

## POLYMORPHISM, DYNAMICS AND ULTRASTRUCTURE OF STEROIDOGENIC CELLS OF CAPRINE CORPUS LUTEUM

BATRA, S.<sup>1</sup> AND SHARMA, R. K.<sup>2</sup>

<sup>1</sup>Department of Zoology, University College, Kurukshetra University, Kurukshetra, 136119

<sup>2</sup>Reproductive Biology laboratory, Department of Zoology, Kurukshetra University, Kurukshetra 136119 (Haryana). E. mail: [soniabatra2dec@rediffmail.com](mailto:soniabatra2dec@rediffmail.com)

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**Abstract:** Seven categories of corpora lutea i.e. small (1-5 days), medium (6-10 days), large (11-15 days), regressing (16-21 days) and of previous cycle, penultimate cycle and pregnancy (<30 days) of goat (*Capra hircus*) were analyzed for cellular polymorphism, dynamics and ultrastructural variations. Histologically, corpus luteum was comprised of two types of steroidogenic cells. The diameter of granulosa luteal cells increased from 33.00  $\mu$ m in small to 42.00  $\mu$ m in large category. The theca luteal cells dimensions increased from 19.25 to 28.00  $\mu$ m. The cell diameters of both these categories of cells showed maxima during pregnancy whereas corpora lutea of previous and penultimate cycles revealed the minimum values. The relative abundance of different types of steroidogenic and non-steroidogenic cells were studied in different reproductive phases of the goat. Blood capillaries and the endothelial cells showed statistically significant variations in different phases. Ultrastructurally, both theca and granulosa luteal cells revealed variations in the relative abundance of different of cell organelles. Mitochondria, smooth endoplasmic reticulum, Golgi complex and secretory granules increased with the increase in granulosa cell diameter during pregnancy. The lysosomes, lipid droplets and vesicles were maximum in the theca luteal cells showing minimum values of diameters in penultimate cycle.

**Key words:** Goat ovary, Corpus luteum

### INTRODUCTION

The corpus luteum, a dynamic endocrine gland [1,2] is comprised of steroidogenic cells and non-steroidogenic cells which show variations in their dimensions and numbers [3-6]. The steroidogenic cells i.e. granulosa luteal cells and theca luteal cells have been implicated for the production of progesterone hormone for maintaining pregnancy [3]. The corpus luteum of small and large ruminants show variations in its activity. During its functional life span, it undergoes differentiation, grows in size and regresses to corpus albicans. Morphology (including histochemistry and ultrastructure) of rodents corpus luteum have been documented [7]. Limited information on cellular components of bovine corpus

luteum is focussed on cows and buffaloes [3,8]. In goat and sheep fragmentary information on morphology of corpus luteum of luteal phase of estrous cycle have been recorded [9-11]. Keeping in view, these gaps in literature, present investigation was undertaken to investigate the cellular changes of steroidogenic cells of caprine corpus luteum during different phases of oestrous cycle and pregnancy.

### MATERIALS AND METHODS

Goat (*Capra hircus*) ovaries were collected from the slaughter house of Eidgah Delhi and brought to laboratory at 0°C in ice bucket. Corpora lutea were dissected out and classified into seven categories on the basis of its morphology (Table 1). The corpora

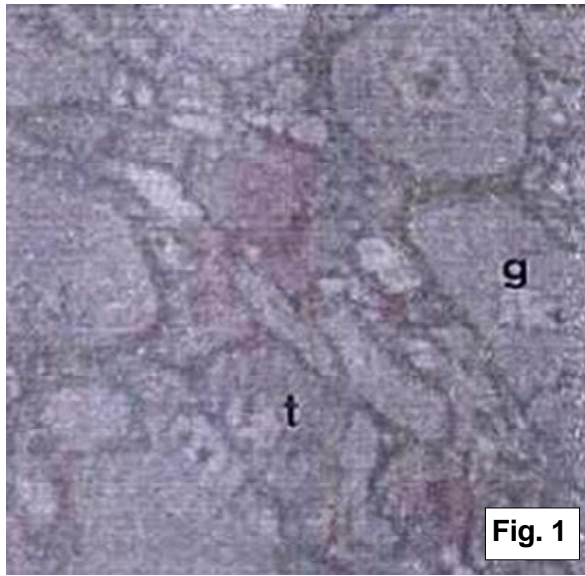


Fig. 1

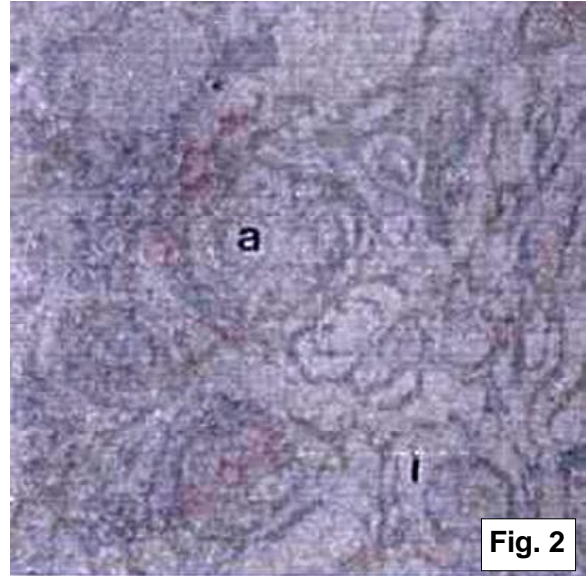


Fig. 2

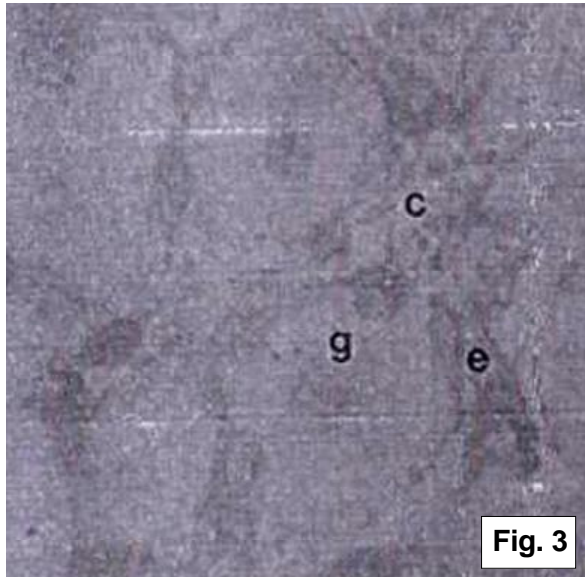


Fig. 3

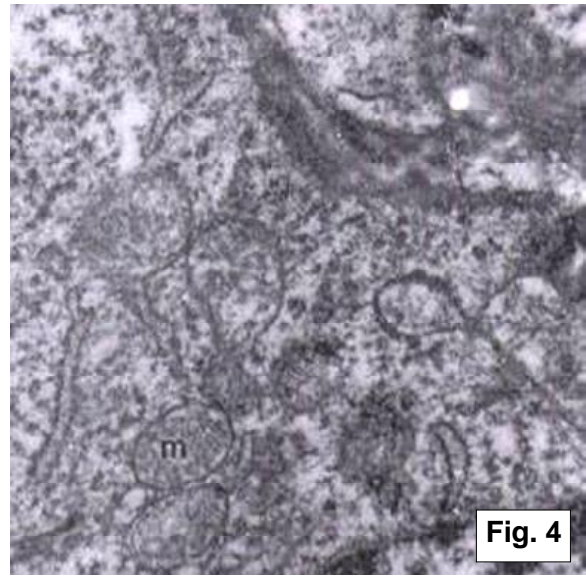


Fig. 4

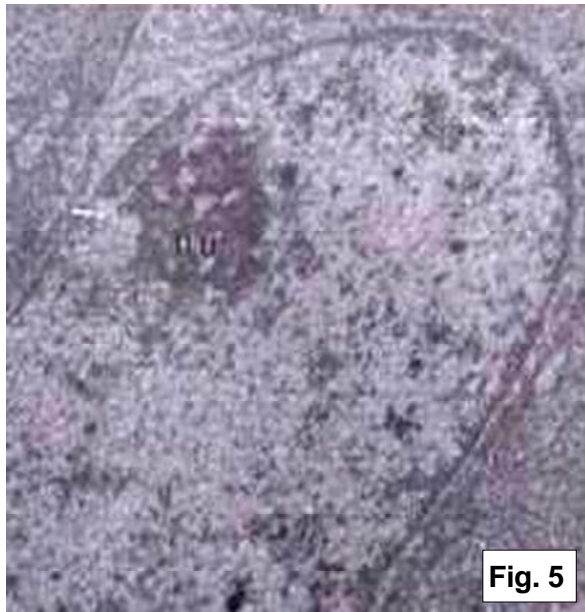


Fig. 5

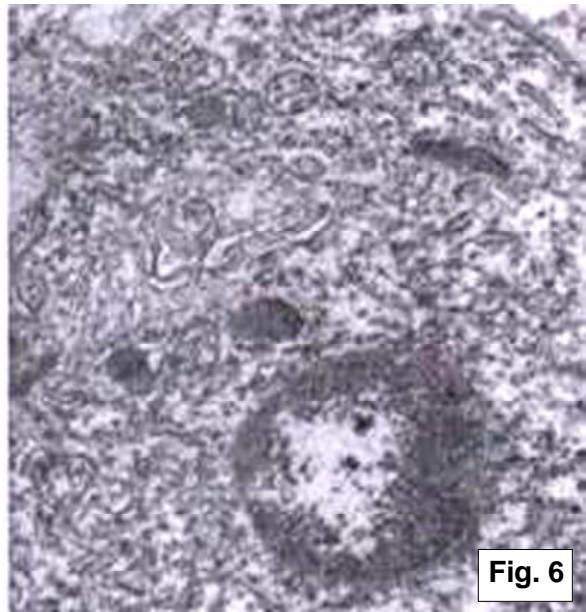
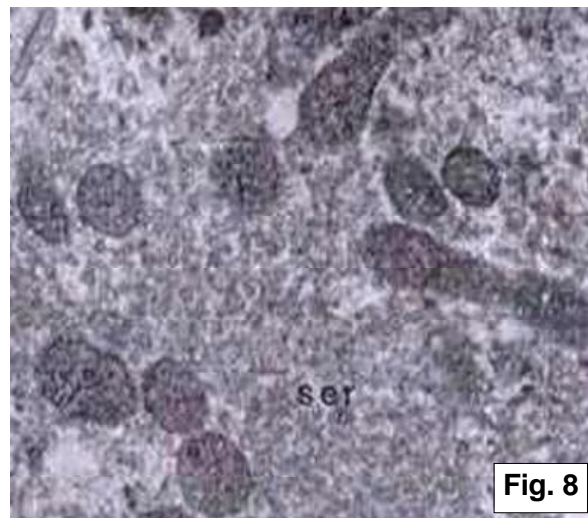
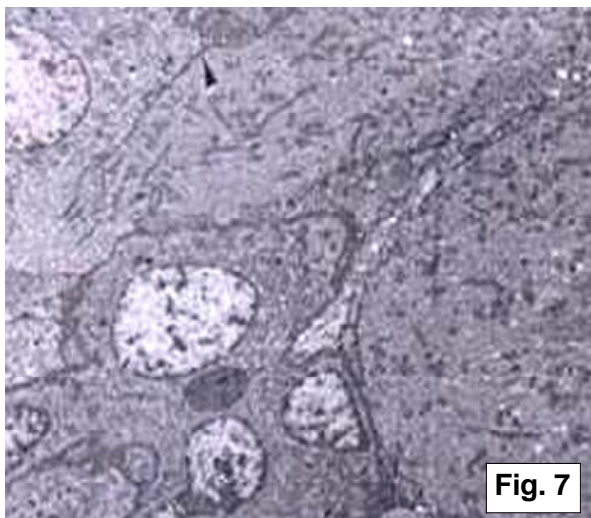


Fig. 6



lutea of different categories were subjected to histological and ultrastructural studies.

For histological studies, corpora lutea were fixed in alcoholic Bouins fixative for 24 hours and were processed following the technique of Pearse [12]. The sections were cut at 7 $\mu$ m thickness and stained with haematoxylin and eosin stain.

For ultrastructural studies, corpus luteum was fixed in Karnovsky fixative in 0.1M phosphate buffer (pH 7.2 to 7.4) at 4°C for 24 hours. The tissue was processed by the method of Zamboni [13]. The sections were cut at 60-90nm mounted on 100 mesh grids and were stained with uranyl acetate followed by lead citrate [14]. The sections were examined and photographed under electron microscope, CM-10 Philips installed at All India Institute of Medical Sciences, New Delhi.

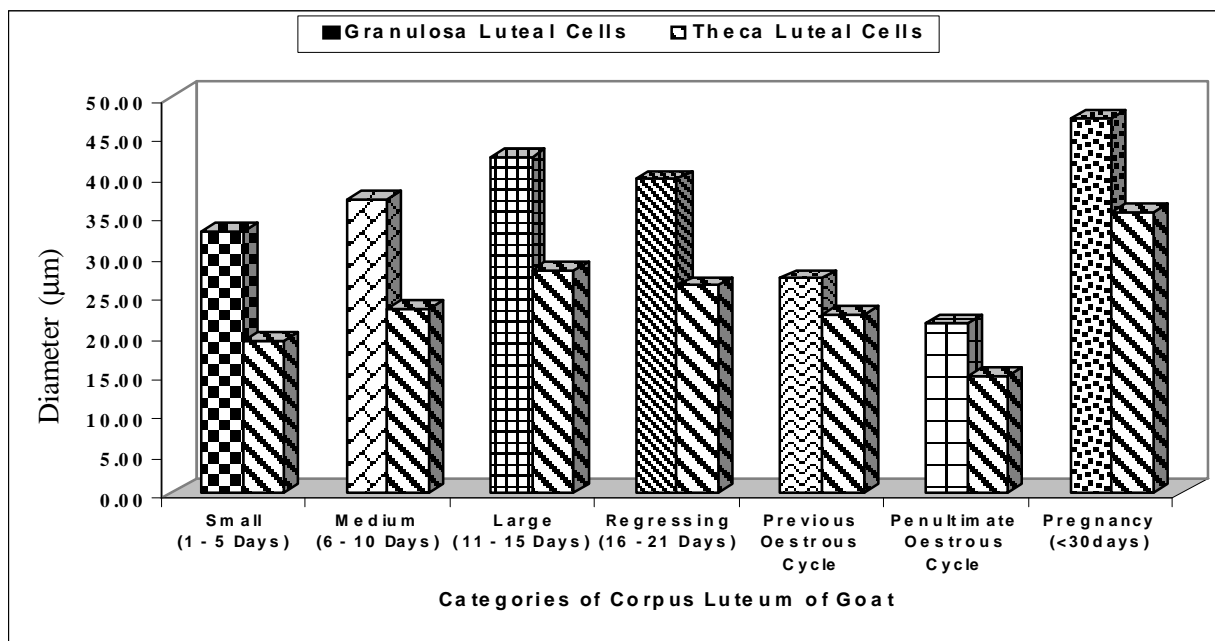
## RESULTS AND DISCUSSION

The corpus luteum of goat ovary was comprised of two types of steroidogenic cells i.e. small theca luteal cells and large granulosa luteal cells and several other types of cells such as endothelial cells, pericytes, smooth muscle cells, fibroblasts, macrophages, leucocytes and plasma cells (Fig. 1). The dimensions and relative abundance of different type of cells present in the corpus luteum varied with the phase of oestrous cycle and pregnancy (Table 2,3; Figs. 9,10). During regression, stellate to irregular in shapes of steroidogenic cells were observed. A large number of phagocytic cells were observed in corpus luteum (Fig. 2).

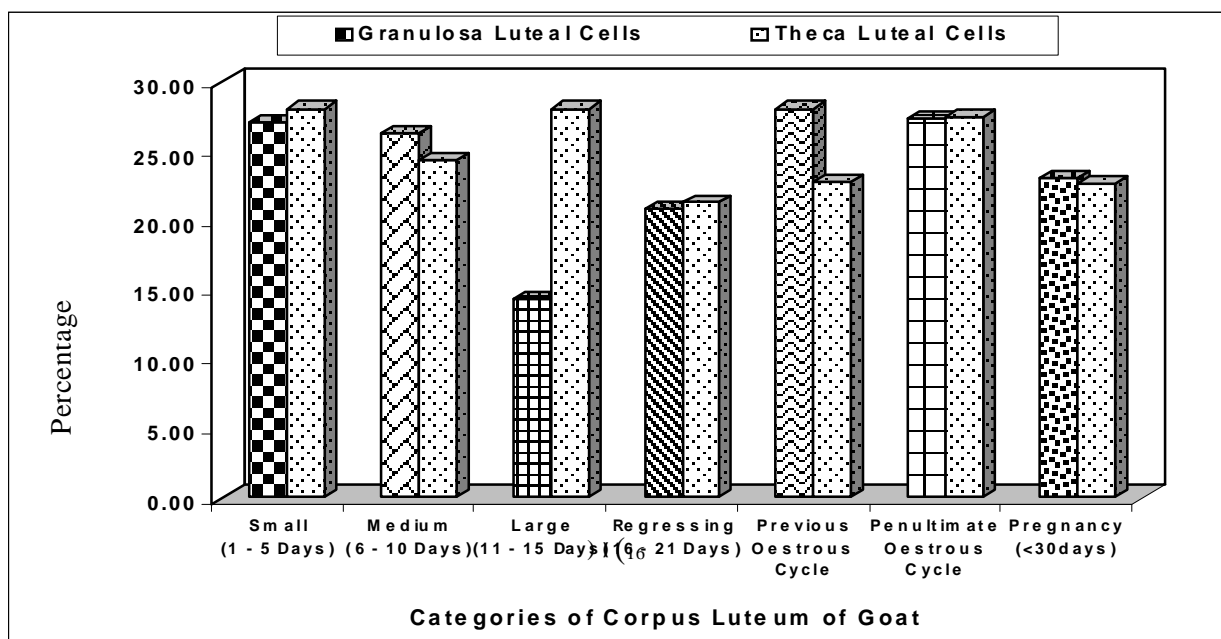
During pregnancy, large granulosa luteal cells with small spherical nucleus were observed whereas small theca luteal cells were characterized by large oval

### Explanation of figures:

**Fig.1:** A magnified view of a portion of the corpus luteum of large category (11-15 days) showing large granulosa luteal cells (g) with spherical nucleus possessing prominent nucleoli and pale cytoplasm. The small theca luteal cells (t) having an elongated nucleus and dense cytoplasm. X600. **Fig. 2:** T.S. of corpus luteum of regressing category (16-21 days) revealing a large number of autophagocytotic bodies (a) and irregular shaped steroidogenic cells (i). X600. **Fig. 3:** A portion of the corpus luteum of pregnancy (< 30 days) showing large granulosa luteal cell (g) with small spherical nucleus. The small sized endothelial cell (e) in the connective tissue (c) are enclosing the luteal cells. Note the hypertrophy of luteal cells. X600. **Fig. 4:** A magnified view of granulosa luteal cell of large category (11-15 days) of corpus luteum exhibiting rough endoplasmic reticulum, mitochondria (m) and lipid droplets (l). X26460. **Fig. 5:** Electronmicrograph of theca luteal cell from large category of corpus luteum (11-15days) revealing oval shaped nucleus with eccentric nucleolus (nu), abundant smooth endoplasmic reticulum, lipid droplets (l). X15960. **Fig. 6:** Ultramicrophotograph of granulosa luteal cell from 16-21 days of corpus luteum showing swollen, disintegrating rough endoplasmic reticulum, fewer swollen, vacuolated mitochondria (m) and a few secretory granules (arrow), lysosomes. X26460. **Fig. 7** Fine photograph of granulosa luteal cell of corpus luteum of pregnancy (< 30 days) revealing uneven contour of cell membrane, undulating nuclear membrane (arrow), abundant oval to spherical shaped mitochondria with crecentric cristae, secretory granules and fewer number of lipid droplets. X3654 **Fig. 8:** Electronmicrograph of theca luteal cell of corpus luteum of pregnancy (< 30 days) revealing abundant smooth endoplasmic reticulum (ser) and spherical to oval shaped mitochondria with well developed cristae. Note a number of elongated to dumble shaped mitochondria revealing the division of mitochondria. X35280



**Fig. 9:** Histogram showing variations in the diameter (µm) of granulosa luteal cells and theca luteal cells in different categories of corpus luteum of goat.



**Fig. 10:** Histogram showing variations in the percentage of number of granulosa luteal cells and theca luteal cells in different categories of corpus luteum of goat.

nucleus. The dimensions of luteal cells were maximum during pregnancy. The relative percentage of blood capillaries and blood vessels were more during pregnancy as compared to rest of the categories (Tables 2,3; Fig. 3). The diameter of granulosa luteal cells, theca luteal cells, endothelial cells and that of blood vessels and blood capillaries revealed the maximum values during pregnancy.

Ultrastructural studies of caprine corpus luteum has revealed the presence of rough and smooth ER in

large granulosa luteal cells. Mitochondria were spherical to oval in shape with well developed cristae (Fig. 4). Spherical nucleus with well spread chromatin material in homogenous nucleoplasm, Golgi complexes and abundant secretory granules were observed. On the contrary, small theca luteal cells were characterized by having oval shaped nucleus with eccentric nucleolus, abundant smooth endoplasmic reticulum, lipid droplets and few mitochondria. Their cytoplasm was devoid of rough endoplasmic reticulum and secretory granules (Fig. 5).

During regression, pycnotic nucleus, apoptotic granules, dispersed nuclear membrane with uneven outline were observed. The clump of vacuolated and deeply condensed chromatin material was seen adhering to the nuclear membrane and was heterogeneously dispersed within the nucleoplasm (Fig. 6). The lipid droplets, lysosomes and the clumps of vacuolated mitochondria with reduced cristae were observed. Degenerating swollen rough endoplasmic reticulum was irregularly dispersed in the cytoplasm (Fig.6).

The corpus luteum of pregnancy, the cellular details were clear and distinct. In large granulosa luteal cells, the nuclear membrane was thrown into a number of undulations and folds. These cells possessed rough endoplasmic reticulum, secretory granules, oval and spherical shaped mitochondria with crecentric cristae. The lipid droplets were fewer in number whereas secretory granules were abundant and were randomly placed in the cytoplasm (Fig. 7). In small theca luteal cells, abundant smooth endoplasmic reticulum,

spherical, oval and dumble shaped mitochondria with well developed cristae were observed (Fig. 8). The presence of steroidogenic and non steroidogenic cells in the caprine corpus luteum during different phases of reproduction suggest that cellular archite-cture of caprine corpus luteum shows great similarity with other mammalian species [3,6,8,11,15]. The maximum diameters of steroidogenic cells observed in corpus luteum during pregnancy while a minimum values recorded in corpus luteum of penultimate cycle correspond to the progesterone production in these phases of reproduction [11,16-19]. The variations in luteal cell dimensions is a direct indication of their steroidogenic activity as already reported in ewe [20].

Ultrastructurally, the presence of rough endoplasmic reticulum and smooth endoplasmic reticulum in granulosa luteal cells indicate that these cells have exceptional steroidogenic and protein synthesizing cells as reported in sheep and other bovine species [3,6,21-23] Besides usual organelles, steroidogenic specific components like abundant smooth endoplasmic reticulum, lipid droplets, lysosomes and multivascular bodies observed in pregnant and mid-luteal caprine corpus luteum show resemblance largely with that of sheep corpus luteum [21,24]. The increased meshwork of smooth endoplasmic reticulum and mitochondria in small luteal cells in addition to the usual cell organelles indicate that these cells are largely steroidogenic in function as observed in other bovine species [3,21,24]. The presence of Golgi complexes with stacks of cisternae and nascent

**Table 1:** Classification of Corpus luteum

Sr.No.	Size (mm) and colour	Name of category		Stage
1.	<2 Pink with red blood clot	Small	I	1-5 days
2.	2-5 Pink	Medium	II	6-10 days
3.	>5 Red	Large	III	11-15 days
4.	2-4 Brown	Regressing	IV	16-21 days
5.	≈ 2 Yellow	Previous	V	Pr
6.	<2 White	Penultimate	VI	Pn
7.	> 6 Dark Red	Pregnancy	VII	<30 days

Cell Types Parameters	Categories of Corpus Luteum						
	I Small (1-5 days)	II Medium (6-10 days)	III Large (11-15days)	IV Regressing (16-21 days)	V Previous (Pr)	VI Penultimate (Pn)	VII Pregnancy (< 30 days)
Granulosa Luteal Cells	33.00 ± 4.65 (15.0-35.0)	37.00 ± 4.97 (17.5-40.0)	42.38 ± 6.82 (22.5-55.0)	39.50 ± 7.89 (20.0-45.0)	27.13 ± 2.88 (22.5-30.0)	21.38 ± 4.57 (15.0-27.5)	47.25 ± 11.77 (27.5-60.0)
Theca Luteal Cells	19.25 ± 3.88 (10.0-22.5)	23.13 ± 2.84 (12.5- 25.0)	28.00 ± 3.59 (17.5-30.0)	26.25 ± 2.90 (15.0-27.5)	22.50 ± 3.54 (17.5-27.5)	14.63 ± 3.09 (10.0-20.0)	35.38 ± 5.77 (25.0-40.0)
Endothelial Cells	6.25 ± 1.25 (5.0-7.5)	8.50 ± 1.66 (5.0-10.0)	12.75 ± 1.92 (10.0-15.0)	10.38 ± 1.63 (7.5-12.5)	7.88 ± 0.89 (7.5-10.0)	5.75 ± 1.15 (5.0-7.5)	15.25 ± 2.07 (12.5-17.5)
Blood Capillaries	14.25 ± 1.60 (10.0-15.0)	17.75 ± 3.70 (12.5-22.5)	26.38 ± 4.77 (17.5-35.0)	22.62 ± 5.61 (15.0-30.0)	20.75 ± 4.62 (12.5-27.5)	16.00 ± 1.84 (12.5-17.5)	34.50 ± 6.30 (20.0-40.0)
Blood Vessels	108.00 ± 13.66 (50.0-112.5)	115.38 ± 15.64 (67.5-125.0)	135.38 ± 24.25 (82.5-180.0)	121.25 ± 22.30 (70.0-140.0)	109.88 ± 14.69 (80.0-125.0)	91.88 ± 16.18 (70.0-112.5)	52.50 ± 46.55 (85.0-212.5)

**Table 2:** Variations in the diameter of different categories of corpus luteum of goat (*Capra hircus*), (Range in Parenthesis)

Parameters	Categories of Corpus Luteum						
	I Small (1-5 days)	II Medium (6-10 days)	III Large (11-15days)	IV Regressing (16-21 days)	V Previous (Pr)	VI Penultimate (Pn)	VII Pregnancy (< 30 days)
Granulosa Luteal Cells	27.11	26.32	14.26	20.80	28.03	27.30	23.06
Theca Luteal Cells	28.07	24.39	28.08	21.29	22.75	27.54	22.61
Endothelial Cells	43.00	46.00	53.30	53.77	46.95	44.01	48.75
Blood Capillaries	1.38	2.22	3.25	3.11	1.47	0.84	3.90
Blood Vessels	0.42	1.07	1.10	1.01	0.80	0.31	1.68

**Table 3:** Relative percentage of number of different types of cells of corpus luteum in different phases of oestrous cycle and pregnancy.

granules associated with concave side of Golgi revealed a close functional relatedness of goat corpus luteum with that of cow [3,25]. Stacks of rough endoplasmic reticulum observed in both the categories of steroidogenic cells have already been reported in cows [3]. The higher frequency of secretory granules and their organization in large clusters are similar to those reported in other domestic animals [3,25,26]. Ultrastructurally, large and small luteal cells of goat corpus luteum revealed a great similarity to those of other bovine species [3,27,28]. During regression, the steroidogenic cells of caprine corpus luteum revealed apoptotic specific degenerating features similar to those reported in cow [24,29-31]. Thus it is evident from the present studies that caprine corpus luteum and bovine corpus luteum have close evolutionary relationship and common regulatory mechanism.

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