to mid luteal phase but then greatly increased in late luteal phase CL (Fig. 3 and Table 4).

Table 5 shows that the vascular space was similar in human and bovine CL from early luteal phase. In mid and late luteal phases, however, human CL contained a greater amount of vascular space than bovine CL. In both species, luteal vascular space increased from early to mid luteal phase, followed by a considerable decline in late luteal phase (Table 5). The vascular space in human corpora albicantia and pregnancy human and bovine CL was not quantified.

TABLE 5. Distribution of vascular space in human and bovine CL from various reproductive states.

Reproductive state	Vascular space, μm²/1 000 μm² (x̄ ± SEM)*	
	Human	Bovine
Early luteal phase	61.4 ± 0.8[a]	68.6 ± 0.8[a]
Mid luteal phase	96.3 ± 0.4[b]†	86.2 ± 0.2[b]
Late luteal phase	47.6 ± 0.8[c]†	36.7 ± 0.7[c]

*Vascular space in twenty 1 000-μm² areas of each CL was measured.
†The values in horizontal rows with dagger symbol (†) indicate differences ($p < 0.05$) compared to corresponding luteal phase of bovine CL.
[a-c]The values in vertical columns with different superscript letters are different, $p < 0.05$.

DISCUSSION

Human and bovine CL are known to contain small and large luteal cells as well as nonluteal cells. However, these cells in human CL have never been quantified in situ and in bovine CL they have been quantified only during the mid luteal phase [23]. The results of the present in situ quantitative studies showed that the cell composition of human and bovine CL was similar in some respects but different in others. The differences may reflect species differences in the length of each luteal phase and consequent hormonal and/or developmental variations during the menstrual versus estrous cycle. The similarities may reflect common underlying mechanisms regarding the follicular origin of cells and their hormonal and developmental regulation. The similarities suggest that bovine CL may be used as an animal model to study the regulation of human luteal cell composition.

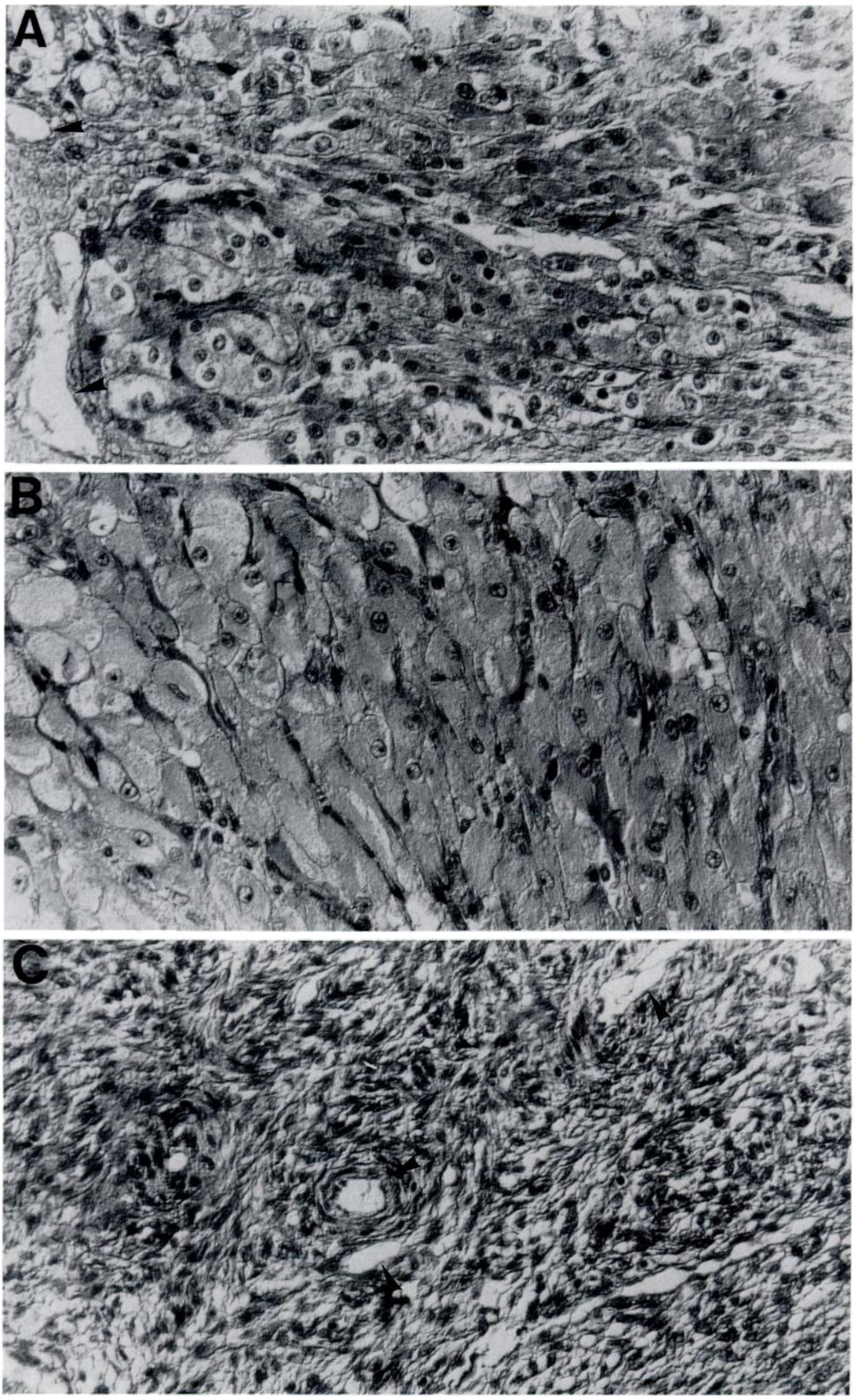
The proportion of small to large bovine luteal cells was lower than in previous studies [18, 20, 21, 23, 29]. One reason for this discrepancy could be that selective losses of large luteal cells may occur during the cell dispersion and purification procedures [18, 20, 21, 29], resulting in higher ratios. Other reasons such as breed differences, whether CL used were obtained from natural or estrus-synchronized cycles, and type of compounds and regimens used for estrus synchronization, etc. [23] are also possible.

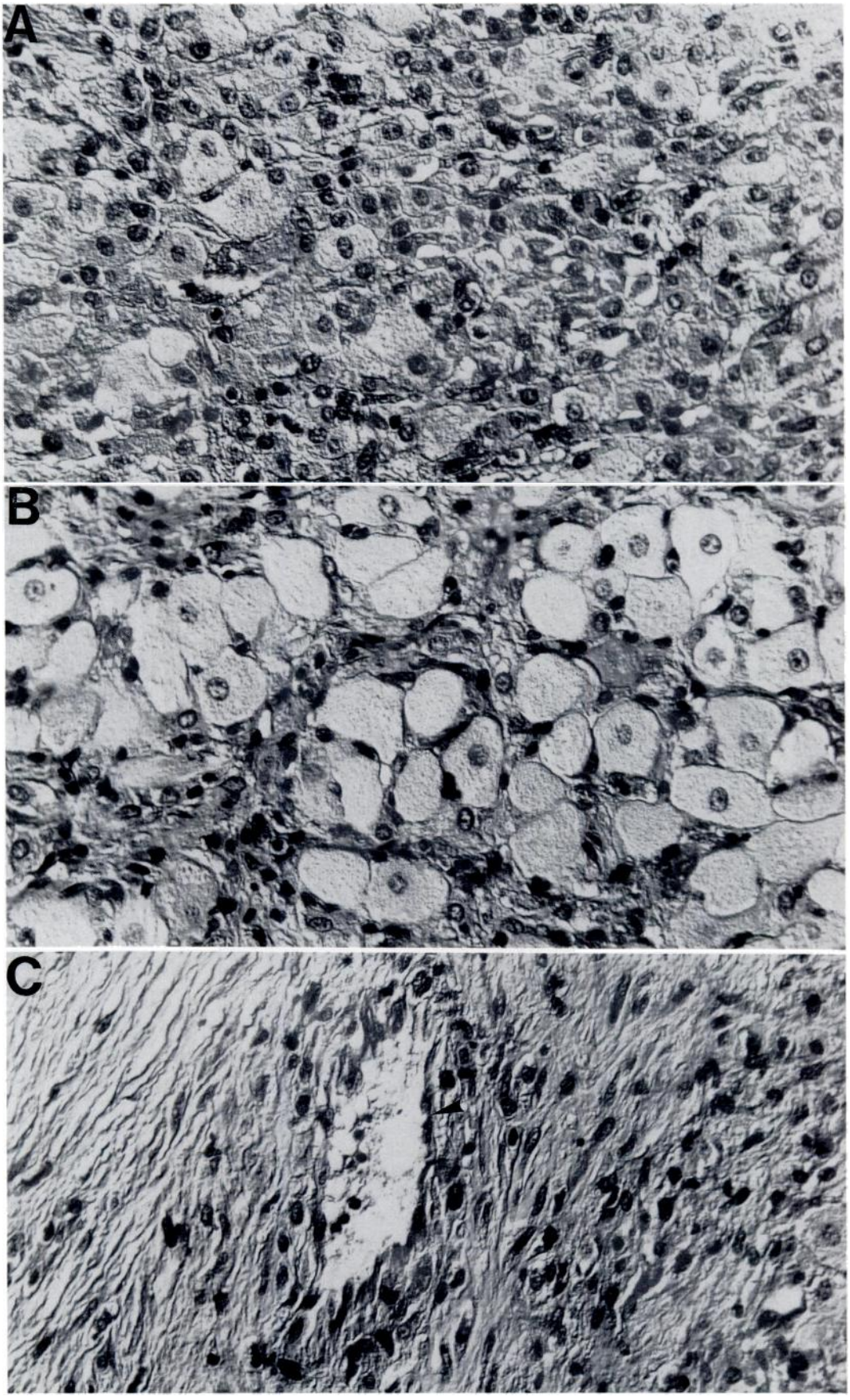
The changes of luteal versus nonluteal cells and small versus large luteal cells are inversely related. This inverse

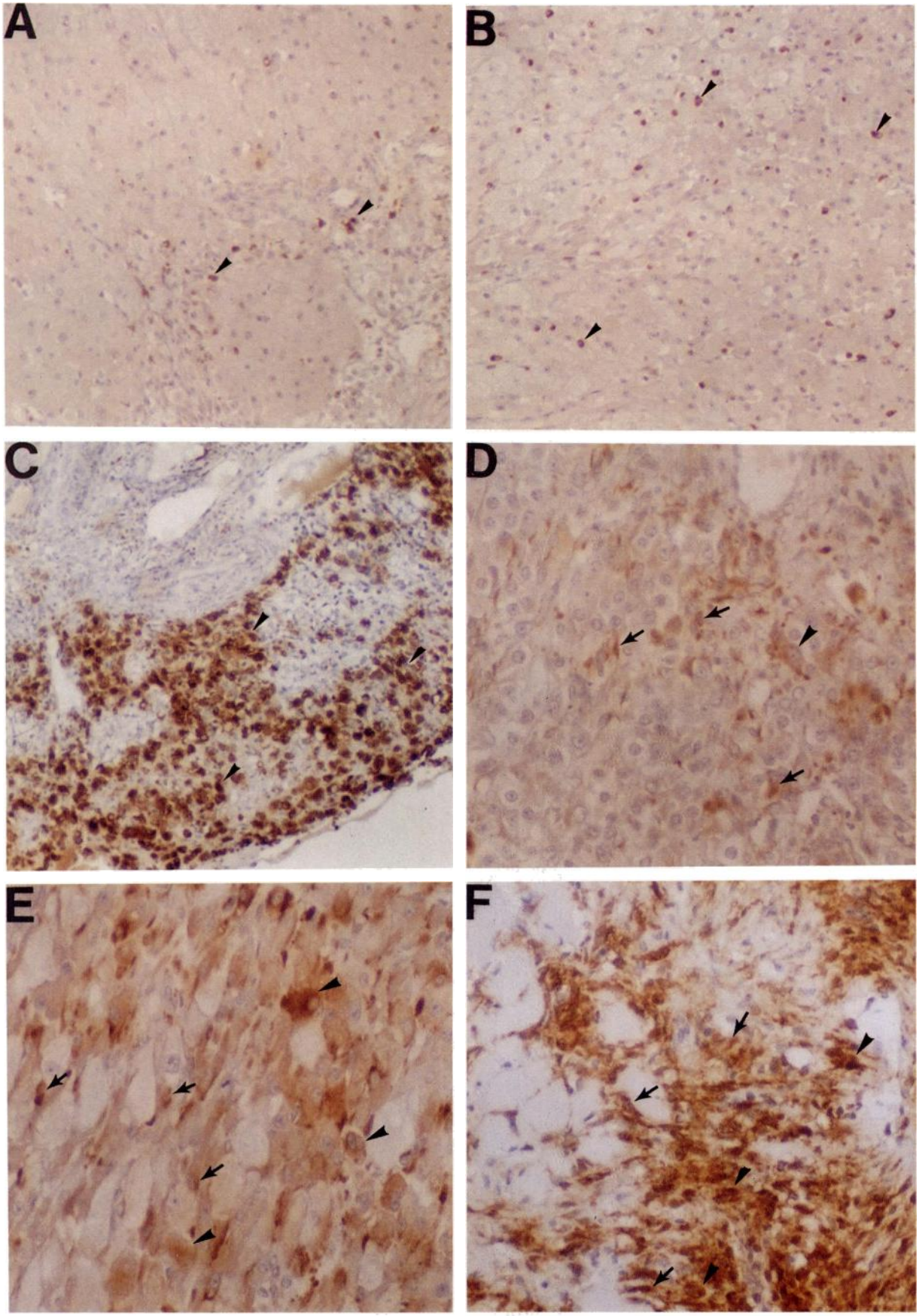
FIG. 1. Phase-contrast photographs showing predominantly small luteal (A), large luteal (B), and nonluteal (C) cells in mid-luteal phase human CL. Blood vessels can be seen traversing the luteal (A) and nonluteal (C) cells (arrowheads). ×1 000.

FIG. 2. Phase-contrast photographs showing predominantly small luteal (A), large luteal (B), and nonluteal (C) cells in mid-luteal phase bovine CL. A large blood vessel can be seen traversing nonluteal cells (arrowhead). ×1 000.

FIG. 3. Immunocytochemical staining for macrophages (A–C) and fibroblasts (D–F) in early (A, D), mid (B, E), and late (C, F) luteal phase human CL. Several immunostained macrophages are indicated by arrowheads in panels A and C, and some immunostained fibroblasts are indicated by arrows in panels D–F. Some large luteal cells in all three luteal phases also showed immunostaining with the vimentin antibody, as indicated by arrowheads in panels D–F. ×500.







relationship between nonluteal and luteal cells suggests that the population of nonluteal cells may decrease or increase as a result of cell migration, cell death, cell division, and/or vascular invasion. The inverse relationship between small and large luteal cells is compatible with independent follicular origin of small and large luteal cells and some of the small luteal cells differentiating into large luteal cells during the later stages of the cycle and in pregnancy. The overall changes in luteal and nonluteal cells and luteal vascular space are consistent with the changing luteal function in different reproductive states in both species. It is conceivable that some of the luteal dysfunctional states result from abnormal hormonal and/or developmental regulation of cell composition, which may not necessarily be reflected in discernible changes in hormone production.

Nonluteal cells represent many different cell types, of which only macrophages and fibroblasts have been identified and quantified in human CL. Of these two resident cell types, macrophages in CL have previously been studied in detail. These studies have shown that macrophages may have luteotropic as well as luteolytic roles [30–32]. Increasing numbers of macrophages are found in regressing CL [31–36]. Macrophages are phagocytic in nature and they contain remnants of luteal cells in degenerating CL [31]. To our knowledge, however, none of the previous studies have quantified macrophages in human CL from different luteal phases. The present data showing the different distribution patterns as well as increasing numbers from early to late luteal phase suggest that macrophages could play dual functional roles, i.e. luteotropic in early and mid luteal phases and luteolytic in late luteal phase.

The number luteal fibroblasts decreased in maximally functioning CL and then greatly increased in regressing CL. The role(s) that fibroblasts play in luteal maintenance or demise is unknown. The high numbers of fibroblasts and macrophages in late luteal phase suggest that they could serve as cellular markers of luteal regression. What initiates their increase and whether such an increase is a consequence or cause of luteal regression are unknown at the present time.

Some of the differences in cell composition may explain biochemical differences between human and bovine CL. For example, human and bovine CL bind ^{125}I-hCG with about equal affinity [37, 38]; but, the amount of binding per milligram protein is lower in human than in bovine CL [37, 38; unpublished data]. This lower binding could be due to the presence of fewer luteal cells bearing gonadotropin receptors in human CL than in bovine CL.

The present data show that the quantity of luteal and nonluteal cells in term pregnancy human CL is very similar to that of mid luteal phase. This is consistent with term pregnancy human CL being functionally active as determined by ultrastructural and biochemical signs, secretion of progesterone and relaxin, specific binding of hCG/LH, and in vitro response to hCG in terms of production of C-19 steroid hormones [2–10, 39]. Even though functionally active, human CL at term pregnancy may still be in an overall decline, as small and large luteal cell composition at term pregnancy is very similar to that in late luteal phase. In support of this possibility was the finding that term pregnancy and late luteal phase CL contain a lower number of hCG/LH receptors [10, 37].

Bovine CL are functional throughout pregnancy [11, 40, 41]. Therefore, as expected, the cell composition of early pregnancy bovine CL is very similar to that of maximally functioning mid lutea phase CL.

In summary, nonluteal cells were in the majority in human and bovine CL. Among luteal cells, small cells exceeded the large cells in quantity. Human CL contained fewer luteal and more nonluteal cells with smaller average cell diameters and a greater amount of vascular space than bovine CL. In both species, the cell composition changed, which was consistent with the changing luteal function during various reproductive states.

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REFERENCES

1. Speroff L, Glass RH, Kase NG. Clinical Gynecologic Endocrinology and Infertility. Baltimore: Williams & Wilkins; 1989; 91–121.
2. Nielson MH, Warren JC. TPNH generation in the human ovary. Acta Endocrinol 1965; 49:58–64.
3. Green JA, Garcilazo JA, Maqueo M. Ultrastructure of the human ovary. II. The luteal cell at term. Am J Obstet Gynecol 1967; 99:855–863.
4. Guraya SS. Histochemical study of human corpus luteum at term. Am J Obstet Gynecol 1968; 102:219–221.
5. LeMaire WJ, Conly PA, Moffet A, Cleveland WW. Plasma progesterone secretion by the corpus luteum of term pregnancy. Am J Obstet Gynecol 1970; 108:132–134.
6. Caspo AI, Pulkkinen MO, Ruttner B, Sauvage JP, Wiest WG. The significance of the human corpus luteum in pregnancy maintenance. Am J Obstet Gynecol 1972; 112:1061–1067.
7. Crisp TM, Dessouky DA, Denys FR. The fine structure of the human corpus luteum of term pregnancy. Am J Obstet Gynecol 1973; 115:901–911.
8. Marsh JM, LeMaire WJ. Cyclic AMP accumulation and steroidogenesis in the human corpus luteum: effect of gonadotropins and prostaglandins. J Clin Endocrinol Metab 1974; 38:99–106.
9. Weiss G, O'Byrne EM, Steinetz BG. Relaxin: a product of the human corpus luteum of pregnancy. Science 1976; 194:948–949.
10. Rao ChV, Sanfilippo J, Carman FR Jr. Gonadotropin receptors in human corpora lutea of term pregnancies. Am J Obstet Gynecol 1978; 132:581–583.
11. Estergreen VL, Frost OL, Gomes WR, Erb RE, Bullard JF. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. J Dairy Sci 1967; 50:1293–1295.
12. Corner GW. The histological dating of the human corpus luteum of menstruation. Am J Anat 1956; 98:377–401.
13. Crisp TM, Dessouky DA, Denys FR. The fine structure of the human corpus luteum of early pregnancy and during the progestational phase of the menstrual cycle. Am J Anat 1970; 127:37–70.
14. Donaldson L, Hansel W. Histological study of bovine corpora lutea. J Dairy Sci 1965; 48:905–909.
15. Niswender GD, Schwall RH, Fitz TA, Farin CE, Sawyer HR. Regulation of luteal function in domestic ruminants: new concepts. Rec Prog Horm Res 1985; 41:101–151.

16. Webley GE, Richardson MC, Smith CA, Masson GM, Hearn JP. Size distribution of luteal cells from pregnant and nonpregnant marmoset monkeys and comparison of the morphology of marmoset luteal cells with those from the human corpus luteum. J Reprod Fertil 1990; 90:427–437.
17. Alila HW, Hansel W. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 1984; 31:1015–1025.
18. Ursely J, Leymarie P. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. J Endocrinol 1979; 83:303–310.
19. Koos RD, Hansel W. The large and small cells of the bovine corpus luteum: ultrastructural and functional differences. In: Schwartz NB, Hunzicker-Dunn M (eds.), Dynamics of Ovarian Function. New York: Raven Press; 1981: 197–203.
20. Chegini N, Ramani N, Rao ChV. Morphological and biochemical characterization of small and large luteal cells during pregnancy. Mol Cell Endocrinol 1984; 37:89–102.
21. Weber DM, Fields PA, Romrell LJ, Tumwasorn S, Ball BA, Drost M, Fields MJ. Functional differences between small and large luteal cells of the late pregnant vs. nonpregnant cow. Biol Reprod 1987; 37:685–697.
22. Ohara A, Mori T, Taii S, Ban C, Narimoto K. Functional differentiation in steroidogenesis of two types of luteal cells isolated from mature human corpora lutea of menstrual cycle. J Clin Endocrinol Metab 1987; 65:1192–1200.
23. O'Shea JD, Rodgers RJ, D'Occhio MJ. Cellular composition of the cyclic corpus luteum of the cow. J Reprod Fertil 1989; 85:483–487.
24. Hansel W. Survival and gonadotropin responsiveness of luteal cells in vitro. In: Diczfalusy E (ed.), In Vitro Methods in Reproductive Cell Biology, 3rd Symposium. Copenhagen: Bogtrykkeriet; 1971: 295–312.
25. Smith RD, Pomerantz AJ, Beal WE, McCann JP, Pilbeam TE, Hansel W. Insemination of holstein heifers at a preset time after estrous cycle synchronization using progesterone and prostaglandin. J Anim Sci 1984; 58:792–800.
26. Bioquant TM, System IV. Documentation. Nashville, TN: R&M Biometrics, Inc.; 1985.
27. Rao ChV, Chegini N, Lei ZM. Immunocytochemical localization of 5- and 12-lipoxygenases and cyclooxygenase in nonpregnant human uteri. In: Zor U, Naor Z, Danon A (eds.), Leukotrienes and Prostanoids in Health and Disease. New Trends in Lipid Mediators Research. Basel: Karger; 1989: 3:283–286.
28. Steel RG, Torrie JH, Principles and Procedures of Statistics. New York: McGraw Hill; 1960.
29. Hansel W, Alila HW, Dowd JP, Yang Z. Control of steroidogenesis in small and large bovine luteal cells. Aust J Biol Sci 1987; 40:331–347.
30. Kirsch TM, Friedman AC, Vogel RL, Flickinger GL. Macrophages in corpora lutea of mice: characterization and effects on steroid secretion. Biol Reprod 1981; 25:629–638.
31. Paavola LG. Cellular mechanisms involved in luteolysis. Adv Exp Med Biol 1979; 112:527–530.
32. Bagavandoss P, Kunkel SL, Wiggins RC, Keyes PL. Tumor necrosis factor-α (TNF-α) production and localization of macrophages and T lymphocytes in the rabbit corpus luteum. Endocrinology 1988; 122:1185–1187.
33. Bulmer D. The histochemistry of ovarian macrophages in the rat. Am J Anat 1964; 98:313–319.
34. Gilliam SW, Christensen AK, McLennan CE. Fine structure of the human menstrual corpus luteum at its stage of maximum secretory activity. Am J Anat 1969; 126:409–428.
35. Paavola LG. The corpus luteum of the guinea pig. Fine structure at the time of maximum progesterone secretion and during regression. Am J Anat 1977; 150:565–604.
36. Paavola LG. The corpus luteum of the guinea pig. Fine structure of macrophages during pregnancy and post partum luteolysis, and phagocytosis of luteal cells. Am J Anat 1979; 154:337–364.
37. Rao ChV. Properties of gonadotropin receptors in cell membranes of bovine corpus luteum. J Biol Chem 1974; 249:2864–2872.
38. Rao ChV, Griffin LP, Carman FR Jr. Gonadotropin receptors in human corpora lutea of the menstrual cycle and pregnancy. Am J Obstet Gynecol 1977; 128:146–153.
39. Savard K, Marsh JM, Rice BF. Gonadotropins and ovarian steroidogenesis. Rec Prog Horm Res 1965; 21:285–365.
40. Mills RC, Morrissette MC. Progesterone synthesis by perfused bovine ovaries of early and late pregnancy. J Reprod Fertil 1970; 22:435–440.
41. Rao ChV. The presence of gonadotropin and prostaglandin E receptors in the cell membranes of bovine corpora lutea throughout pregnancy. Fertil Steril 1975; 26:1185–1189.