International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; SP-8(6): 155-157

Received: 08-10-2020
Accepted: 22-11-2020

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Histological and micrometrical exploration of corpus Luteum in cyclic Nellore sheep

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DOI: https://doi.org/10.22271/chemi.2020.v8.i2c.11094

Abstract
Ovaries from twenty two apparently healthy, non-pregnant and cyclic Nellore sheep was taken with the aim to explore histological and micrometrical aspects of the corpus luteum in ovary of adult Nellore sheep. The sections were cut at 5-6µ thick and histological procedures were carried out. From capsule, various trabeculae descend into the substance of corpus luteum. Thickness of the trabeculae which descends from the capsule was almost equal in thickness to that of capsule at the periphery and tapers towards the centre. The capsule mainly comprised of collagen and reticular fibres. The occurrence of two fully developed corpora lutea in the same ovary was also observed.
On the basis of shape and the size, three basic types of lutein cells were observed: large (16.38-28.17µm), small (7.95-27.06µm) and spindle shaped (15.73-29.57µm) cells. Moderate PAS reaction was seen in Capsule, Trabeculae and mild reaction was seen in luteal cells of corpus luteum.

Keywords: Nellore sheep, corpus Luteum, histology, ovary

Introduction
The Nellore breed of sheep was an important, popular meat breed and widely distributed in Andhra Pradesh. The mutton of Nellore breed sheep was tasty and has good demand. Considering the paramount importance and bright prospects of Nellore sheep, production level should be maintained properly by increasing fertility and conception rate. The ovary is dynamic reproductive organ and formation of corpus luteum is a commonly occurring physiological phenomenon. The corpus luteum appeared as a typical encapsulated gland in non-pregnant cyclic animals which secretes progesterone during pregnancy (Dellmann and Eurell, 1998) [2]. Some works on the morphology of corpus luteum of the sheep (Hadek, 1958; Rajput and Sharma, 1996) [4, 9], goat (Shalini and Sharma, 2003, Saleem *et al*., 2017) [10, 11] have been reported. But no comprehensive study has yet been undertaken on the corpus luteum of Nellore sheep in India. Hence, the present work has been undertaken.

Materials and Methods
The study was conducted in the laboratory of the Department of Anatomy, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, India. The ovaries (22) were transferred to and allowed to fix in Neutral Buffered Formalin (NBF) for a period of three days. The ovaries were further processed for routine paraffin technique (Bancroft, 2008) [1]. The sections were cut at 5-6µ using microtome (Leica RM2125RTS) and histological procedures were carried out (Luna, 1968) [5]. Sections were stained by Hematoxylin and Eosin for routine histomorphology, Von Gieson and Verhoeff's method to differentiate Collagen and elastic fibres, Gridley's method to demonstrate reticular fibres, Masson's Trichrome technique to demonstrate Collagen fibres and Crossman's modification of Mallory's triple stain. For histochemical studies the sections were stained by Periodic acid Schiff method (PAS) to demonstrate carbohydrate.
The micrometical parameters were recorded using calibrated ocular micrometer duly calibrated with stage micrometer to elucidate various components of corpus luteum. All the recorded data were put to Standard Statistical procedures (Snedecor and Cochran, 1994) [12] to find out Students "t" test using 16.0 version of SPSS software.

Results and Discussion

The occurrence of two fully developed corpora lutea in the same ovary in Nellore sheep was observed as reported earlier by Rajput and Sharma (1996) [9] in Gaddi sheep. The corpus luteum of adult Nellore sheep was a typical encapsulated gland having stroma and parenchyma. The wall of the ovulated follicle collapsed and thrown into folds, as a result of evacuation of liquor folliculi as described earlier by Hadek in sheep (1958) [4]. The cells of granulose were drawn in along with the connective tissue and cells of theca interna. The theca externa of the follicle changed into thick fibrous capsule of the corpus luteum. It was measured 97.45 - 234.57µm in thickness with a mean of 177.35 ±4.89µm (Table 1).

The capsule sent several trabeculae into the substance of corpus luteum (Fig. 1, 2, 3, 4). The capsule and trabeculae were richly vascular. The capsule was dense mainly comprised of collagen fibres (Fig 2, 3). Elastic fibres were not seen in capsule but were present in tunica intima of the blood vessels (Fig. 3). The reticular fibers were abundant in the capsule and interwoven between the individual luteal cells and also formed the boundary of capillaries and sinusoids (Fig.4). The traberculae divided the parenchyma into several lobes and lobules. The thickness of trabeculae varies between 59.81–123.65µm with the mean of 104.5±3.73µm. These observations were in congruent with those of Rajput and Sharma (1996) [9], Shalini and Sharma (2003) [11] and Gupta *et al.* (2006) [3] in Gaddi sheep, Gaddi goats and goats respectively. Saleem *et al*., (2017) [10] observed the thickness of trabeculae vary between 33 – 110µm with the mean of 60.13 ± 3.90µm in Bakerwali goat.

Moderate PAS reaction was seen in capsule and trabeculae. Blood vessels in capsule of corpus luteum were highly reactive to Alcian Blue. On the contrary, Prasad *et al.* (1979) [8] have described PAS and AMPS negative theca lutein cells in buffalo.

The cells of theca interna were transformed into theca lutein cells. They were seen along the periphery of the gland; these were smaller in size and had darker staining nuclei than the granulosa lutein cells. The lutein cells of granulosa and thecal origin often mingled together (Fig.5). These results were similar to those reported by O' Shea *et al.* (1990) [6] and Ozen, *et al.* (2007) [7] in cows. But on the basis of their shape and the size, these could be classified into three basic types: large cells, small cells and spindle shaped cells (Fig. 5). Shalini and Sharma (2003) [11] and Gupta *et al.* (2006) [3] in goats reported all three types of luteal cells whereas Ozen, *et al.* (2007) [7] reported only small and large cells in cow.

The large luteal cells were of two types *viz*., light and dark types. These were more in number in the centre of the lobule. The large dark cells varied from 17.57 to 28.17µm in diameter with nuclear diameter of 5.77 – 7.94µm. The large light cells varied from 16.38 to 19.78µm in diameter with nuclear diameter of 4.37 – 5.97µm (Table.1). The large cells had a heterochromatic nucleus with clear nucleoli. Their cytoplasm was acidophilic in H&E stained sections.

The small luteal cells were also of two types *viz*., light and dark types. The small dark cells were 11.9 – 27.06µm in diameter with nuclear diameter of 3.28 - 5.37µm. The small light cells were 7.95-11.25µm in diameter with nuclear diameter of 3.05 – 4.07µm (Table.1). Their cytoplasm was also acidophilic. These were more in number near the periphery and less in the centre. However, Gupta *et al.* (2006) [3] also reported almost similar observations i.e., 23.95 ± 2.31µm and 11.67 ± 0.85µm as diameter of granulosa and theca lutein cells in goat respectively.

Although all the cell types occurred in a mingled manner but the concentration of small and spindle shaped cells were always near the trabeculae and capsule. The spindle shaped cells were 15.73-40.57µm in length with nuclear length of 6.01 – 8.25µm (Table.1). Chromatin was coarser, more heterochromatic and darkly stained. These observations were in line with those of adult Bakerwali goats. The spindle shaped cells were 23.8 – 42.84 µm in length with nuclear length of 7.14 – 9.52 µm in Bakerwali goats (Saleem *et al*., 2017) [10].

Table 1: Micrometry of various components of Corpus luteum in Adult Nellore sheep.

Parameter	Mean ± S.E	Range
Thickness of Capsule (µm)	177.35 ±4.89	97.45 - 234.57
Thickness of Trabeculae (µm)	104.5±3.73	59.81 - 123.65
Diameter of Large dark lutein cells (µm)	24.26±0.60	17.57-28.17
Diameter of Large light lutein cells (µm)	18.43± 0.18	16.38-19.78
Diameter of Small dark lutein cells (µm)	22.60±0.689	11.9-27.06
Diameter of Small light lutein cells (µm)	10.29±0.14	7.95-11.25
Length of Spindle shaped cells (µm)	23.79±0.67	15.73-29.57
Nuclear diameter of Large dark lutein cells (µm)	7.24±0.11	5.77-7.94
Nuclear diameter of Large light lutein cells (µm)	5.20±0.07	4.37-5.97
Nuclear diameter of Small dark lutein cells (µm)	4.63±0.09	3.28-5.37
Nuclear diameter of Small light lutein cells (µm)	3.62±0.05	3.05-4.07
Nuclear length of Spindle shaped cells (µm)	7.44±0.09	6.01-8.25

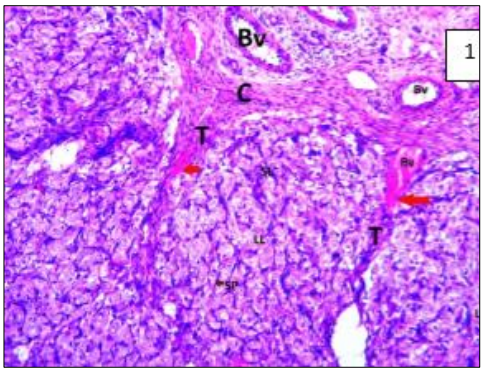

Fig 1: Photomicrograph showing capsule and Traberculae of corpus Luteum having rich blood supply (Arrow) (H&E 40X)

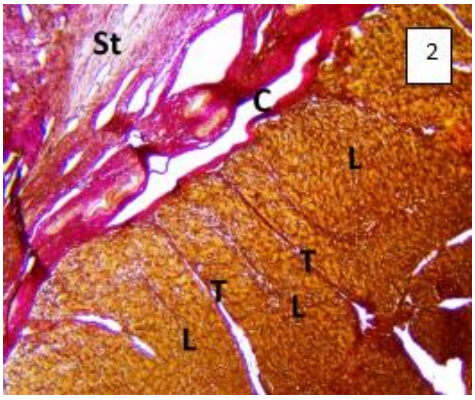

Fig 2: Photomicrograph showing capsule and its Traberculae rich in collagen fibres (Vangieson's,100x)

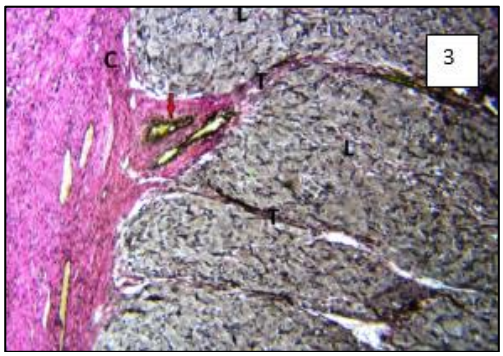

Fig 3: Photomicrograph showing capsule and its traberculae dividing paryncyma into lobes, elastic fibres of trabercular blood vessels (arrow) (Verhoff's, 100x)

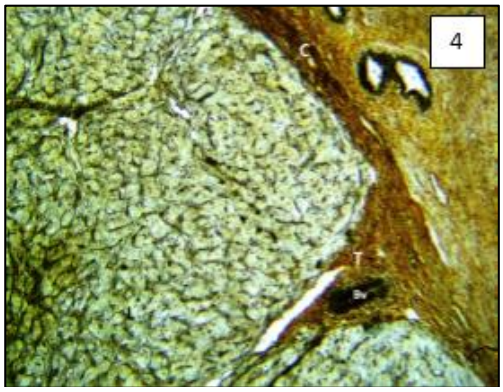

Fig 4: Photomicrograph showing reticular fibre distribution in capsule, traberculae and parenchyma (Gridley's, 100x)

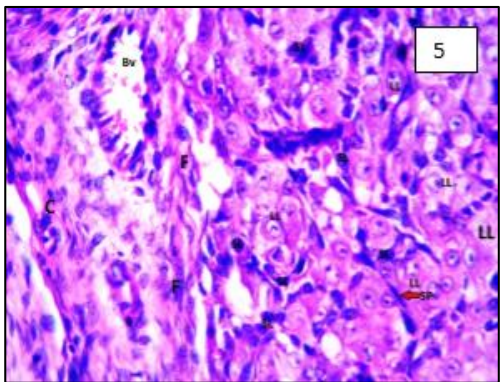

Fig 5: Photomicrograph showing large, small luteal cells and spindle shaped cells present in the corpus luteum (H&E, 400X)

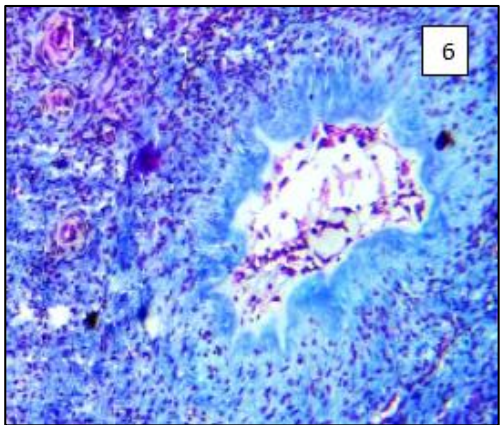

Fig 6: photomicrograph showing regressing corpus Luteum (Crossmon's modification of Mallory's triple stain, 100x).C: Capsule, T: Traberculae, L: Lobe, F: fibroblast, Bv: Blood vessel, LL: Large luteal cell, SL: small luteal cell, SP: Spindle shaped cell

Regressing corpus luteum

Regressing corpus luteum was measured 750-980µm in diameter. Its hyalinised capsule thickness was 75-147µm. The capsule stained blue in Crossmon's modification of mason's trichome stain. In the centre of the regressing corpus luteum mononuclear, dendritic cells were observed with fuscinophilic granules. Rajput and Sharma (1996) [9] in Gaddi sheep observed similar findings. The corpora albicantia developed due to the replacement of luteal cells of the corpora lutea by infiltration of fibroblasts and macrophages. The luteal cells were vacuolated with indistinct cell boundaries. The nuclei were displaced and showed karryorrhexis and karyolitic changes. Macrophages phagocytised the dead and degenerated luteal cells, the latter were finally replaced by the fibroblasts. Similar findings were observed by Gupta *et al.* (2006) [3] in goat. Hadek (1958) [4] reported some pigment cells in corpora albicantia of sheep. Such cells were not reported in the present study. It showed moderate to strong reaction for PAS. Similar findings were encountered by Gupta *et al.* (2006) [3]. However, Rajput and Sharma (1996) [9] in Gaddi sheep encountered negative reaction for PAS and positive reaction for AMPS.

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