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

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Abstract

The present study was conducted on uterus of 18 healthy female Kendrapada sheep from the Kendrapada district, Odisha minimum six each of 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 uterus. Gross studies revealed that the uterus was a hollow, elongated, musculo-membranous tubular organ. By means of the broad ligament (of uterus) it was attached dorso-laterally to the pelvic wall. The uterus was bicornuate and bipartite type. It was comprised of a pair of horns, body and the neck or cervix. Biometrical observations revealed that the left uterine horn was longer in the animal. The weight of the left uterine horn was more than the right one. Histological studies revealed that the wall of the uterus was comprised of four tunics such as tunica mucosa, tunica submucosa, tunica muscularis (myometrium) and tunica serosa (perimetrium). The tunica mucosa and submucosa are together known as endometrium. The uterus was lined by pseudostratified columnar epithelium. In uterine horns, the mucosal folds lie in close apposition to each other as compared to uterine body. Numbers of endometrial glands were more in uterine body than uterine horn. The cervical glands were simple coiled tubular mucus type that were housed in the propria-submucosa of cervix. The tunica muscularis comprised of two layers namely inner circular and outer longitudinal layer of smooth muscles. Tunica serosa comprised of dense irregular fibrous connective tissue which contained loosely arranged collagen fibres lined by mesothelial lining having blood vessels and lymphatics. The micrometrical, histochemical and histoenzymatic studies of uterus revealed variable results pertaining to their functional status and genetic potential at pre-pubertal, pubertal and post-pubertal stages of Kendrapada sheep.

Keywords: Histological, histochemical, uterus, 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 the uterus of Kendrapada sheep at pre-pubertal, pubertal and adult stages.

Materials and methods

The samples of the uterus 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^[15].

After recording the gross parameters, the specimen were fixed at 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^[8]. 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

Gross anatomy

The uterus was a hollow, elongated, musculo-membranous tubular organ. It consisted of two uterine cornuae (horns of uterus), corpus uteri (body of uterus) and a cervix uteri (neck of uterus). The uterus was situated in the pelvic cavity and related dorsally to rectum, ventrally to urinary bladder and covered by the pelvic peritoneum. The reflection of peritoneum that attaches the uterus to rectum was known as recto-genital pouch and the reflection of peritoneum that attaches the uterus to urinary bladder was known as genito-vesical pouch. By means of the broad ligament (of uterus) it was attached dorso-laterally to the pelvic wall. Blood vessels and nerves passed through the broad ligament to supply the uterine wall. The uterus was bicornuate and bipartite type. The cornuae were well developed. Similar findings were also reported by^[5 and 14] in goat. The left uterine horn was longer than the right in all the age groups of the animal. The length of left and right uterine horns in pre-pubertal age group was found to be 8.7 \pm 0.42 cm and 5.3 \pm 0.23 cm respectively (Table 1). The length of left and right uterine horns in pubertal age group was found to be 12.5 \pm 1.1 cm and 9.7 \pm 0.87 cm respectively. The lengths of left and right uterine horns in post-pubertal age group was found to be 16.6 \pm 1.12 cm and 11.1 \pm 0.73 cm respectively. It was close to measurements recorded by^[2 and 7] in goat and^[23] in sheep and goat, which indicated that the left uterine horn was more capacious in all the stages in small ruminants.

The weight of the left uterine horn was more than the right in all the age groups of the animal. The weight of left and right uterine horns in pre-pubertal age group was found to be 2.208 \pm 0.31 gm and 1.709 \pm 0.17 gm respectively. The weight on the left and right uterine horns in the pubertal age group was found to be 9.876 \pm 0.79 gm and 7.325 \pm 0.51 gm respectively. The weights of left and right uterine horns in post-pubertal age group was found to be 12.395 \pm 1.25 gm and 9.26 \pm 1.18 gm respectively. The width of left and right uterine horns at utero-tubal junction in pre-pubertal stage was recorded to be 0.65 \pm 0.03 cm and 0.56 \pm 0.03 cm respectively. The width of left and right uterine horns at utero-tubal junction in pubertal stage was recorded to be 1.0 \pm 0.02 cm and 0.9 \pm 0.04 cm respectively. The width of left and right uterine horns at utero-tubal junction in post-pubertal stage was recorded to be 1.4 \pm 0.03 cm and 1.5 \pm 0.09 cm respectively. The width of left and right uterine horns in the middle in pre-pubertal stage was recorded to be 0.71 \pm 0.04 cm and

0.74 \pm 0.03 cm respectively. The width of left and right uterine horns at the middle in pubertal stage was recorded to be 1.3 \pm 0.04 cm and 1.4 \pm 0.05 cm respectively. The width of left and right uterine horns in the middle in post-pubertal stage was recorded to be 5.4 \pm 0.12 cm and 3.5 \pm 0.57 cm respectively. The width of left and right uterine horns at the junction between the horn and uterine body in pre-pubertal stage was recorded to be 0.79 \pm 0.03 cm and 0.84 \pm 0.02 cm respectively. The width of left and right uterine horns at the junction between the horn and uterine body on pubertal stage were recorded to be 1.8 \pm 0.11 cm and 2.7 \pm 0.06 cm respectively. The width of left and right uterine horns at the junction between the horn and uterine body in post-pubertal stage were recorded to be 6.5 \pm 0.29 cm and 4.2 \pm 0.88 cm respectively.

The weight of the body of the uterus was measured to be 0.91 \pm 0.05 gm, 4.563 \pm 0.91 gm and 21.944 \pm 2.82 gm in pre-pubertal, pubertal and post-pubertal stages of Kendrapada sheep respectively. Similarly, the length of the body of the uterus was measured to be 1.7 \pm 0.12 cm, 3.1 \pm 0.18 cm and 17.1 \pm 1.23 cm in pre-pubertal, pubertal and post-pubertal stages of Kendrapada sheep respectively. The width of body of uterus at the cranial end, middle and caudal end were found to be 1.2 \pm 0.07 cm, 1.1 \pm 0.03 cm and 0.9 \pm 0.02 cm respectively in the pre-pubertal stage of the sheep. The width of body of uterus at the cranial end, middle and caudal end were found to be 3.7 \pm 0.24 cm, 3.8 \pm 0.25 cm and 3.6 \pm 0.27 cm respectively in the pubertal stage of the sheep. The width of body of uterus at the cranial end, middle and caudal end were found to be 10.3 \pm 0.79 cm, 10.4 \pm 1.14 cm and 7.5 \pm 1.23 cm respectively in the post-pubertal stage of the sheep. The weight of cervix was found to be 0.052 \pm 0.01 gm, 0.106 \pm 0.03 gm and 1.272 \pm 0.09 gm in the pre-pubertal, pubertal and post-pubertal stages of the sheep respectively. The length of cervix was found to be 0.5 \pm 0.02 cm, 1.2 \pm 0.08 cm and 1.3 \pm 0.04 cm in the pre-pubertal, pubertal and post-pubertal stages of the sheep respectively. The width of cervix was found to be 0.3 \pm 0.01 cm, 0.8 \pm 0.03 cm and 1.1 \pm 0.02 cm in the pre-pubertal, pubertal and post-pubertal stages of the sheep respectively.

Histology and histochemistry

The wall of uterine horn and corpus uteri were comprised of four tunics from within outwards, they were tunica mucosa, tunica submucosa, tunica muscularis (myometrium) and tunica serosa (perimetrium) respectively. The tunica mucosa and submucosa together were known as endometrium. Similar findings were also reported by^[14, 22] in buffalo and^[9] in goat.

The tunica mucosa was the thickest layer of uterine horn and body. It comprised of lamina epithelialis and propria-submucosa. The uterine cavity irrespective of horns and body was lined with pseudostratified columnar epithelium (Fig. 1). The epithelium contained the columnar cells, goblet cells and basal cells over the basement membrane. Similar observations were recorded in Egyptian buffalo^[4, 17, 20] in buffalo and^[9] in goat reported pseudostratified columnar epithelium. The mean height of the epithelium and nucleus in the uterine horn at post-pubertal stage was recorded as 8.443 \pm 0.79 μm and 2.402 \pm 0.21 μm respectively (Table 2). However,^[18] reported mean epithelial height of uterus as 28.70 \pm 3.56 μm and 28.15 \pm 5.83 μm in follicular and luteal phase respectively in Gaddi goat. The mucosal folds were in close opposition to each other in the uterine horns as compared to the corpus uteri. At some places epithelium was seen invaginating into propria submucosa forming endometrial glands. These endometrial glands were more in the corpus uteri than in cornuae, which might be helpful in the production of

histotroph during pregnancy to supply nutrition to the developing foeti. Similar findings were reported by ^[3, 21] in other domestic animals.

The propria-submucosa comprised of loose connective tissue that housed the glands as also reported by ^[14, 19, 22] in buffalo in Gaddi goat. The endometrial stroma consisted of mainly collagen fibres which also surrounds the endometrial glands (Fig. 2). Reticular fibres were also observed forming fine network around the glands. The connective tissue fibres were also more and densely arranged in uterine body as compared to horns which could be attributed to the weight bearing capacity of the uterine body for multiple foeti in the Kendrapada sheep. The lining epithelium of uterine horn showed weak acid and alkaline phosphatase activities (Fig. 3). The lining epithelium also showed positive results for presence of lipids under Sudan III stain. The propria-submucosa of uterine horn was also found positive for lipids, which might be considered as an energy source. The endometrium of uterus showed moderate alkaline phosphatase activity. The histochemical and histoenzymatic reactions were similar in both uterine horns and body.

The endometrial glands were simple, coiled tubular glands arising as invaginations of the endometrial lining epithelium as reported by ^[3, 21] in other domestic animals. They were lined by simple columnar epithelium (Fig. 32). This was in agreement with the observations of ^[9, 12, 14] in goat. The glands reached up to the inner circular muscle layer of tunica muscularis. In some cases these glands penetrated and reached stratum vasculare for better circulation of the secretion of these glands and also for its utilisation. The collagen and reticular fibres were found around the glands. Similar findings were also reported by ^[19] in Gaddi goat. The longitudinal glandular diameter, transverse glandular diameter, longitudinal luminal glandular diameter, transverse luminal glandular diameter, glandular epithelial height and glandular nuclear height of endometrial glands of uterine body at post-pubertal stage were recorded as $104.256 \pm 4.88 \mu\text{m}$, $81.577 \pm 4.23 \mu\text{m}$, $60.492 \pm 3.92 \mu\text{m}$, $44.510 \pm 3.01 \mu\text{m}$, $21.504 \pm 1.67 \mu\text{m}$ and $8.495 \pm 0.84 \mu\text{m}$ respectively (Table 3). ^[12] in sheep and ^[19] in Gaddi goat observed increased glandular epithelium height, luminal and glandular diameter in uterine horn and body during the luteal phase. The endometrial glands showed weak positive results for AB-PAS having the presence of acid mucopolysaccharide (Fig. 4). On the contrary, ^[9] showed moderate to intense activity of PAS-Alcian blue (2.5pH) in mucosal and glandular epithelium of horn and body of the uterus. Contrary to this, ^[17] observed a broader zone of PAS positive material during the follicular phase than in the luteal phase and superficial glands were strongly reactive as compared to basal glands in bovines.

The tunica muscularis comprised of two layers namely inner circular and outer longitudinal layer of smooth muscles. The inner circular layer in its outer third contained a very well developed vascular layer (stratum vasculare). This was in agreement with findings of ^[3, 21] in other domestic animals and ^[22] in buffalo. It was well developed, thicker and more prominent in the uterine body. The muscle bundles were arranged in the form of several interwoven sheets. Collagen fibres were present in between the smooth muscle bundle of the outer longitudinal layer. Reticular fibres were very few in number and form fine network in between the muscle bundles. The outer longitudinal layer composed of bundles of smooth muscles with collagen fibers. The thickness of tunica muscularis of uterine horn at post-pubertal stage was recorded as $307.881 \pm 24.67 \mu\text{m}$. The tunica muscularis of uterine horn showed positive result for AB-PAS having presence of the

acid mucopolysaccharide. It also showed positive results for lipids under Sudan III stain. The tunica muscularis of uterus showed presence of the neutral mucopolysaccharide. The histochemical and histoenzymatic reactions were similar in both uterine horns and body. The well developed tunica muscularis along with the presence of stratum vasculosa, collagen fibres, reticular fibres which showed positive result for acid mucopolysaccharide and lipid indicated the contracting power and weight bearing capacity of the uterus in the Kendrapada sheep.

The tunica serosa comprised of loose connective tissue layer covered by mesothelial cell layer. The fibro-architecture comprised mainly of collagen fibres with few reticular fibres. Similar findings were also reported by ^[3, 21] in other domestic animals. The thickness of tunica serosa of uterine horn at post-pubertal stage was recorded as $22.251 \pm 3.52 \mu\text{m}$. On contrary, ^[1] showed increased thickness of perimetrium in follicular phase. The tunica serosa of uterine horn showed positive result for AB-PAS having presence of acid mucopolysaccharide (Fig. 5). It also showed positive results for lipids under Sudan III stain as it required more energy for more stability and power for being as an independent organ. Similar findings were observed by ^[11] in Gaddi sheep. The intima of blood vessels showed reactivity with Alcian blue. Weak alkaline phosphatase activity was noted in the perimetrium of uterine horn. The histochemical and histoenzymatic reactions were similar in both uterine horns and body.

The cervix uteri was the caudal most part of the uterus, also known as the neck of the uterus. The cervix or the neck of the uterus was thick walled, highly muscular and tubular structure. It had a narrow lumen the *canalis cervicis uteri*. The latter communicated the corpus uteri through *ostium uteri internum* and with vagina through the *ostium uteri externum*. It was comparable with the earlier reports of ^[3, 21] in other domestic animals. The cervix uteri had the usual coats as tunica mucosa, submucosa, tunica muscularis (Myometrium) and tunica serosa (Perimetrium) from inside to outside. The tunica mucosa and submucosa were together called as endometrium. Similar findings were also described by ^[3, 10 and 21] in domestic animals.

The cross-sections of the cervix revealed that the mucosa was thrown into several finger like projections called *plicae longitudinales* as described by ^[12] in sheep, ^[3] in domestic animals and ^[13] in Gaddi goat and ^[9] in goat. The longitudinal folds (cervical crypts) of opposite sides lie in close apposition with each other as observed by ^[13] in Gaddi goat. The lining epithelium of cervical crypts was pseudostratified columnar type having various cell populations. At some points the primary folds were branched to give rise to secondary folds. The primary folds were variably thick. The secondary folds invariably invaginated into the propria submucosa resulting into the formation of cervical glands. The core of folds comprised of thick collagen fibres reinforced with fine reticular fibres. Many engorged blood vessels were also seen in the core of cervical crypts. Similar findings were also observed in Gaddi goats ^[13]. The presence of folds could be attributed to the opening and closing of the cervical canal in different stages of oestrus, pregnancy and parturition. The lining epithelium of cervical crypts was pseudostratified columnar type having various cell populations. Contrary to this, ^[11, 12, 13] in Gaddi goat reported tall columnar epithelium with goblet cells. However ^[22], in neonatal buffalo calf and ^[9] in goat had observed pseudostratified columnar epithelium, which indicated the secretory activity of the epithelium of cervical canal in different stages. The lamina propria-

submucosa comprised of dense collagen fibres extended up to the tip of cervical crypt as described by [3]. The cervical glands were formed due to the invaginations of the lining epithelium into the propria submucosa. These glands were simple coiled tubular mucus type as observed by [6, 12]. They were lined with simple tall columnar epithelium. The nuclei were oval and located basally as observed by [16] in buffalo. The glands were surrounded by dense collagen fibres and fine reticular fibres.

The muscular tunic of the cervix consisted of inner circular and outer longitudinal muscle layer which was infiltrated with large number of blood vessels and nerves as also described by [3] in domestic animals. Coarse collagen fibres and fine reticular fibres were found encircling individual muscle bundle as reported by [21] in other domestic animals, which might be contributing to the power of contraction and dilatation of cervical canal. The tunica serosa comprised of dense irregular fibrous connective tissue which contained loosely arranged collagen fibres. However [13, 14], in Gaddi goat, [12] in buffalo and [11] in sheep observed that tunica serosa comprised of loose connective tissue lined by mesothelial lining having blood vessels and lymphatics. This difference in type of connective tissue could be due to species or breed difference.

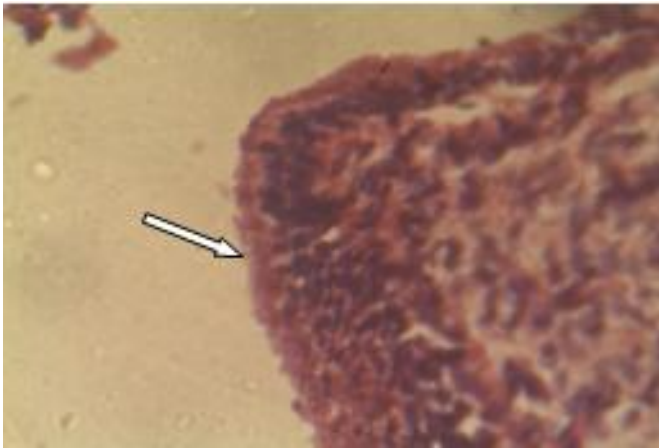


Fig 1: Photomicrograph of uterine horn showing pseudo-stratified columnar epithelium (arrow) the tunica mucosa at pre-pubertal stage of Kendrapada sheep. H&E X 400.

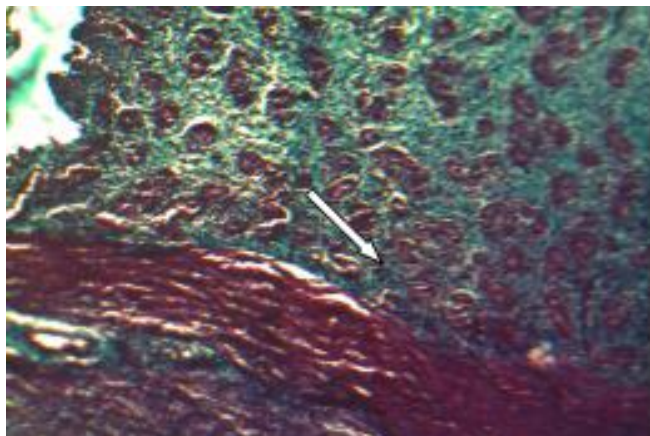


Fig. 2: Photomicrograph of uterine horn showing presence of collagen fiber around the endometrial glands (arrow) at post-pubertal stage of Kendrapada sheep. Masson's trichrome X 100

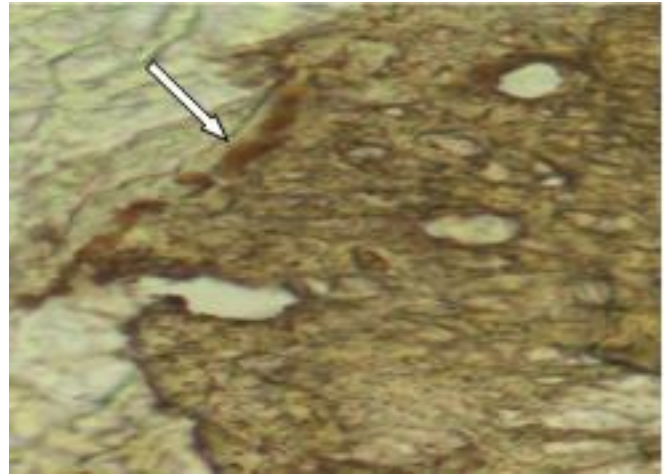


Fig 3: Photomicrograph of uterine horn showing presence of alkaline phosphatase activity (arrow) in the lining epithelium at post-pubertal stage of Kendrapada sheep. Azo dye X 100.

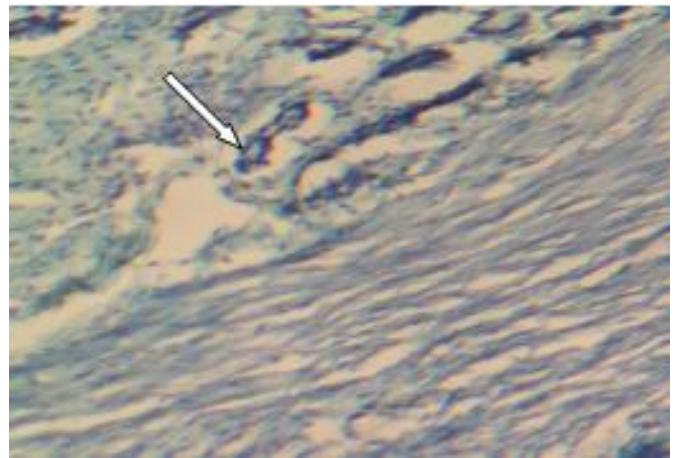


Fig 4: Photomicrograph of uterine horn showing presence of acid mucopolysaccharide in the endometrial glands (arrow) at post-pubertal stage of Kendrapada sheep. AB-PAS X 100.

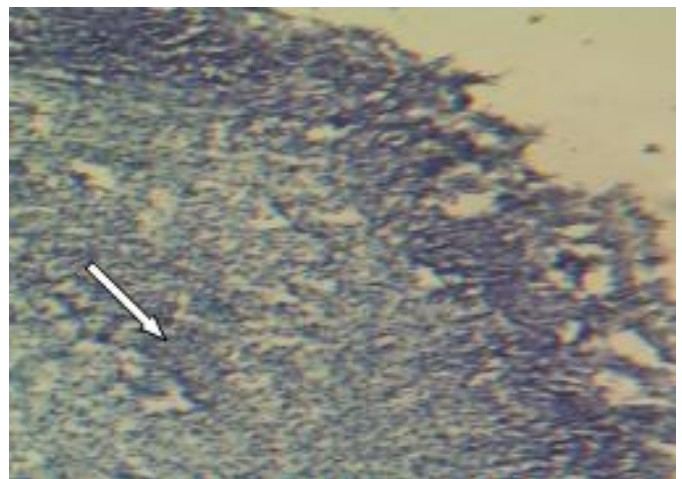


Fig 5: Photomicrograph of uterine horn showing presence of acid mucopolysaccharide (arrow) in tunica muscularis at post-pubertal stage of Kendrapada sheep. AB-PAS X 100.

Table 1: Gross biometrical observations of different parts of Uterus of Kendrapada sheep at different reproductive stages (Mean \pm S.E).

Parameters	Age groups					
	Pre-pubertal		Pubertal		Post-pubertal	
	Left	Right	Left	Right	Left	Right
Length of uterine horn (cm)	8.7 \pm 0.42	5.3 \pm 0.23	12.5 \pm 1.1	9.7 \pm 0.87	16.6 \pm 1.12	11.1 \pm 0.73
Weight of uterine horn (gm)	2.208 \pm 0.31	1.709 \pm 0.17	9.876 \pm 0.79	7.325 \pm 0.51	12.395 \pm 1.25	9.26 \pm 1.18
Width of uterine horn at utero-tubal junction (cm)	0.65 \pm 0.03	0.56 \pm 0.03	1.0 \pm 0.02	0.9 \pm 0.04	1.4 \pm 0.03	1.5 \pm 0.09
Width of uterine horn at the middle (cm)	0.71 \pm 0.04	0.74 \pm 0.03	1.3 \pm 0.04	1.4 \pm 0.05	5.4 \pm 0.12	3.5 \pm 0.57
Width of uterine horn at the junction between horn and uterine body (cm)	0.79 \pm 0.03	0.84 \pm 0.02	1.8 \pm 0.11	2.7 \pm 0.06	6.5 \pm 0.29	4.2 \pm 0.88
Weight of the body of uterus (gm)	0.91 \pm 0.05		4.563 \pm 0.91		21.944 \pm 2.82	
Length of the body of uterus (cm)	1.7 \pm 0.12		3.1 \pm 0.18		17.1 \pm 1.23	
Width of body of uterus at cranial end (cm)	1.2 \pm 0.07		3.7 \pm 0.24		10.3 \pm 0.79	
Width of body of uterus at the middle (cm)	1.1 \pm 0.03		3.8 \pm 0.25		10.4 \pm 1.14	
Width of body of uterus at caudal end (cm)	0.9 \pm 0.02		3.6 \pm 0.27		7.5 \pm 1.23	
Weight of the body of cervix (gm)	0.052 \pm 0.01		0.106 \pm 0.03		1.272 \pm 0.09	
Length of the cervix (cm)	0.5 \pm 0.02		1.2 \pm 0.08		1.3 \pm 0.04	
Width of the cervix (cm)	0.3 \pm 0.01		0.8 \pm 0.03		1.1 \pm 0.02	

Table 2: Micrometrical observations of different components of Uterine horn in Kendrapada sheep at post-pubertal stage (Mean \pm S.E) under 100X magnification

Parameters	Mean \pm S.E (Range)
Epithelial height (μ m)	8.443 \pm 0.79
Nuclear height (μ m)	2.402 \pm 0.21
Thickness of Tunica muscularis (μ m)	307.881 \pm 24.67 (under 40X)
Thickness of Tunica serosa (μ m)	22.251 \pm 3.52 (under 40X)

Table 3: Micrometrical observations of Glands of Uterine horn in Kendrapada sheep at post-pubertal stage (Mean \pm S.E) under 400X magnification.

Parameters	Mean \pm S.E (Range)
Longitudinal Glandular Diameter (μ m)	104.256 \pm 4.88
Transverse Glandular Diameter (μ m)	81.577 \pm 4.23
Longitudinal Luminal Diameter (μ m)	60.492 \pm 3.92
Transverse Luminal Diameter (μ m)	44.510 \pm 3.01
Glandular epithelial height (μ m)	21.504 \pm 1.67
Glandular nucleus height (μ m)	8.495 \pm 0.84

Conclusion

The uterus of Kendrapada sheep was a hollow, elongated, musculo-membranous tubular organ. It was bicornuate and bipartite type. It was comprised of a pair of horns, body and the neck or cervix. The left uterine horn was longer in the animal. The uterus was lined by pseudostratified columnar epithelium. In uterine horns, the mucosal folds lie in close apposition to each other as compared to uterine body. Numbers of endometrial glands were more in uterine body than uterine horn. The cervical glands were simple coiled tubular mucus type that were housed in the propria-submucosa of cervix. The tunica muscularis comprised of two layers namely inner circular and outer longitudinal layer of smooth muscles. Tunica serosa comprised of dense irregular fibrous connective tissue which contained loosely arranged collagen fibres lined by mesothelial lining having blood vessels and lymphatics.

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