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Gross, histological and histochemical studies on the ovary and oviduct of Kendrapada sheep (*Ovis aries*) at different age groups

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Abstract

The present study was conducted on ovary and oviduct of 18 healthy female Kendrapada sheep from the Kendrapada district, Odisha minimum six each from three age groups viz. pre-pubertal (4-6 months), pubertal (8 to 10 months) and adult animals (11 months and above). After conducting gross studies, histological, micrometrical and histochemical observations were made from sections obtained from different parts of the ovary and oviduct. Gross studies revealed that the ovaries of Kendrapada sheep were firm and oval in shape. They were held in positions by the broad ligament known as mesovarium. The oviducts were tortuous and had three parts as infundibulum, ampulla and isthmus. Biometrical observations revealed that the weight of left ovary and oviduct were more than the right ones in pubertal and post-pubertal stages. The left oviduct was longer in the animal. Histological studies revealed that the ovary consisted of an outer cortex and an inner medulla. Further, the cortex consisted of the superficial epithelium or germinal epithelium, tunica albuginea, primary follicles, secondary follicles, matured Graafian follicles and atretic follicles. The medulla had Hilar cells, blood vessels, collagen and reticular fibers. The wall of the oviduct comprised of four layers viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The oviduct was lined by pseudo-stratified columnar epithelium. Mucosal folds were maximum in the tubal part of infundibulum, less in ampulla and least in isthmus in all stages of the animal. The propria-submucosa was comprised of loose connective tissue with blood vessels, abundant amount of collagen fibres and few reticular fibers. It housed oviductal glands that were simple tubulo-alveolar type. The micrometrical, histochemical and histoenzymatic studies of ovary and oviduct revealed variable results pertaining to their functional status and genetic potential at pre-pubertal, pubertal and post-pubertal stages of Kendrapada sheep.

Keywords: Gross, histological, histochemical, ovary, oviduct, Kendrapada sheep

Introduction

Kendrapada sheep is native to the coastal Jagatsinghpur and Kendrapada districts of Odisha and has been declared as 'rare and singular species' by the government of India. It is locally called as 'Kuji mendha' and has been registered as a breed of sheep in the National Bureau of Animal Genetic Resources (NBAGR). It is a fast breeder and gives multiple births. So, it becomes a profitable livelihood source. It is identified to carry FecB mutation gene that is responsible for multiple births or prolificacy. It has received registration number India_Sheep_1500_Kendrapada_14042. It became the 42nd sheep breed in India to receive such tag. They are well adapted to high ambient temperature, high humidity and heavy rain. As it is a new breed in Odisha registered at the National level, this present study was taken up to establish the reproductive database of uterus with reference to its capacity for multiple births. So far no work has been done in this context. Hence, the present work was undertaken to study the gross, histological and histochemical characteristics of ovary and oviduct of Kendrapada sheep at pre-pubertal, pubertal and adult stages.

Materials and methods

The samples of ovary and oviduct were procured from 18 healthy female Kendrapada sheep from the Kendrapada district, Odisha minimum six each from three age groups viz. pre-pubertal (4-6 months), pubertal (8 to 10 months) and adult animals (11 months and above) immediately after they are slaughtered. The approximate age of the animals was determined from their dentition pattern. The body weight will be measured by using the formula – $L \times G^2/300$ in lbs., where L = Body length of the animal and G – heart girth measured just caudal

to the fore limb. The specimen was brought to the laboratory in ice-pack as soon as possible. Gross biometrical parameters were recorded for each organ separately with the help of Vernier Calliper, scale and non-stretchable thread and the data were subjected to statistical analysis [17].

After recording the gross parameters, the specimen were fixed in 10% Neutral Buffered Formalin (NBF) for 24-48 hours and subsequently tissues from ovary, oviduct, cornu, corpus and cervix from 18 animals (six from each group) were processed for routine paraffin block preparation by alcohol-xylene schedule. Tissue sections of 5-6 μm thick were obtained from these blocks on clean glass slides with the help of digital rotary microtome. The sections were then subjected to various histological and histochemical methods [7]. Further, some tissue samples were fixed in 10% formol calcium at 4 $^{\circ}\text{C}$ for 24 hours immediately after their collection from 18 animals (six from each group). Subsequently, 10-12 μm thick tissue sections were cut in the Cryostat and processed for histoenzymatic study.

Results and discussion

The results have been critically analyzed with reference to the ovary and oviduct of the Kendrapada sheep at different age groups under study. The internal female genitalia of Kendrapada sheep consisted of Ovaries (Left and right), Oviducts (Left and right) and uterus.

Ovary

Gross anatomy

The ovaries of Kendrapada sheep were firm and oval in shape. They were held in positions by the broad ligament known as mesovarium. They were attached to the fimbriae of the oviduct on either side. Similar findings were reported in cow by [10]. Biometrical results revealed that the weight of left ovary was more than the right one in pubertal and post-pubertal stages, but opposite results were recorded in pre-pubertal stages of Kendrapada sheep. The weight of left and right ovaries in pre-pubertal stage was 0.120 ± 0.02 gm and 0.176 ± 0.03 gm respectively (Table 1). The weight of left and right ovaries in pubertal stage was 1.335 ± 0.05 gm and 1.253 ± 0.05 gm respectively. The weight of left and right ovaries in post-pubertal stage was 1.5 ± 0.08 gm and 0.95 ± 0.03 gm respectively.

Histology and histochemistry

The histomorphological examination of ovary in Kendrapada sheep revealed that it consisted of an outer cortex and inner medulla (Fig. 1).

Cortex

The cortex consisted of the superficial epithelium or germinal epithelium, tunica albuginea, primary follicles, secondary follicles, matured Graafian follicles (Fig. 2) and atretic follicles which were in agreement with those reported by [3]. The thickness of cortex at post-pubertal stage was recorded to be 69.359 ± 3.68 μm (Table. 2). The cortex was positive for AB-PAS showing presence of neutral mucopolysaccharide and also showed weak alkaline phosphatase activity.

The ovary of Kendrapada sheep was lined with stratified cuboidal epithelium. Similar findings were observed by [6 and 15] in goats, [12] in sheep and [2] in buffalo. On the contrary, [14] observed stratified type of epithelium in senile Gaddi goats. This observation of the surface epithelium of ovary in the present study could be attributed to breed difference. The thickness of superficial epithelium or germinal epithelium at

post-pubertal stage was recorded to be 22.049 ± 1.58 μm (Table. 3). It was reinforced with collagenous fibres as seen in Masson's trichrome stain. The superficial epithelium was moderately positive for AB-PAS positive showing presence of acid mucopolysaccharide (Fig. 3). It also showed weak acid and alkaline phosphatase activities, especially in the post-pubertal stage (Fig. 4). It was found having the presence of lipids under Sudan III. Occurrence of lipids has been described in the germinal epithelium of various mammalian ovary [11]. Similar observations were also recorded by [14] in Gaddi goats.

The tunica albuginea comprised of densely woven connective tissue fibres, which was particularly rich in collagen (Fig. 5) for more strength of ovarian wall and for multiple ovulation. Contrary to this [2], found compact reticular fibres, mesenchymal cells differentiating into fibroblasts and smooth muscles in buffaloes. [14] observed presence of elastic fibers in the tunica albuginea in Gaddi goats. This might be due to species difference. It was highly vascular having blood vessels of varying diameters. Similar findings were observed by [13] in the ovary of cattle. Its thickness at post-pubertal stage was recorded to be 42.067 ± 2.12 μm , where large developing follicles approached the ovarian surface. The tunica albuginea was moderately positive for AB-PAS stain showing the presence of neutral mucopolysaccharide. The blood vessels of this layer showed weak alkaline phosphatase activity and there was also presence of acid mucopolysaccharide.

The ovaries presented a number of follicles along their surface. The follicles were more on left ovary than on right ovary. It was compared with the findings of [14] in Gaddi goats. The primary follicle consisted of an ovum covered with a layer of low cuboidal or flattened follicular cells. Similar findings were reported by [3] in domestic animals and [2] in buffalo. The follicular cells enclosed an immature ovum. The ovum was spherical in shape and had a large vesicular shaped nucleus with the deeply stained chromatin material. The nucleolus was distinct. The follicles were located mainly in the outer cortical region of the ovary, which were in line with the findings of [5]. The longitudinal and transverse diameters of primary follicle at post-pubertal stage were recorded to be 18.763 ± 1.23 μm and 12.794 ± 1.12 μm respectively. The size of the follicles corresponded with those of the goats' [6] and Gaddi sheep [12]. The cytoplasm of the secondary follicle was finely granular and eosinophilic. A thin reticulum separated the follicle from the ovarian stroma. The ovum was surrounded by 2-6 layers of follicular cells. Similar reports were given by [3, 19]. The zona pellucida marked off the ovum from the surrounding follicular cells. The theca was poorly defined. The theca externa and interna were not distinguished. Similar findings were reported by [6] in goats. Many secondary follicles were seen in various stages of atresia, which could be attributed to high genetic potential or prolificacy of Kendrapada sheep as development of more numbers of follicles resulted in formation many secondary follicles, which further might develop into Graafian follicle. The wall of Graafian follicle had a well-defined membrana granulosa, theca interna and externa. The oocyte was placed eccentrically in the follicle. The oocyte was surrounded by vitelline membrane, zona pellucida and corona radiata. A group of granulosa cells extended up to the corona radiata layer of oocyte forming cumulus oophorus. Graafian follicle had cavity known as antrum filled with liquor folliculi. Sometimes, these follicles occupied the major portion of the cortex. The transverse luminal diameter of the Graafian

follicle at post-pubertal stage was recorded to be $120.016 \pm 5.12 \mu\text{m}$. Similar findings were found by [2 and 13]. The transverse luminal diameter of Graafian follicle at post-pubertal stage was recorded to be $120.016 \pm 5.12 \mu\text{m}$. However, [2] reported that the diameter of the Graafian follicle of buffalo measured between $5126.01 \pm 666.70 \mu\text{m}$, which might be due to species difference. It pressed against the tunica albuginea and bulged on the surface of the ovary. A thick basement membrane surrounded the membrana granulosa and separated it from the theca. The thickness of membrana granulosa at post-pubertal stage was recorded as $10.743 \pm 2.22 \mu\text{m}$. The theca layer was clearly identifiable into the outer theca externa and inner theca interna layers. The thickness of theca interna and theca externa at post-pubertal stage was recorded to be $6.171 \pm 1.38 \mu\text{m}$ and $9.143 \pm 1.11 \mu\text{m}$ respectively. The antrum particularly large sized cavities or vesicles contained acidophilic liquor folliculi which appeared homogenous gel like fluid. Similar findings were also observed by [2] in buffalo. Further, most of the secondary and vesicular follicles which failed to discharge ovum had undergone atresia, showing degenerative changes either in the ovum or in the granulosa cells [6]. Had also observed atresia in different stages of follicular development. The cells of membrana granulosa showed nuclear degenerative changes i.e. pyknosis. In some follicles ova were not found. Similar findings were observed by [20] in goat.

Medulla

The cortico-medullary boundary was not clearly demarcated. The only identification of medulla was that it was rich in blood vessels of varying diameter. The medullary area was richly collagenous with a rarer network of coarse reticulin. Similar findings were reported by [15 and 18] in goats. The medulla was positive for AB-PAS showing presence of acid mucopolysaccharide. The tunica intima of blood vessels of the medulla was also positive for AB-PAS showing the presence of acid mucopolysaccharide. The blood vessels showed weak alkaline phosphatase activity and some blood vessels were also found having acid phosphatase activity. The medulla was found positive for presence of lipids under Sudan III stain. These results under present study indicated the better functional status of ovary with less degenerative changes. Hilar cells were ovoid or polyhedral in shape having relatively small and condensed nuclei which were masked with cytoplasmic granules. The cytoplasm of hilar cells was granular. They were found scattered singly or in groups particularly between the blood vessels in medullary region.

2. Oviduct

Gross anatomy

The oviduct was a long tubular structure which was coiled variably along its length. The infundibulum was attached to the tubal extremity of ovary by tubal fimbriae. The ampullary region was quite tortuous and the isthmus was a comparatively less coiled part of the oviduct. These findings were similar to those reported in ruminants [4] in other domestic animals and [13] in cattle. It forms a shape of "S" or "U" before terminating into the uterine horns. The termination was more or less abrupt from oviduct to uterine horns, which was in agreement with [12] in Gaddi sheep, but in contradiction with the findings of [13] in ruminants, which might be due to species difference. The weight of left oviduct was more than the right one in pubertal and post-pubertal stages, but opposite results was obtained in the pre-pubertal stage of the animal. The weight of left and right oviducts in pre-pubertal stage was

found to be $0.120 \pm 0.04 \text{ gm}$ and $0.214 \pm 0.03 \text{ gm}$ respectively. The weight of left and right oviducts in pubertal stage was found to be $0.77 \pm 0.02 \text{ gm}$ and $0.636 \pm 0.02 \text{ gm}$ respectively. The weight of left and right oviducts in post-pubertal stage was found to be $0.7 \pm 0.02 \text{ gm}$ and $0.337 \pm 0.02 \text{ gm}$ respectively.

Further, the left oviduct was longer than the right one in all the age groups of Kendrapada sheep. The length of left and right oviducts in pre-pubertal group was recorded to be $11.6 \pm 0.21 \text{ cm}$ and $10.4 \pm 0.12 \text{ cm}$ respectively. The length of left and right oviducts in the pubertal group was recorded to be $12.3 \pm 0.27 \text{ cm}$ and $10.2 \pm 0.18 \text{ cm}$ respectively. The lengths of left and right oviducts in post-pubertal group were recorded to be $19.5 \pm 0.23 \text{ cm}$ and $15.5 \pm 0.24 \text{ cm}$ respectively (Table 1). The width of left infundibulum was less than the right one in all the age group of the sheep. The width of left and right infundibula in the pre-pubertal stage was found to be $0.6 \pm 0.02 \text{ cm}$ and $0.9 \pm 0.03 \text{ cm}$ respectively. The width of left and right infundibula in the pubertal stage was found to be $0.9 \pm 0.03 \text{ cm}$ and $1.3 \pm 0.05 \text{ cm}$ respectively. The width of left and right infundibula in the post-pubertal stage was found to be $1.2 \pm 0.07 \text{ cm}$ and $1.7 \pm 0.06 \text{ cm}$ respectively (Table 1). The width of left ampulla was less than the right one in all the age group of the sheep. The widths of left and right ampulla in the pre-pubertal stage were found to be $0.3 \pm 0.03 \text{ cm}$ and $0.5 \pm 0.01 \text{ cm}$ respectively. The width of left and right ampulla in the pubertal stage was found to be $0.6 \pm 0.01 \text{ cm}$ and $0.8 \pm 0.02 \text{ cm}$ respectively. The width of left and right ampulla in the post-pubertal stage was found to be $0.8 \pm 0.02 \text{ cm}$ and $1.2 \pm 0.04 \text{ cm}$ respectively (Table 1). The width of left isthmus was more than the right one in all the age groups of the sheep. The width of left and right isthmus in the pre-pubertal stage was found to be $0.4 \pm 0.02 \text{ cm}$ and $0.3 \pm 0.01 \text{ cm}$ respectively. The width of left and right isthmus in the pubertal stage was found to be $0.6 \pm 0.02 \text{ cm}$ and $0.4 \pm 0.01 \text{ cm}$ respectively. The width of left and right isthmus in the post-pubertal stage was found to be $0.8 \pm 0.03 \text{ cm}$ and $0.6 \pm 0.02 \text{ cm}$ respectively (Table 1).

Histology and histochemistry

The oviduct of Kendrapada sheep was divided into the funnel shaped portion whose anterior end was thrown as finger like projections over the ovarian surface known as "infundibulum", the expanded intermediate segment is "ampulla" and the narrow portion near the uterus called "isthmus". The wall of the oviduct comprised of four layers viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The tunica mucosa was further subdivided into lamina epithelialis and lamina propria. Lamina propria and tunica submucosa were blended together because of absence of distinct lamina muscularis mucosae to form propria-submucosa. This was in agreement with the earlier descriptions in goat [6], sheep [16] and other domestic animals [3, 13, 19].

Tunica mucosa of infundibulum was characterized by the presence of longitudinal folds throughout the length of the oviduct. Similar findings were reported by [19] in other domestic animals and [8] in buffalos, which increased the surface area of lumen for easy passage and accommodation of more active epithelial surface. The lining epithelium of fimbria was pseudostratified columnar type. The lamina propria was very thin containing few collagen and reticular fibres. The tunica mucosa of infundibulum was highly folded and the folds were categorised into primary, secondary and tertiary folds. The epithelium consisted of ciliated and secretory cells. The infundibulum consisted of more number

of ciliated cells than the secretory cells. Similar findings were reported by [6] in goat. The epithelium consisted of ciliated and secretory cells. Nayak and Ellington (1977) also reported bovine infundibular epithelium contained both secretory and ciliated cells. The ciliated cell had basally located oval nucleus, whereas the nucleus of the secretory cells were round. The epithelial apical border was irregular with numerous cytoplasmic projections. Similar findings were also reported by [14] in Gaddi goat, which were meant for transportation of reproductive materials. The lamina propria continued with the submucosa constituting propria submucosa as there was no intervening lamina muscularis mucosae. It comprised of loose connective tissue with blood vessels and an abundant amount of collagen fibres. The collagen fibres were present throughout the propria-submucosa forming a network. Similar observations were reported by [3 and 13] in ruminants. Contrary to this, [1] in sheep observed that this layer was primarily comprised of dense connective tissue with reticular fibres oriented parallel to the basement membrane, which could be attributed to species difference. The reticular fibres were found in the basement membrane. In the primary and secondary folds the core contained mainly collagen fibres reinforced with reticular fibres which appeared standing up from the tunica muscularis towards the tip of folds. Tunica muscularis was thin and composed of circular muscles only. In between muscle bundles collagen and reticular fibres were also interspersed within the tunica muscularis. This was in agreement with the findings of [15] in goat and [19] in other domestic animals. The presence of the collagen and reticular fibres with blood vessels increased the functional performance of this layer. Tunica serosa comprised of mainly collagen and reticular fibres. Fine blood vessels were also observed. Similar findings were also reported by [3] in other domestic animals and [8] in buffalo. It showed positive results for AB-PAS having the presence of acid-mucopolysaccharide. The tunica intima of blood vessels also showed similar results for AB-PAS. Similar findings were observed by [15] in Gaddi goats. On the contrary [8], in buffalo reported mild reaction with PAS and Alcian Blue that could be due to breed difference.

The tunica mucosa of ampulla was highly folded. The thickness of tunica mucosa at post-pubertal stage was recorded as $45.81 \pm 3.48 \mu\text{m}$ (Table 4). The mucosal folds were lined by pseudostratified ciliated columnar epithelium. The basement membrane was distinct. The epithelium also consisted of ciliated and secretory cells like the infundibulum with similar characteristics. The average height of the epithelium and nucleus at post-pubertal stage were recorded as $4.314 \pm 0.61 \mu\text{m}$ and $3.00 \pm 0.29 \mu\text{m}$ respectively. The stroma was formed by loose connective tissue similar to infundibulum. Collagen and reticular fibres were present throughout the propria submucosa. The tunica muscularis has similar characteristics like that of infundibulum, i.e. it consisted of inner circular (Fig. 6) and outer longitudinal smooth muscle layer. But the numbers of muscle fibre layers were more. The thickness of tunica muscularis at post-pubertal stage was recorded as 24.083 ± 2.69 . Fine reticular fibres as well as bundles of collagen fibres were seen in tunica muscularis along with small blood vessels. The tunica serosa of ampulla shared similar characteristics with that of infundibulum. The thickness of post-pubertal stage was recorded as $20.709 \pm 1.84 \mu\text{m}$.

The mucosa of isthmus did not have as many folds as in the ampulla. Secondary folds were rarely seen. The mucosal folds

were lined with pseudostratified columnar epithelium, mostly non-ciliated type. The propria submucosa comprised of loose connective tissue. Fine and coarse collagen and reticular fibres were present throughout the propria submucosa forming network around the oviductal glands. Tunica muscularis comprised of a thick circularly arranged smooth muscle fibres with a few muscle cells oriented longitudinally. It was well vascularised. The collagen and reticular fibres were present in between muscle bundles. Tunica serosa was mainly composed of collagen fibres with blood vessels and nerves. The thickness of tunica muscularis was increased gradually towards the isthmus. Similar trends were observed by [8] in buffalo. The trend of increase of thickness towards isthmus could be due to more developed muscularis in the next continuing part, i.e. the uterus.

The oviductal glands were mainly found in tubal part of infundibulum, ampulla and isthmus. The glands were simple tubulo-alveolar type formed by the invagination of lining epithelium into propria-submucosa. The lining epithelium of glands was simple columnar type. The longitudinal glandular diameter, transverse glandular diameter, glandular epithelial height and nuclear height at post-pubertal stage were recorded as $51.642 \pm 3.24 \mu\text{m}$, $30.106 \pm 2.88 \mu\text{m}$, $11.747 \pm 1.69 \mu\text{m}$ and $6.181 \pm 0.96 \mu\text{m}$ respectively. Intense acid phosphatase activity was observed in the oviductal glands.

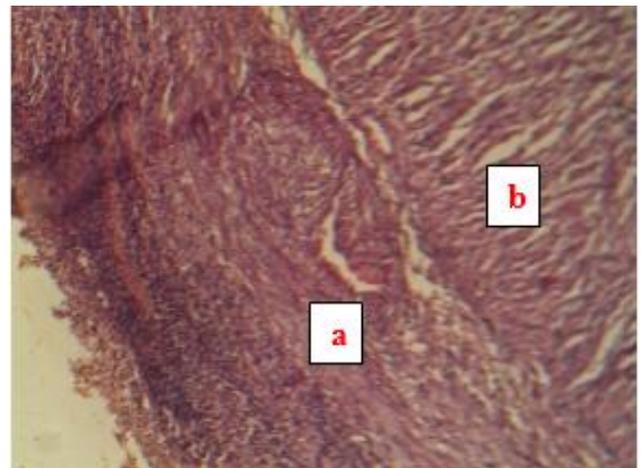


Fig. 1: Photomicrograph of Ovary showing a) Cortex and b) Medulla at pubertal stage of Kendrapada sheep. H&E X 100.

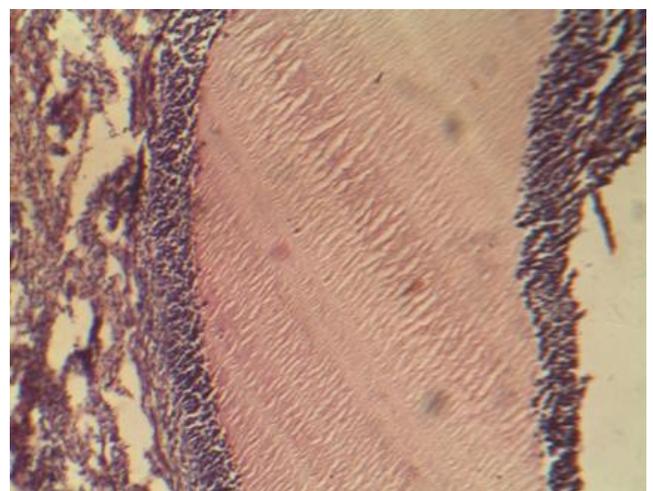


Fig 2: Photomicrograph of Ovary showing matured Graafian follicle in cortex at pubertal stage of Kendrapada sheep. H&E X 100

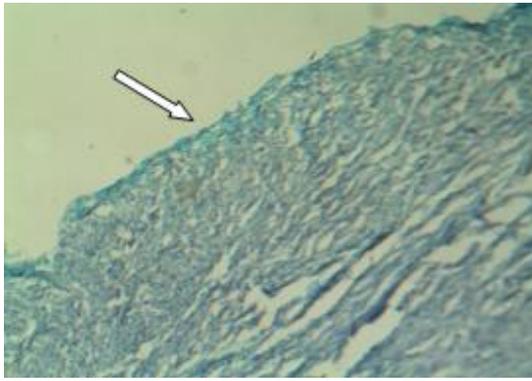


Fig 3: Photomicrograph of Ovary showing presence of acid mucopolysaccharide (arrow) in germinal epithelium at post-pubertal stage of Kendrapada sheep. AB-PAS X 100.



Fig 4: Photomicrograph of Ovary showing acid phosphatase activity (arrow) in germinal epithelium at post-pubertal stage of Kendrapada sheep. Azo dye X 100



Fig 5: Photomicrograph of Ovary showing presence of collagen fiber (arrow) in the tunica albugenia at post-pubertal stage of Kendrapada sheep. Masson's trichrome X 100.

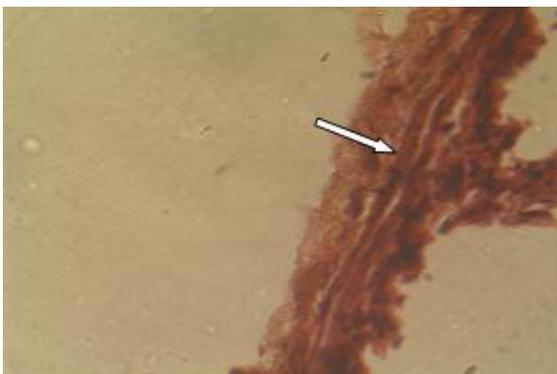


Fig 6: Photomicrograph of ampulla showing presence of circular smooth muscle layer (arrow) in the tunica muscularis at pubertal stage of Kendrapada sheep. H&E X 400

Conclusion

The ovaries of Kendrapada sheep were firm and oval in shape. The oviducts were tortuous and comprised of infundibulum, ampulla and isthmus. The weight of left ovary and oviduct were more than the right ones in pubertal and post-pubertal stages. The left oviduct was longer in the animal. The ovary consisted of an outer cortex and an inner medulla. Further, the cortex consisted of the superficial epithelium or germinal epithelium, tunica albuginea, primary follicles, secondary follicles, matured Graafian follicles and atretic follicles. The medulla consisted of Hilar cells, blood vessels, collagen and reticular fibers. The mucosal folds were maximum in the tubal part of the infundibulum, less in ampulla and least in isthmus in all stages of the animal. The propria-submucosa had oviductal glands and was comprised of loose connective tissue with blood vessels, abundant amount of collagen fibres and few reticular fibers.

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