

# Anatomical Studies on the Reticulum of Adult Non-Descript Sheep of Jammu Region

Jasvinder Singh Sasan\*, Shalini Suri, Kamal Sarma

## ABSTRACT

Present study was carried out on six stomach samples from adult apparently healthy non-descript sheep. Reticulum was spherical and was separated from rumen by rumino-reticular groove ventrally. Mucosa was pale brown in colour. Reticular cells were subdivided by secondary and tertiary crests. Mean empty weight of reticulum was 13.11% of the total empty weight of the stomach whereas the capacity was 6.04% of the total volume of the stomach. The dorso-ventral height of reticulum was greater than its cranio-caudal width. Histologically, reticulum consisted of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Lamina epithelialis was lined by keratinized stratified squamous epithelium. Lamina muscularis mucosae was present only towards the tip of primary reticular crests. Tunica muscularis had inner circular and outer longitudinal layers of smooth muscle. Thickness of outer muscular layer, length of reticular crest and width of reticular crest showed significant difference ( $p < 0.05$ ) and were higher at greater curvature. Histochemically, stratum corneum showed strong PAS reaction, stratum granulosum showed strong reaction for Alcian blue (pH 2.5). Distribution of glycogen was moderate in the layers of lamina epithelialis mucosae, except stratum corneum. Lamina epithelialis mucosae and tunica muscularis showed strong reaction for basic proteins. Stratum corneum showed moderate to strong reaction for lipids.

**Keywords:** Anatomy, Histology, Histochemistry, Reticulum, Sheep

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## INTRODUCTION

Sheep farming is popular among India's economically disadvantaged people and is seen as a "mortgage lifter" for poor farmers (Poonia *et al.*, 2012). They are vital to the livelihoods of many small and marginal farmers as well as landless labourers, who work in the sheep industry. On the basis of foraging behaviour, ruminants can be classed as grazers, browsers, or intermediate grazers (Gordon, 2003). Sheep prefer to graze, but goats prefer to browse (Mosavat and Chamani, 2013). The digestive system of ruminants is unique in a way that it allows them to use energy from fibrous plant material better than other herbivores. Ruminant forestomach includes rumen, reticulum and omasum. Forestomach have a unique sorting function which ensures that large ingesta particles are regurgitated and remasticated (Fritz *et al.*, 2009). This mechanism facilitates a high digestive efficiency (Clauss *et al.*, 2009).

Literature is available on the histology of forestomach of cattle, buffalo and sheep (Scala *et al.*, 2011), gross morphology and biometry of forestomach of Black buck (Kumari *et al.*, 2013), and morphology of stomach of Swamp buffalo (Nurliani *et al.*, 2015), macroscopic anatomy of reticulum in wild ruminant species (Clauss *et al.*, 2009). Data is also available on non-descript goats (Sasan *et al.*, 2022) and Bakerwali goat (Sasan *et al.*, 2023) of Jammu region. However, meager information is available on the anatomy of reticulum of non-descript sheep of Jammu region. Hence, the present study was planned and executed.

## MATERIALS AND METHODS

For the study, six stomach samples from adult apparently healthy non-descript sheep were collected from slaughter

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houses in and around Jammu region. Immediately after collection, the stomach was cleaned with running water and brought to the laboratory for recording the gross morphological and biometrical studies. The parameters recorded were: total empty weight (gm) of stomach and weight (gm) of reticulum was recorded with help of Monopan balance. Capacity (mL) of entire stomach and reticulum was determined by filling with water and measuring with help of measuring cylinder. Circumference (cm) of reticulum was recorded as longitudinal and transverse circumference from the middle of the reticulum. Similarly, dorso-ventral height (cm) and cranio-caudal width (cm) of reticulum was noted. Length (cm) of honey-comb cells was taken by measuring maximum distance between two angles of large sized cells at floor (greater curvature) of reticulum as well as towards reticular groove (lesser curvature). Width (cm) of honey-comb cells was taken by measuring maximum distance between two crests of large sized cell at floor (greater curvature)

of reticulum as well as towards reticular groove (lesser curvature).

After recording the gross and biometrical parameters, tissue samples were collected from two sites, *i.e.*, lesser curvature (close to reticular groove) and greater curvature (floor of reticulum) and preserved in 10% Neutral Buffered Formalin (NBF) solution (Luna, 1968). The tissue samples were processed and tissue sections of 5  $\mu\text{m}$  thickness were obtained. The sections were stained with Haematoxylin and Eosin for routine histomorphology, Von Gieson and Verhoeff's and Gomori stains for connective tissue fibers, and Ayoub-Shklar method for keratin (Luna, 1968). For histochemistry, Best Carmine, Bromophenol blue, PAS-AB and Sudan Black B methods were used for demonstration of glycogen, basic proteins, neutral & acidic mucopolysaccharides and lipids, respectively. The micrometrical observations were taken, *i.e.*, thickness ( $\mu\text{m}$ ) of lamina epithelia, lamina-propriasubmucosa, tunica muscularis and tunica serosa of reticulum, length ( $\mu\text{m}$ ) and width ( $\mu\text{m}$ ) of primary reticular crest. All the data was subjected to standard statistical analysis (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

### Gross Morphology and Biometry of Reticulum

Reticulum was most cranially located compartment of sheep stomach similar to the findings of Sasan *et al.* (2022) in non-descript goats and Sasan *et al.* (2023) in Bakerwali goat. It was somewhat spherical in outline (Fig. 1), located in between diaphragm and rumen. However, the reticulum of Marsh deer was of pyriform shape (Machado and Oliveira, 2008). It consisted of parietal/left and visceral/right surface both of which were convex. Dorsally, it continued with the cranial sac of rumen whereas ventrally a distinct rumino-reticular groove was seen separating reticulum from rumen. Similar observation was made by Mahendrakumar (2017) in Surti goat and Sasan *et al.* (2022) in non-descript goats. The parietal and visceral surfaces of reticulum of non-descript sheep presented very prominent blood vessels (Fig. 1). Interiorly, reticulum communicated with oesophagus and rumen through reticular groove and with omasum via reticulo-omasal orifice.

The mucosa of the reticulum of non-descript sheep was pale brown in colour similar to the findings of Sasan *et al.* (2022) in non-descript goats and formed permanent crests known as *cristae reticuli* which intersect to form 4-6 sided cells known as *cellulae reticuli* thus giving the interior of reticulum a typical honey-comb appearance (Fig. 2). However, in Bakerwali goat, the mucosa was dark brown in colour (Sasan *et al.*, 2023). The *cellulae reticuli* were mostly rectangular in outline (Fig. 2) and in non-descript goats, the cells were more hexagonal (Sasan *et al.*, 2022) whereas in Bakerwali goat, the cells were variable in outline (Sasan *et al.*, 2023). Colville and Bassert (2008) stated that 4-6 sided structures of the honeycomb served to increase the surface area of the

reticulum and thus increased the absorptive surface. Reece (2005) observed that the crests were papillated in sheep but smooth in cattle. These cells were smaller near lesser curvature as compared to the cells towards greater curvature. Similar observation was made by Nurliani *et al.* (2015) in swamp buffalo and Mahendrakumar (2017) in Surti goat. The cells were further subdivided by secondary and tertiary crests (Fig. 3) both towards greater curvature as well as lesser curvature. In adult Bakerwali goat, few *cellulae reticuli* towards greater curvature presented only the secondary crests whereas *cellulae reticuli* towards lesser curvature were devoid of both secondary and tertiary crests (Sasan *et al.*, 2023). In non-descript goats, the cells towards greater curvature were subdivided by both secondary as well as tertiary crests (Sasan *et al.*, 2022). Both the crests and floor of cells were studded with small conical papillae as also observed by Konig and Liebich (2009) in ruminants.

Various biometrical parameters of reticulum are presented in Table 1. The mean empty weight of reticulum ( $126.03 \pm 9.15$  gm) was  $13.11 \pm 0.31\%$  of the total empty weight of the stomach. It was close to the findings of Mahendrakumar (2017) in Surti goat but less than the findings of Sasan *et al.* (2023) in Bakerwali goat. The capacity of reticulum ( $766.67 \pm 78.17$  mL) was  $6.04 \pm 0.51\%$  of the total volume of the stomach. It was much less than in Bakerwali goat (Sasan *et al.*, 2023). The longitudinal circumference ( $29.47 \pm 0.85$  cm) was greater than transverse circumference ( $25.87 \pm 2.07$  cm), which was in approximation with the findings of Mahendrakumar (2017) in Surti goat. In Black buck, the longitudinal diameter was  $17.7 \pm 0.72$  cm and transverse diameter was  $14.4 \pm 0.72$  cm (Kumari *et al.*, 2013).

**Table 1:** Various biometrical parameters of reticulum of non-descript sheep of Jammu region

Parameter	Mean $\pm$ SE
Empty weight (gm) of stomach	913.30 $\pm$ 78.84
Empty weight (gm) of reticulum	126.03 $\pm$ 9.15
% Reticulum weight	13.11 $\pm$ 0.31
Total capacity (mL) of stomach	10770 $\pm$ 575.64
Capacity (mL) of reticulum	766.67 $\pm$ 78.17
% Reticulum capacity	6.04 $\pm$ 0.51
Longitudinal circumference (cm)	29.47 $\pm$ 0.85
Transverse circumference (cm)	25.87 $\pm$ 2.07
Dorso-ventral height (cm) of reticulum	12.76 $\pm$ 0.26
Cranio-caudal width (cm)	10.78 $\pm$ 0.72
Length (cm) of honeycomb cells towards greater curvature	2.19 $\pm$ 0.04
Width (cm) of honeycomb cells towards greater curvature	1.45 $\pm$ 0.05
Length (cm) of honeycomb cells towards lesser curvature	1.34 $\pm$ 0.09
Width (cm) of honeycomb cells towards lesser curvature	0.97 $\pm$ 0.07

The dorso-ventral height of reticulum ( $12.76 \pm 0.26$  cm) was greater than its cranio-caudal width ( $10.78 \pm 0.72$  cm). These values were slightly lesser than in Bakerwali goat (Sasan *et al.*, 2023). Perez and Vazques (2012) recorded slightly lower values in Brown Brocket deer. Perez *et al.* (2015) calculated the height and cranio-caudal length of reticulum of deer as  $19.2 \pm 0.8$  cm and  $11.6 \pm 1.2$  cm, respectively. In Dorcas Gazelle, dorso-ventral height of reticulum was in approximation with cranio-caudal width (Jerbi *et al.*, 2016). Length ( $2.19 \pm 0.04$  cm) and width ( $1.45 \pm 0.05$  cm) of honeycomb cells towards the greater curvature of reticulum was greater than the length ( $1.34 \pm 0.09$  cm) and width ( $0.97 \pm 0.07$ ) of cells present towards lesser curvature of reticulum. Similar pattern was observed in non-descript goats (Sasan *et al.*, 2022) and Bakerwali goat (Sasan *et al.*, 2023).

**Histomorphology and Micrometry of Reticulum**

Histologically, reticulum consisted of four layers namely tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The lamina epithelia consisted of keratinized stratified squamous epithelium (Fig. 4) having stratum basale, stratum spinosum, stratum granulosum and stratum corneum. Similar observations were made by Sasan *et al.* (2022) in non-descript goats of Jammu region. The cells of stratum basale were columnar, stratum spinosum were polyhedral and larger than the basal cells. The stratum granulosum was one to three cells thick. The upper keratinized layer protect against abrasion by the rough, fibrous diet as also quoted by Dyce *et al.* (2002). In yak, the mucosal epithelium of reticulum was not keratinized (Wang *et al.*, 2014). In the present study, the epithelial mucosa of reticulum was thrown into luminal extensions as primary reticular crests with lateral border presenting prominent conical ridges. The epithelium formed epidermal pegs which were more prominent in reticular crests. Lamina propria consisted of dense irregular connective tissue as also observed by Ramkrishna and Gadre (2004) in domestic animals and contained abundant collagen fibers with few reticular and elastic fibers (Fig. 5). Smaller vessels, lymphatics and capillaries were also seen. Lamina muscularis mucosae was present only towards the tip of primary reticular crests as also reported earlier by Sultana *et al.* (2021) in sheep. Secondary reticular crests were devoid of muscularis mucosae layer. Due to absence of muscularis mucosae layer, the lamina

propria merged with tunica submucosa forming lamina propria submucosa rich in collagen and elastic fibers (Fig. 5). Lamina epithelialis was separated from underlying lamina propria submucosa by basement membrane consisting of reticular fibers (Fig. 6). Eurell and Frappier (2006) reported that basement membrane is composed of reticular fibers.

Tunica muscularis consisted of two layers of smooth muscle fibers, namely inner circular and outer longitudinal layers (Fig. 5) as also reported by Malik (2014) in Gaddi sheep. Numerous coarse reticular fibers reinforced the muscle bundles of tunica muscularis. Loose connective tissue and blood vessels were also recorded in between. Nerve plexus and parasympathetic ganglionic cells were seen between layers of tunica muscularis (Fig. 7). Outer most layer was tunica serosa which consisted of loose connective tissue, various blood vessels and nerve fibers as also reported by Mahendrakumar (2017) in Surti goat. There was mixed distribution of collagen and elastic fibers in this layer.

Various micrometrical parameters of reticulum are presented in Table 2. Thickness of outer muscular layer, length of reticular crest and width of reticular crest showed significant difference ( $p < 0.05$ ) and were higher at greater curvature. In non-descript goats, the primary crests were longer and wider at greater curvature than at lesser curvature (Sasan *et al.*, 2022).

**Table 2:** Various micrometrical parameters of reticulum of non-descript sheep of Jammu region (Mean  $\pm$  SE)

Parameter ( $\mu$ )	Towards lesser curvature	Towards greater curvature
Thickness of lamina epithelialis mucosae	69.34 $\pm$ 2.32	65.67 $\pm$ 7.40
Thickness lamina propria submucosa	398.84 $\pm$ 40.98	441.63 $\pm$ 52.67
Inner muscle layer	896.73 $\pm$ 82.62	755.89 $\pm$ 48.50
Outer muscle layer	399.15 <sup>a</sup> $\pm$ 35.55	601.14 <sup>b</sup> $\pm$ 40.32
Tunica muscularis	1332.01 $\pm$ 88.10	1479.98 $\pm$ 73.67
Tunica serosa	245.21 $\pm$ 29.74	254.35 $\pm$ 33.08
Primary crest per field at 40x	1.50 $\pm$ 0.34	1.17 $\pm$ 0.17
Length of primary crest	1588.35 <sup>a</sup> $\pm$ 200.76	1960.41 <sup>b</sup> $\pm$ 97.16
Width of primary crest	432.35 <sup>a</sup> $\pm$ 72.17	652.57 <sup>b</sup> $\pm$ 75.93

Mean value with same superscript within row do not differ significantly ( $p > 0.05$ )

**Table 3:** Histochemical observations of reticulum of adult non-descript sheep

Parameters	Stain	Lamina epithelialis					LP-S	TM	TS
		SC	SG	SS	SB	BM			
Carbohydrates	PAS	+++	-	-	-	++	++	++	-
Glycogen	Best carmine	-	++	++	++	-	-	+	-
Basic proteins	Bromphenol blue	+++	+++	+++	+++	-	-	+++	-
Lipids	Sudan Black B	+++ / ++	-	-	-	-	-	-	-

Strong: +++, Moderate: ++, Weak: +, Negative: -

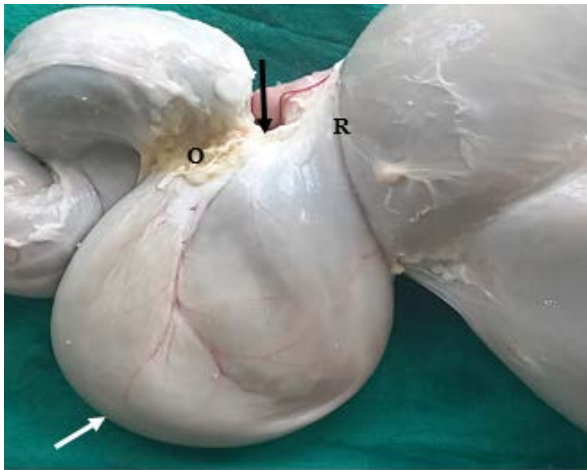
SC: Stratum corneum, SG: Stratum granulosum, SS: Stratum spinosum, SB: Stratum basale, BM: Basement membrane, LP-S: Lamina propria-submucosa, TM: Tunica muscularis, TS: Tunica serosa

**Histochemistry**

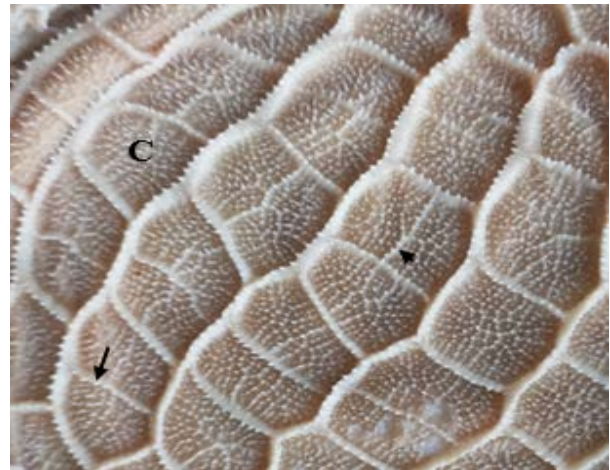
The histochemical distribution of neutral and acid mucopolysaccharides, basic proteins, glycogen and sudanophilic lipids in the reticulum of non-descript sheep is summarized in Table 3. Strong positive PAS reaction was seen in the stratum corneum of lamina epithelialis mucosae whereas basement membrane exhibited moderate PAS reaction. Lamina propria-submucosa and tunica muscularis also showed moderate PAS reaction (Fig. 8). Lakshmishree (2014) in sheep, goat and buffalo observed strong reaction of stratum corneum for carbohydrates. Basement membrane,

lamina muscularis mucosae and tunica muscularis showed moderate reaction, however Malik (2014) in Gaddi sheep observed mild PAS reaction in lamina propria-submucosa.

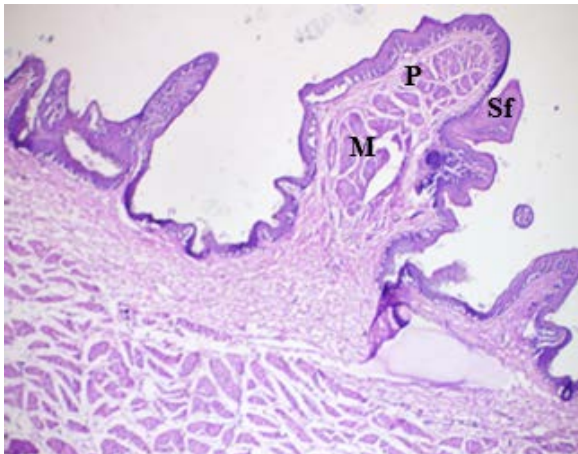
The inter-cellular spaces in stratum spinosum layer were moderately positive for acid mucopolysaccharides. Cells of stratum granulosum showed strong reaction whereas sub-epithelial connective tissue showed moderate reaction for Alcian blue (pH 2.5) (Fig.8). However, Pachpande (2008) observed weak reaction in epithelium and tunica serosa, moderate in tunica submucosa and absent in tunica muscularis with PAS-AB in goats of 90-120 days of age contrary to the findings of Malik (2014) in Gaddi sheep.



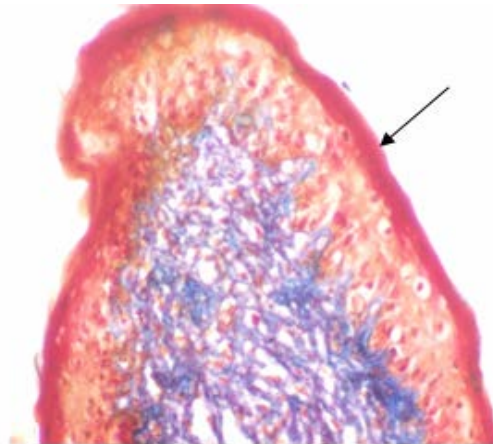
**Fig. 1:** Photograph showing reticulum of adult non-descript sheep showing lesser curvature (arrow), greater curvature (white arrow), ruminal extremity (R) and omasal extremity (O). Blood vessels were prominent



**Fig. 2:** Photograph showing interior of reticulum having reticular cells (C) of mostly rectangular in outline. Secondary (arrow) and tertiary crests (arrow head) were present

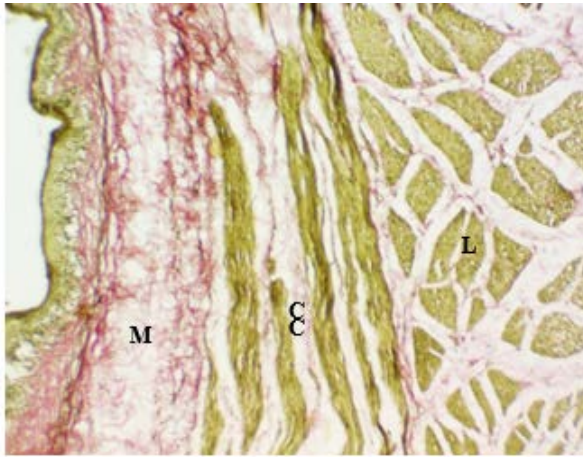


**Fig. 3:** Photograph of reticulum of non-descript sheep showing primary reticular crests (P) containing smooth muscle bundles (M) whereas secondary folds (Sf) were devoid of muscle bundles. x40 H&E stain.

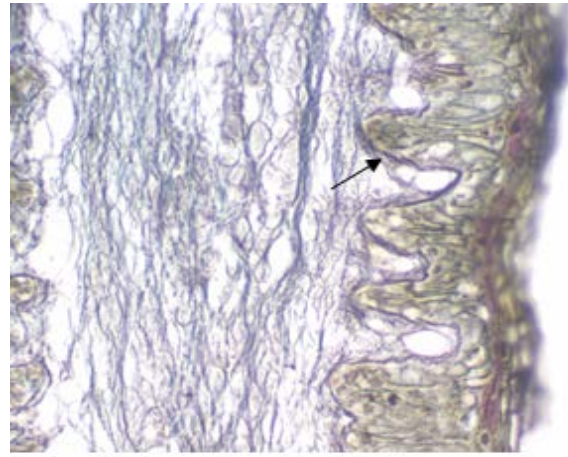


**Fig. 4:** Photograph of reticulum of non-descript sheep showing keratinized epithelium (arrow). x400 Ayoub-Shklar method

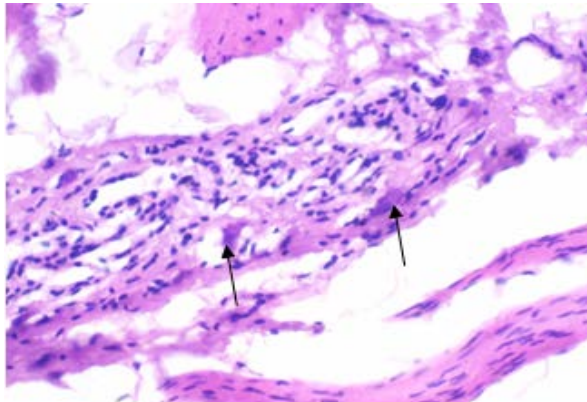




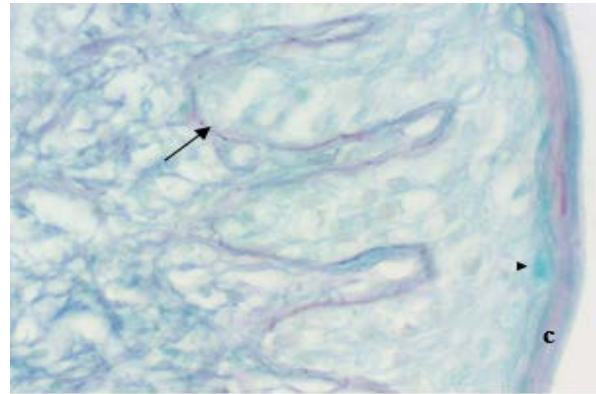
**Fig. 5:** Photomicrograph of reticulum (at greater curvature) showing collagen fibers (pink colour) in Lamina propria submucosa (M). Inner circular (C) and outer longitudinal (L) muscle layers are evident. x100 Von Geison Verhoeff stain



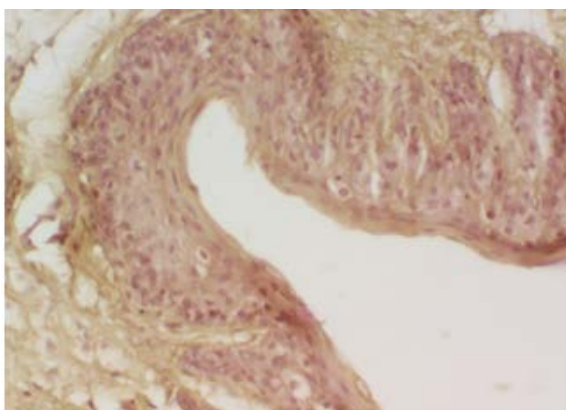
**Fig. 6:** Photomicrograph showing presence of reticular fibers (arrow) in basement membrane. x400 Gomori's stain.



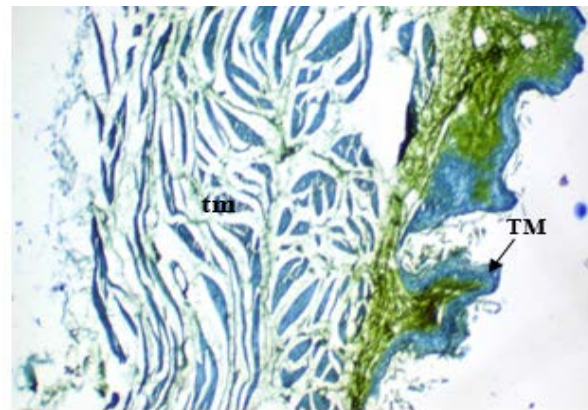
**Fig. 7:** Photomicrograph of reticulum showing presence of parasympathetic ganglionic cells (arrow). x400 H&E stain



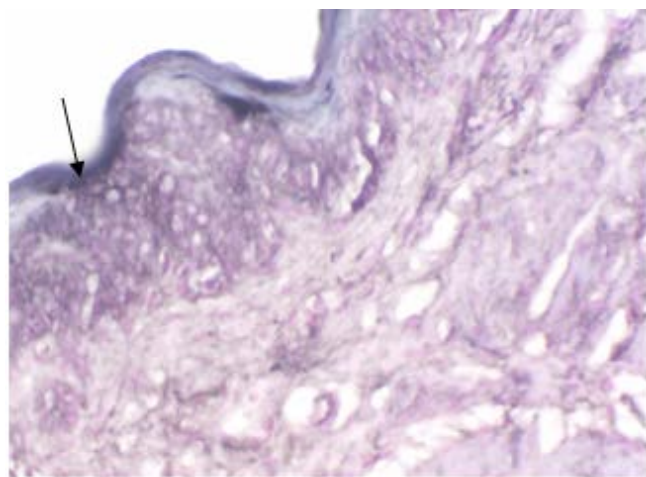
**Fig. 8:** Photomicrograph of reticulum of adult non-descript sheep. Stratum granulosum (arrow head) showing strong reaction for Alcian Blue. Stratum corneum (c) and basement membrane (arrow) showing reactivity for PAS (magenta colour). x1000 PAS-AB stain



**Fig. 9:** Photomicrograph of reticulum of adult non-descript sheep. Stratum corneum showed negative reaction whereas other epithelial layers showed moderate reaction for glycogen. x400 Best Carmine stain



**Fig. 10:** Photomicrograph of reticulum of adult non-descript sheep. Only Tunica mucosa (TM) and tunica muscularis (tm) showed strong reaction. x40 Bromophenol Blue stain



**Fig. 11:** Photomicrograph of reticulum of adult non-descript sheep. Stratum corneum showed moderate to strong reaction. x400 Sudan Black B stain

The distribution of glycogen was moderate in the layers of lamina epithelialis mucosae except stratum corneum. Tunica muscularis reacted weakly for glycogen with Best Carmine stain. However, lamina propria-submucosa and tunica serosa showed negative reaction for glycogen (Fig. 9). However, Lakshmishree (2014) in sheep, goat and buffalo observed moderate glycogen reaction of lamina muscularis mucosae and tunica muscularis.

Lamina epithelialis mucosae and tunica muscularis showed strong reaction for basic proteins whereas all the other layers showed negative reaction for basic proteins (Fig. 10). Moderate to strong lipid reaction was observed in stratum corneum only as also observed by Malik (2014) in Gaddi sheep (Fig. 11).

## CONCLUSION

The present research extends the depth of the knowledge regarding anatomy of reticulum in non-descript sheep which prefers grazing. The detailed anatomical study further adds to the literature. When compared with available literature on goat which prefers browsing, significant gross anatomical differences existed which can be correlated to their digestive physiology.

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