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**Supriya B**

Ph.D., Scholar, Department of Anatomy, Veterinary College, Bengaluru, Karnataka, India

**KV Jamuna**

Professor and Head, Department of Anatomy, Veterinary College, Bengaluru, Karnataka, India

**P Jagapathi Ramayya**

Professor and Head, Department of Anatomy, College of Veterinary Science, Tirupati, India

**RV Prasad**

Professor, Department of Anatomy, Veterinary College, Bengaluru, Karnataka, India

**ML Sathyanarayana**

Professor and Head, Department of Veterinary Pathology, Veterinary College, Bengaluru, Karnataka, India

**AVN Siva Kumar**

Assistant Professor, Department of Veterinary Physiology, College of Veterinary Science, Tirupati, India

**Corresponding Author:****Supriya B**

PhD Scholar, Department of Anatomy, Veterinary College, Bengaluru, Karnataka, India

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# Morphologic and morphometric exploration of the ovarian follicles in the Nellore sheep (*Ovis aries*)

**Supriya B, KV Jamuna, P Jagapathi Ramayya, RV Prasad, ML Sathyanarayana and AVN Siva Kumar**

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### Abstract

Ovary plays the dynamic role in the reproductive biology of female animals. With the aim to study the ovarian and follicular morphometry of Nellore sheep, both right and left ovaries were collected separately from the slaughter houses of Tirupati. For each of the specimens, gross parameters such as weight, length, width and thickness were recorded. Then they were processed and stained with H&E, Masson's trichome, Verhoff's and Gomori's stains for histology and histomorphometry. The right ovary weighed (0.37±0.06g) more the left (0.30±0.03g). The length of the right ovary (1.020±0.12cm) was lower than the left (1.13±0.07cm) but the width of the right (0.93±0.10cm) was greater than the left (0.83±0.02cm). The diameter of ovarian follicles in the cortex was measured as primordial (29.74±1.30µm), primary (47.27±2.06µm), secondary or early antral (140.65±15.90µm), small vesicular or antral (584.43±23.9µm) and large vesicular/antral (1413.61±102.94µm) of ovary. In the large antral follicle, the thickness of granulosa cell layer was 80.08±1.43µm, theca interna 51.03±1.86µm, theca externa 41.02±0.92µm and the oocyte diameter was 118.9±3.64µm. These results will be helpful to manipulate ovarian functions in small ruminants.

**Keywords:** Ovary, Nellore sheep, follicle, antral follicle, atresia

### 1. Introduction

The Nellore breed of sheep a popular meat breed and is widely distributed in Andhra Pradesh. This breed is one of the tallest among all other breeds in India. The mutton of Nellore breed sheep is 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. Some works on the morphology and physiology of reproductive organs of the sheep (Hadek, 1958; Rajput and Sharma, 1996; Shehan *et al.*, 2015; Sahu *et al.*, 2017) [7, 15, 19, 16] have been reported. But no comprehensive study has yet been undertaken on the ovary of Nellore sheep in India. Therefore, the study was designed to describe the morphology and morphometry of the ovary of Nellore breed of sheep. The knowledge of the present study will contribute significantly in the reproductive biology and biotechnology of small ruminants.

### 2. Materials and Methods

#### 2.1 Gross morphology

##### 2.1.1 Collection and transportation of ovaries

Both the right and the left ovaries of the Nellore breed of sheep were collected from the slaughter houses of Tirupati. The animals were apparently healthy, non pregnant and cyclic. The ovaries were then kept in collection vial containing phosphate buffered saline in a thermos flask at 38 °C and transported to the laboratory within 2 to 3 h of slaughter. The ovaries were then transferred to sterilized Petri dishes and rinsed thoroughly with physiological saline at 38 °C before further processing.

##### 2.1.2 Measurement of weight, length and width

After separating, the right and left ovaries were weighed with the help of an electric balance. The length, breadth and thickness were measured with the help of digital vernier callipers. After taking measurements the medulla was scooped out from each half of the ovary.

The numbers of follicles were counted in the cortical slice using stereo zoom microscope.

**2.1.3 Statistical Analysis:** The data generated from this experiment was organized and analysed using student's t-test in SPSS 20 software.

## 2.2 Histomorphology

The study was conducted in the laboratory of the Department of Anatomy, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, India. The ovaries (20 pairs) 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 5-6 $\mu$  using microtome (Leica RM2125RTS) and histological procedures were carried out (Luna, 1968) [11]. The histological sections were subjected to standard Haematoxylin-Eosin, Masson's trichrome, vangieson's, Verhoeff's and Gomori's staining techniques (Bancroft, 2008) [1]. The diameter of ovarian follicles, oocytes and thickness of follicular layers and the thickness of zona pellucida were measured by inbuilt software with micaps proseries 1080 HDMI camera.

## 3. Results and Discussion

### 3.1. Gross morphology

The shape of the ovary of Nellore sheep ranged from spherical, oval to almond. Some ovaries showed large corpus luteum or translucent large vesicular follicles on their surface. Biometrical results revealed that the right ovary (0.37 $\pm$ 0.06 g) weigh more than the left one (0.30 $\pm$ 0.03 g). None of the gross parameter of the ovary varied significantly between the right and left ovaries (Table.1). In case of wild goat the weight of the right (0.66 $\pm$ 0.16 g) and left (0.52 $\pm$ 0.20 g) ovaries is very high (Dogan *et al.*, 2019) [4].

**Table 1:** Biometrical measurements of right and left ovaries of Nellore sheep ( $P < 0.01$ )

Measurements	ovary		Level of significance
	Right	Left	
Weight(g)	0.37 $\pm$ 0.06	0.30 $\pm$ 0.03	NS
Length(cm)	1.020 $\pm$ 0.12	1.13 $\pm$ 0.07	NS
Width(cm)	0.93 $\pm$ 0.10	0.83 $\pm$ 0.02	NS
Thickness(cm)	0.63 $\pm$ 0.04	0.60 $\pm$ 0.02	NS

### 3.2. Histomorphology

Histologically the longitudinal section of ovary showed outer parenchymatous cortex and inner vascular medulla.

### 3.3 Cortex

The cortex consisted of primordial follicles, primary follicles, secondary follicles (early antral), tertiary follicles (antral and large antral), atretic follicles and corpus luteum. The ovaries presented a number of follicles along their surface. The follicles were more on right ovary (on an average 20.9) than on left ovary (on an average 15.2). The cortical stroma consisted of higher proportion of fibroblast like cells, and fibres oriented in different directions. The cells were spindle shaped with darkly stained oval nucleus. The biometrical values pertaining to diameter of various stages of follicles, the diameter of oocyte, and the thickness of various layers of graafian or antral follicles were presented in Table: 2.

### 3.4 Germinal epithelium

The ovary of Nellore sheep was lined with simple squamous

to simple cuboidal epithelium (Fig: 1A, 1B). Similar findings were observed in sheep (Rajput and Sharma, 1996, Shehan *et al.*, 2015) [15, 19], goats (Joshi *et al.*, 1977) [10], cyclic buffalo (Bharadwaj and Roy, 2006) [3], and Anatolian wild goat (Dogan *et al.*, 2019) [4]. At some places breach of surface epithelium was observed that may have resulted from ovulation. In some specimens from old individuals, stratified cuboidal epithelium was observed. At few locations stratification was observed in squamous, cuboidal and columnar cells in the ovary of old cow (Sharma *et al.*, 1974) [18] and goat (Singh and Prakash, 1988) [20]. The thickness of superficial epithelium was 5-8 $\mu$ m. The height of the surface epithelium was comparable with that of cyclic Indian buffalo (Bharadwaj and Roy, 2006) [3].

### 3.5 Tunica albuginea

The thickness of tunica albuginea was 81.82 $\pm$ 1.30 $\mu$ m. The thickness of the tunica albuginea was not even but reduced at contact places with large antral follicles (Fig. 2D). The tunica albuginea was highly vascular having blood vessels of varying diameters, fibroblast cells with dark staining nucleus (Fig: 1B), and comprised of densely woven connective tissue fibres particularly rich in collagen fibres (Fig: 3B). Big network of reticular fibres are also visible in tunica albuginea of Nellore sheep stained with Gomori's (Fig. 2A). These findings were in character with the findings of Bharadwaj and Roy (2006) [3] in buffaloes. Contrary to this Sahu *et al.*, (2017) [16] stated that the ovary of kendrapada sheep do not consist of reticular fibres. Shalini and Sharma (2004) [17] observed the presence of elastic fibres in the tunica albuginea in Gaddi goats which might be due to species variation.

According to Gupta *et al.*, (2010) [6], in young goat ovary the tunica albuginea was made up of mainly reticular fibres along with few sparsely arranged collagen fibres. Interspersed between the fibres there were few fibroblast like cells. In adult and senile animals this layer chiefly contained collagen and reticular fibres.

### 3.6 Primordial follicle

The primordial follicles appeared either singly, distributed evenly in peripheral cortex of some ovaries and in some specimens they formed groups, egg nets just underneath the tunica albuginea. These findings were in accordance with Banks, (1993) [2]. They consisted of single layer of flat follicular cells (4-8) surrounding the primary oocyte (Fig.1A). The ovum was spherical in shape and consisted of vesicular nucleus with the deeply stained chromatin material. The cytoplasm of oocyte was granular and eosinophilic. These findings were in line with Paramasiva and Sharma (2003) [14] in ovary of Gaddi sheep.

In the present study multioocyte follicles were detected (Fig. 1D) and this observation was in line with earlier reports (Hadek, 1958 and Oliveira *et al.*, 2017) [7, 13]. Such occurrence of polyovular follicle and polynuclear ova is related with age and sexual cycle in goats (Joshi *et al.*, 1976) [9]. Fine reticular fibres surrounded the primordial follicles which were clearly visible with Gomori's stain (Fig.2A). Paramasiva and Sharma (2003) [14] and Joshi *et al.*, (1977) [10] measured the follicular diameter of primordial follicle as 25-35  $\mu$ m and 24-48  $\mu$ m in Gaddi sheep and goat ovaries respectively. In the present study measurement of primordial follicle (Table: 2) coincides with these earlier reports.

### 3.7 Primary follicle

The primary follicle consisted of primary oocyte surrounded

by 8-15 cuboidal follicular cells. The ovum was spherical in shape and had a large vesicular centrally located nucleus with darkly stained chromatin material. The follicular cells showed prominent oval darkly stained nucleus (Fig.1C). These observations were in conformity with the observations of Paramasiva and Sharma (2003) [14] in Gaddi sheep. The longitudinal and transverse diameters of primary follicle at post-pubertal stage were recorded to be  $18.763 \pm 1.23 \mu\text{m}$  and  $12.794 \pm 1.12 \mu\text{m}$  respectively in kendrapa sheep (sahu *et al.*, 2017) [16]. The measurements of the primary follicles in the present study (Table: 2) corresponded with those of the goats (Joshi *et al.*, 1977) [10] and Gaddi sheep (Rajput and Sharma, 1996, Paramasiva and Sharma, 2003) [15, 14]. But Mahammadpour (2007) [12] stated that the diameter of primary follicle was significantly larger in goats to its counterpart in sheep.

### 3.8 Secondary follicle/Early antral follicle

The secondary follicle consisted of the ovum surrounded by 2–6 layers of follicular cells (Fig. 1A). The cytoplasm of the ovum was finely granular and eosinophilic. The nucleus was eccentrically placed, with dark staining chromatin network and clearly distinguishable nucleolus (Fig. 2B). The follicular cells were cuboidal or polyhedral in shape with dark oval or spherical nucleus. As the follicle grew, vesicles appeared instead of intercellular spaces of the follicular cells. At this point, secondary follicle unveiled a well-developed zona pellucida which appeared thick pink in H&E staining, separating the ovum from the granulosa cells (Fig. 2B). These findings were in accordance with Haque *et al.*, (2016) [8] in black Bengal goats. A clearly distinguishable basement membrane separated the granulosa cells from the surrounding stroma (Fig. 2C). These findings were in accordance with the findings of Sahu *et al.*, (2017) [16] in the ovary of Kendrapada sheep. The theca was poorly defined. The theca externa and interna were not distinguished.

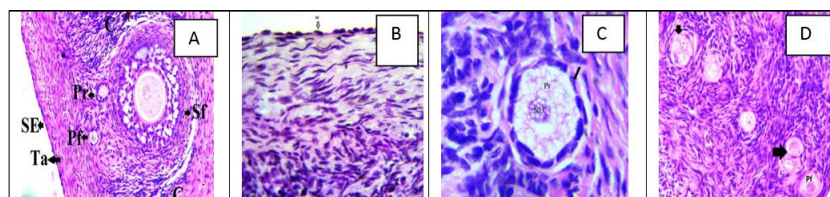
### 3.9 Tertiary follicle/Antral follicle

The wall of tertiary follicle consisted of well-defined membrana granulosa, theca interna and externa. The oocyte was placed eccentrically in the follicle (Fig. 2D). The oocyte was surrounded by vitelline membrane, zona pellucida and

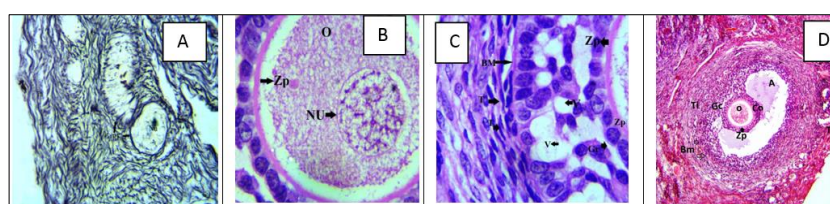
corona radiata. The oocyte showed eccentrically placed nucleus with sparse chromatin network. A group of granulosa cells extended up to and wrap around the corona radiata forming cumulus oophorus. Tertiary follicle showed large cavity known as antrum filled with liquor folliculi (Fig. 2D). The granulosa cell layer was separated by theca interna by a defined reticular basement membrane (Fig. 3C) the cells of granulosa attached to basement membrane were columnar and their nucleus was elongated oval. In the remaining layers the cells were either spherical or polyhedral with distinct deeply stained spherical nucleus (Fig. 3A). These findings were in conformity with the findings of Trautmann and Fiebiger (1957) [21] in domestic animals. The theca layer was clearly identifiable into the outer theca externa and inner theca interna layers, out of which theca interna was thick (Fig.3A). The theca interna consisted of some epitheloid cells and some spindle shaped cells interspersed with reticular (Fig.3C) and collagen fibres (Fig.3B). Numerous blood capillaries of various calibre were observed in theca interna and their concentration was more towards the basement membrane separating the granulosa and thecal layers and also in the junction zone of theca interna with externa. These findings were in congruence with the reports of Gupta *et al.*, (2007) [5] in goat. The theca externa formed chiefly by collagen fibres along with reticular fibres. Several fibroblasts like cells were seen. In Gaddi sheep Rajput and Sharma, (1996) [15] observed a fibrous theca externa. The theca externa was continuous with stroma without much demarcation (Fig.3A). The antrum contained acidophilic liquor folliculi which appeared homogenous gel like fluid (Fig. 2D). The measurements of antral and large antral follicles (Table: 2) corresponds with those of Paramasiva and Sharma (2003) [14] in Gaddi sheep.

### 3.10 Atretic follicles

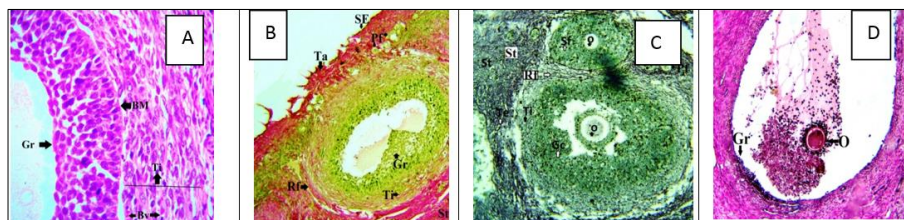
Artesia was observed in different stages of follicular development. The cells of membrana granulosa sloughed off and showed nuclear degenerative changes i.e. pyknosis (Fig: 3D). In some follicles ova was not found. These findings were congruent with Sahu *et al.*, (2017) [16] and they attributed this high follicular atresia to high genetic potential and high prolificacy of kendrapada sheep.



**Fig 1:** A: Photomicrograph showing cortex of ovary (H &E, 100x), B: Photomicrograph showing surface epithelium, tunica albuginea (H &E 1000x), C: Photomicrograph showing primary follicle showing cuboidal pregranulosa cells (arrow) (H &E, 400x), D: Photomicrograph showing multiocyte follicles (arrow) (H &E, 100x). SE: surface epithelium, Ta: tunica albuginea, Pf: primordial follicle, Pr: primary follicle, NU: nucleus



**Fig 2:** A: Photomicrograph showing reticular fibres in the cortex surrounding the follicles (Gomori's, 100x), B: Photomicrograph showing oocyte of secondary follicle (H &E, 1000x), C: Photomicrograph showing vesicle formation in early antral follicle and formation of basement membrane (arrow) (H &E, 100x), D: Photomicrograph showing large antral follicle (H &E, 100x), Ta: tunica albuginea, RF: reticular fibres, St: stroma, O: oocyte, NU: nucleus, Bm: basement membrane, Gr/Gc: granulosa cells, Zp: zona pellucida, Co: corona radiata, V: vesicle, A: antrum, Ti: theca interna, Te: Theca externa, Co: cumulus oophorus



**Fig 3:** A: Photomicrograph showing wall of large antral follicle showing blood vessel between granulosa and theca (H &E, 400x). B: Photomicrograph showing collagen fibre distribution in the ovary (Vangieson's, 100x), C: Photomicrograph showing reticular fibre distribution in the ovary (Gomori's, 400x), D: Photomicrograph showing atretic follicle (Masson's trichrome, 100x), Ta: tunica albuginea, Sf: Secondary follicle, St: Stroma, o: oocyte, Gr: granulosa cells, A: antrum, Ti: theca interna, Te: Theca externa, Bv: Blood vessel

**Table 2:** Micrometrical measurements of certain parameters of various stages of follicles in Nellore sheep ovary

Parameter		Primordial Follicle ( $\mu\text{m}$ )	Primary Follicle ( $\mu\text{m}$ )	Secondary follicle ( $\mu\text{m}$ )	Antral Follicle ( $\mu\text{m}$ )	Large antral Follicle ( $\mu\text{m}$ )
Follicular diameter	Longitudinal	29.74 $\pm$ 1.30	47.27 $\pm$ 2.06	140.65 $\pm$ 15.90	584.43 $\pm$ 23.9	1413.61 $\pm$ 12.94
	Transverse				592.99 $\pm$ 24.72	1441.039 $\pm$ 12.39
Oocyte diameter		17.56 $\pm$ 0.23	27.0 $\pm$ 1.24	50.98 $\pm$ 3.47	81.10 $\pm$ 2.80	118.9 $\pm$ 3.64
Thickness of granulosa		NA	NA	56.39 $\pm$ 3.29	60.27 $\pm$ 1.69	80.08 $\pm$ 1.43
Thickness of theca interna		NA	NA	37.76 $\pm$ 0.96	42.47 $\pm$ 1.38	51.03 $\pm$ 1.86
Thickness of theca externa		NA	NA		33.04 $\pm$ 1.22	41.02 $\pm$ 0.92

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