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Chitralekha Deo
Veterinary Assistant Surgeon Block-Narharpur, Kanker, Chhattisgarh, India

GK Mishra
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and A.H., NDVSU, Jabalpur, Madhya Pradesh, India

RP Tiwari
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and A.H., DSVCKV, Durg, Chhattisgarh, India

Shweta Thawait
Veterinary Assistant Surgeon Veterinary Hospital, Kotmi Chhattisgarh, India

JR Khan
Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and A.H., Anjora, DSVCKV, Durg, Chhattisgarh, India

Tarun Sahu
Veterinary Assistant Surgeon Veterinary Hospital, Kurud, Dhamtari, Chhattisgarh, India

PDS Raghuvanshi
Department of Veterinary Surgery, College of Veterinary Science and A.H., NDVSU, Jabalpur, Madhya Pradesh, India

Megha Katare Pandey
Department of Translation Medicine, AIIMS, Bhopal, Madhya Pradesh, India

Corresponding Author:
Chitralekha Deo
Veterinary Assistant Surgeon Block-Narharpur, Kanker, Chhattisgarh, India

Histomorphological study of goat luteal tissue and association with progesterone hormone

Chitralekha Deo, GK Mishra, RP Tiwari, Shweta Thawait, JR Khan, Tarun Sahu, PDS Raghuvanshi and Megha Katare Pandey

Abstract
The stages of estrous cycle (Early and mid luteal) was determined based on gross ovarian morphometry (color, consistency, vasculature of CL, number and size of follicles), status of genitalia and hormone estimation. The number of small luteal cells were significantly higher at early luteal as compared to mid luteal. Moreover, the number of large luteal cells were found significantly higher at mid luteal as compared to early luteal stage. Significant higher serum estradiol concentration was observed in early luteal than mid luteal stage. There was significant positive correlation of serum progesterone with luteal progesterone concentration during mid luteal stage.

Keywords: Goat, corpus luteum, large luteal cell, small lutealcell, early luteal phase, mid luteal phase, progesterone

Introduction
Goat is a polyestrous animals, bred in a wide range of production systems. Estrous cycle is a cascade of hormonal events which changes the female reproductive system morphology to prepare animal for pregnancy (Fatet *et al*. 2011) [1]. Estrous cycle length of goat is 19 -21 days. Doe are in heat for approximately 30 hours after the onset of estrous and ovulate 33 hours after the onset of estrous. The corpus luteum is a small gland of great importance because its proper functioning determines not only the appropriate course of the estrous/menstrual cycle and embryo implantation, but also the subsequent maintenance of pregnancy (Mlyczyńska*et al*. 2022) [2]. It plays a central role in the regulation of estrous cycle and pregnancy by secreting progesterone, which should be maintained in appropriate levels in mammals in order to achieve a successful reproduction (Stocco *et al*. 2007) [3]. Progesterone acting via nuclear receptors is important for ovulation, embryo development, and preparation of the uterus for implantation. The different cells percentages in the goat corpus luteum are large luteal cell, small luteal cell, endothelial cell or pericyte (Azmi and Bongso 1985) [4]. The luteal cells are derived from theca and granulosa cells that differentiate into Small luteal cells and large luteal cells. The transmutation of these cells with regard to their function has been observed in some species. The small luteal cells are ultrastructurally most equipped for steroidogenic functions, while large luteal cells secrete both steroids and the regulatory peptides. The morphology of CL was used to estimate the estrous cycle stage and to evaluate goat corpus luteum (CL) vascular density (VD) over the estrous cycle (Nascimento *et al*. 2003, Ferreria-Dias *et al*. 2006) [5-6]. Zarkawi and Soukouti (2001) [7] reported that length of oestrous cycle in days 21.2±1.5. The average length of early luteal phase was 2.9±0.8 days, ranging between 2 and 5 days, with a mean progesterone level of 0.69±0.85 (0.00-3.08) (nmol/L) and the length of Luteal phase averaged 15.3±1.4 days (range: 13-20 days), with a mean progesterone level 13.41±4.39 (3.26-27.98) (nmol/L) at different phases of oestrous cycle of Damascus does (n=30). Maximum level of progesterone during this phase was 18.67 nmol/L (range: 14.0027.98 nmol/L) occurring, on average, on day 12.2 of the oestrous cycle). The morphological characteristics of the ovary can also be useful to verify the pathological presence of cysts and tumors as well as to determine the presence of a cyclic ovarian activity and even to estimate the probable cycle phase, allowing one to infer on the animal's fertility status.

Materials and Methods
The present study was aim to histo-morphology of goat luteal tissue and association with

steroid hormones was estimated. Collection of biomaterials from live and slaughter house animals were approved by Institute Animal Ethics Committee as per prescribed CPSCEA, Ministry of Environment**,** Forest and Climate Change, Government of India**.**

Collection of samples

Non-pregnant (n = 12) goat genitalia were procured from the local slaughter house within 10-20 min after exsanguinations and were transported on ice to the laboratory. The stage of estrous cycle early and mid luteal turned into decided based on gross ovarian morphometry (color, consistency, vasculature of CL, number and size of follicles), status of genitalia and hormone estimation. The samples were classified as early luteal (EL) and mid luteal (ML) stage (Rabab *et al*. 2017) [8]. The cyclic ovaries were further classified on the basis of presence of corpus haemorrhagicum (day 1-5) and mature corpus luteum spurium (day 6-13) as early and mid luteal stage (Fig. 1), respectively (Menchaca and Rubianes 2002) [9]. The ovaries containing the luteal tissues of each stage were stored in neutral formalin buffer saline at Room Temperature (RT) for histo-morphological study.

Fig 1: The goat genitalia along with the changes in ovary in early and mid luteal stages Histomorphology of goat luteal tissue

A piece of ovarian tissue containing CL was fixed in 10% neutral buffered formalin saline (NBFS). The fixed tissue samples were taken out and shifted to freshly prepared 10% NBFS for final trimming of the tissues. The tissue samples were washed overnight under tap water and dehydrated through ascending grades of alcohol and tissue clearing was performed with acetone and benzene. The tissues sections were embedded in paraffin blocks. The paraffin block of the tissue was then cut into 4-5 µm thick paraffin sections by microtome. The sections were deparaffinised in xylene and stained with Haematoxylin and Eosin stain (H&E) followed by mounted with DPX. Luteal cells were counted in ten best fields per slide at random and the mean number of cells was counted. Cellular components of corpus luteum and luteal cell population classified as large luteal cells (19-25 µm) and small luteal cells (7-10 µm) was observed under microscope (ECLIPSE-Ni, Nikon, Japan) of stained luteal tissue section and was digitally photographed and recorded.

Hormone assay

4 mL of blood become accumulated from jugular vein in serum vials with clot activator (Novac Polymed, India) at the time of slaughter of the goats and were transported to the laboratory. After clotting of blood, the vials have been centrifuged at 3000 rpm for 5 min. The supernatant serum changed into then transferred to serum collection vials and stored at -20 degree centigrade until hormone estimation. The concentration of serum progesterone (ng/mL) were determined by radio-immuno assay kit supplied by Immunotech, France. Similarly, the luteal tissue progesterone concentration (µg/gm) was estimated to assess the functional status of luteal stage at different stages of estrous cycle using RIA kit (Kubasic *et al*. 1984) [10]. Briefly, the progesterone concentration was measured using kit supplied by the Immunotech France, using I125 GAMA COUNTER IC 4702A as per the protocol, which was supplied by the kit. Moreover, The CL was enucleated from the ovary and washed thrice with fresh phosphate buffer saline (PBS; 0.05 M, pH 7.4). Luteal tissues were chopped and weighed to yield 100.0±0.47 mg tissue that was transferred in 1 ml PBS (0.05 M, pH 7.4) and stored at- 20 °C for hormone assay. On the day of assay, the tissue was thawed and homogenized using pestle and mortar and the total volume of homogenate was made up to 1 mL with PBS. The samples were finally diluted at 1: 40 to make with PBS for progesterone concentration (µg/gm) estimation with the RIA kits (Mondal *et al*. 2004, Mishra *et al*. 2018) [11-12].

Result and Discussion

Histomorphology of goat luteal tissue

Cyclic goat CL was composed of mainly small and large luteal cell (Fig. 7). The mean numbers of small luteal cells (SLC) were 79.50±0.96, and 16.67±0.88 at early (EL) and mid (ML) stage, respectively (Fig. 2). Whereas, the number of large luteal cells (LLC) were 15.67±0.56 and 71.00±0.93 at EL and ML, respectively (Fig.2). The total numbers of luteal cells were 95.17±0.48 and 87.67±1.69 at EL and ML, respectively. The number of small luteal cells were significantly (p<0.01) higher at EL as compared to ML. Moreover, the number of large luteal cells were found significantly (p<0.01) higher at ML as compared to EL. The increase in the number of LLC at ML stage was consistent with the reports of Sangha *et al.* (2002) [13] as goat CL was a discreet population of large polyhedral luteal cells 19-25 µm in diameter with centrally located prominent nucleoli (8 µm).

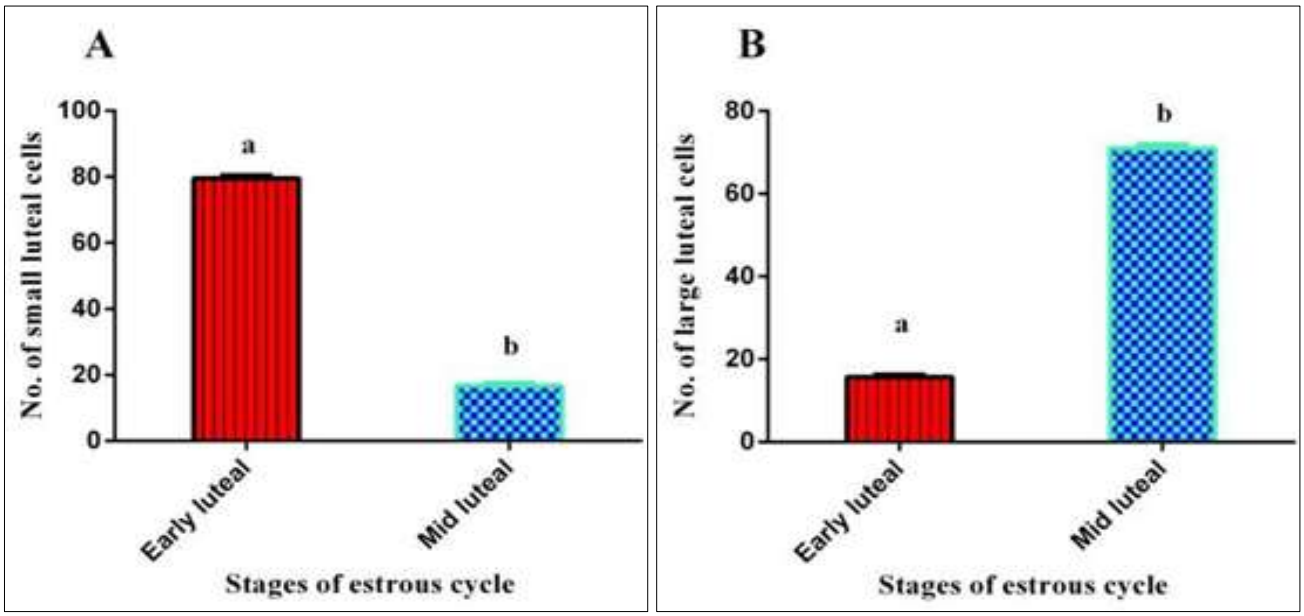

Fig 2: Graphical presentation of number of small (A) and large luteal (B) cells during early and mid luteal stage of estrous cycle in the goat

Our present findings correspond to the reports of Miranda de Moura *et al*. (2010) [14], who observed higher number of large luteal cells on day 12 post ovulation. Similar findings in relation to stage specific increase in number of large luteal cells were also reported by Schawall *et al.* (1986) [15] and Jaglan (2008) [16] in the mid luteal stage of the cyclic buffalo. The percentage of LLC, SLC, endothelial cell and fibroblast in corpus luteum as ~10%, ~25%, ~50% and ~0% respectively and corresponding to our morphological characteristics of the LLC (19-25 µm) and SLC (7-10 µm) of spherical to polyhedral and spindle in shape, respectively. The morphology of CL was used to estimate the estrous cycle stage and to evaluate goat corpus luteum (CL) vascular density (VD) over the estrous cycle.

Serum and luteal progesterone hormone concentration and their correlation

The mean serum progesterone concentration (ng/mL) was 1.31±0.23 (range 1.06-1.56) and 2.17±0.31 (range 2.02-2.38) at EL and ML group, respectively (Table 2). Significant (p<0.01) difference was observed in the serum progesterone concentration between EL and ML. The mean luteal progesterone concentration (µg/gm) was 4.15±0.27 (range 3.25-5.53) and 5.96±0.18 (range 5.19-7.12) at EL and ML group, respectively (Table 2). Significant (p<0.01) difference was observed in the luteal progesterone concentration between EL and ML stage. The correlation between serum and luteal progesterone was 0.39 and 0.96 at EL and ML stage of estrous cycle, respectively (Table 2). There was significant (p<0.958) positive correlation of serum progesterone with luteal progesterone concentration during mid luteal stage.

Table 2: Serum and luteal progesterone concentration and their correlation during early and mid luteal stage of estrous cycle in the goat

Cyclicity	Serum P4 (ng/mL)		Luteal P4 (µg/gm)		Correlation$ between serum and luteal P4
	Mean ± S.Em	Range	Mean ± S.Em	Range	
Early luteal	1.31±0.23a	1.06-1.56	4.15±0.27a	3.25-5.53	0.39
Mid luteal	2.17±0.31b	2.02-2.38	5.96±0.18b	5.19-7.12	0.96
P value	0.001		0.001		

Different superscripts in column differ significantly (p<0.01); $Spearman's rank correlation coefficient's (rho) was calculated

Our reports are in line with the reported plasma progesterone concentration in goat (Bono *et al*. 1983, Gaafar *et al*. 2005, Khanum *et al*. 2008) [17-19] and was associated with higher vascular density during luteal stage post ovulation. Progesterone hormone ranges from 0.58±0.12 to 8.35±2.60 ng/mL during different phase of estrous cycle. The P4level was lowest (0.58±0.12 ng/mL) on the day of the oestrous indicating no luteal activity. It increase gradually after ovulation and reaches to peak value 8.35±2.60 ng/mL on day 14 of the cycle indicating fully developed luteal tissue (corpus luteum) returning again to basal level on the day of next oestrous (0.40±0.15 ng/mL) (Goel *et al*. 2012) [20]. The

present trend in P4 concentration in the luteal tissue further at ML strengthen the earlier findings reported in Indian buffalo (Shah and Mehta, 1992) [21] and in Egyptian buffalo]. The progesterone concentration in the luteal tissue was increased from corpus haemorrhagicum to mature CL however, decrease in the regressive CL, completely undetectable in the corpus albicans in the Egyptian buffalo, significantly higher luteal progesterone concentrations in the mid luteal CL as compare to other luteal phases (El-Sheikh *et al.* 1967) [22]. The progesterone concentration (62 µg/g) was highest on day 13 and decreased to 32.3 µg/g on day 18 in Surti buffalo (Memon *et al.* (1971) [23]. Net progesterone content in the bovine corpus luteum increase progressively from day 2 to day 11 and remained relatively constant up to day 20 and, thereafter, fell at day 0 (Hafs and Armstrong, 1968) [24]. Total progesterone content in corpus luteum was increased from day 7 (26.9 µg/g) today 15 (50.7 µg/g) and then declined at day 17 (9.1 µg/g) Mares *et al.* (1962) [25]. The peak progesterone concentration from corpus luteum was 41.73±6.78 µg/g on day 12 of the cycle in the buffaloes (Roy and Mullick (1964) [26]. These findings are in agreement with the present study as maximum P4 concentration was found in mid CL. As bovine corpus luteum synthesizes more progesterone compared to buffalo corpus luteum, comparatively lower progesterone levels in present study could be due to smaller size and weight of corpus luteum. Moreover, the luteal function was significantly lower (p<0.01) in buffaloes compared to cows throughout the cycle (Mondal and Prakash, 2003) [27].

Conclusions

From the present study, it is concluded that the higher peripheral progesterone concentration is associated with more number of large luteal cells (LLC) present in the CL and positively correlated with the luteal P4 production at ML stage in the goat.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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