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Postnatal Development on Gastric Groove of Small Ruminants

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Abstract: The gastric groove is a vital part of the digestive system of the suckling ruminants. It consisted of two well-developed lips and a floor between them. The floor of the reticular groove had longitudinal folds and macroscopical papillae. These papillae were randomly dispersed in the proximal parts and clustered at the entrance of abomasum at the point of the reticulo-omasal orifice. A comparative qualitative and quantitative studies were applied on the suckling and the adult goat. The same studies were conducted on the suckling and the adult sheep. In the present study, the mucous glands were found only in the adult sheep. These glands were found in the tunica submucosa at the level of the lips-floor junction, then it was found in the tunica muscularis among smooth muscle fibers of the outer longitudinal layer of the lips. Interestingly, no mucous glands were found in the suckling sheep, the suckling goat and the adult goat. Overall, the present study reveals some interesting facts where the individual layers of the reticular groove have a clear and straightforward development from the proximal to the distal parts with prominent increase with age.

Key words: Gastric groove, papillae, mucous glands, age, suckling, longitudinal folds

INTRODUCTION

The Reticular Groove (RG) is a special part of the suckling ruminants fore stomach which transforms itself into a tube to ensure the direct transport of milk from the esophagus to the true stomach (abomasum) via. a vagovagal reflex (Lalatta-Costerbosa *et al*., 2011). The Gastric Groove (GG) consists of a double walled organ, including, both the mucosal and muscular layer. The borders of the two lips gradually approach each other until they touch forming a tube which represents an esophageal extension (Teixeria *et al*., 2009). It passes through the reticulum where it joins the esophagus and the abomasum forming a tube (Ruckebusch, 1989; Pfannkuche *et al*., 2003).

In ruminants, the gastric groove is divided into reticular, omasal and abomasal portions (Kano *et al*., 1988). The reticular groove has papillae which are uniformly distributed near the cardia (Teixeria *et al*., 2009). The entire reticular groove is lined with a stratified squamous epithelium (Lalatta-Costerbosa *et al*., 2011). Muscularis mucosae are few muscle fibers at the lips of the groove (Ramkrishna and Gadre, 2004) where (Eurell and Frappier, 2006) the musclaris mucosa is mostly clear on the lips of the sulcus. It was the continuity of the muscularis mucosae of the esophagus. It appeared as thick fascicles which immersed in the lamina propria (Lalatta-Costerbosa *et al*., 2011).

In sheep, Tunica submucosa contains simple branched tubuloacinar glands which are mucous or mixed type (Ramkrishna and Gadre, 2004). Ergiin *et al*. (2010) and Lalatta-Costerbosa *et al*. (2011) mentioned that mucous glands were found in the connective tissue of the submucosa at the level of the lip-floor junctions where they are densely packed they extended along the entire length of the RG and became more numerous in the distal part where they also extended between the internal and external muscle layers in suckling lambs. The muscle layers of the stomach wall are taking part in the formation of the lips and the floor of the groove (Nickel *et al*., 1979). Tunica musclaris consists of smooth and striated muscle fibers which are longitudinally and transversely arranged. In the floor of the suckling lambs, the tunica muscularis was composed of a thin uncompleted external longitudinal layer and a thick inner circular layer. In the lips, the tunica musclaris of the sulcus consists of smooth muscle fibers coursing parallel to the lips (Eurell and Frappier, 2006). Serosa is composed of loose connective tissue and covered by a mesothelium. A varying amount of fat, blood vessels, lymph vessels and nerves are also located (Ramkrishna and Gadre, 2004). Here, we present new anatomical facts on the reticular grooves of the suckling and the adult sheep and goat.

MATERIALS AND METHODS

Animals and tissue samples: This study were conducted on 32 animals of both sexes. The reticular groove was collected immediately after the slaughter from 6 suckling goats, 10 adult goats, 6 suckling sheep and 10 adult sheep

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of both sexes. The reticular groove from each adult animal was cut into four parts (1st-4th) and that from each suckling animal was cut two parts (1st and 2nd). The specimens were immediately fixed in 10% Neutral Buffered Formalin (NBF) then dehydrated in series of ascending grade of ethanol followed by clearing in xylene (three changes). All specimens were infiltrated with soft melted paraffin in the hot air oven and were embedded in hard paraffin. Using rotary microtome, sections of 5-7 μm thickness were cut, floated on warm water in a floatation bath at 50°C for stretching of the sections and then, the sections were mounted on clean slides using an adhesive (Egg albumin-glycerol) and dried on a slide hot-plate at 37°C (Bancroft and Gamble, 2008).

To best define the histological aspect, the paraffin sections were stained with Harris's Hematoxylin and Eosin (H and E) stain as a routine staining method to demonstrate the general structure. Other stains such as Bromo Phenol Blue (BPB) (for proteins) (Humason, 1979), Alcian blue pH (2.5) (for the acidic mucopolysaccharides), Periodic Acid Schiff (PAS) (for the neutral mucopolysaccharides and some acidic ones), silver impregnation technique (for the reticular fibers), Crossmon's trichrome stain (for the collagen and muscle fibers) (Crossmon, 1937) and Orcein (for the elastic fibers) were also used.

All the stained sections were examined with a standard light microscope (Olympus BX 21, Objective X 4, 10, 40 and Ocular X 10) and photographed by a digital Dsc-W 800 supersteady cyper shot camera (Sony-Japan) (Department of Histology and Cytology, Zagazig University). The tunic thickness of the various parts of the reticular groove from suckling and adult ruminants was determined by measuring lens and then the output was multiplied by some factors according to the magnification power of the objective lens (Salama, 1981). The thickness of all these tunics was expressed as micrometer.

RESULTS

Qualitative aspects: The ruminant's stomach consisted of four compartments rumen, reticulum, omasum and abomasum. The first three parts have a common function where they act as a fermentative chambers. On the other side, the fourth part, the abomasum is the only partition capable of secreting gastric enzyme where it is lined by a secretory mucous membrane.

The reticular groove of the suckling goat was a special part, extending from the cardiac end of the esophagus to the reticulo-omasal orifice and consisted of two lips and a floor (Fig. 1a). The floor of the gastric groove of the adult goat had folds which extended longitudinally from the end of the esophagus to the beginning of the abomasum. In addition to the folds, there

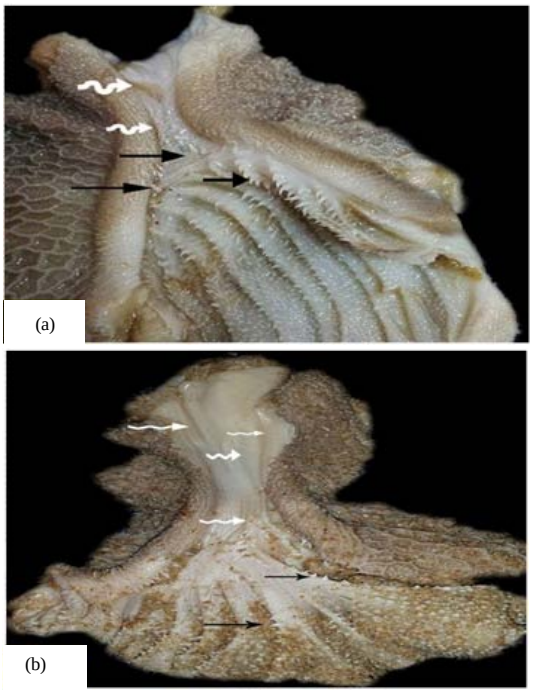

Fig. 1: Comparison of the reticular groove in the suckling kid: a) showing that the floor of the gastric groove had several longitudinal folds "zigzag arrow" and several thorn shape papillae "black arrow" and the adult goat, b) showing several longitudinal folds "zigzag arrow" and several thorn shape papillae "winged arrow" were found on the floor

are a thorn shape papillae which distributes randomly in the proximal parts. In contrast, it is arranged in the form of clusters at the entrance abomasum at the level of the reticulo-omasal orifice (Fig. 1b).

The whole groove in the suckling goat was covered by stratified squamous epithelium in the following order: a wavy basement membrane characterized by the presence of numerous well developed papillary pegs; single layer of columnar cells with oval nuclei called stratum basale; several layers of polyhedral cells with highly active rounded or spherical vesicular nuclei called stratum spinosum; flattened cells with flat nuclei named stratum granulosum and stratum lucidum which appeared in the form of a compact layer taking a reddish color consisted of flattened cells with more flattened nucleus (Fig. 2a). In the case of the adult goat, lamina epithelialis consisted of the same layers as the suckling kid but stratum basale had highly condensed ovally nuclei and stratum spinosum had dark inactive rounded or spherical nuclei (Fig. 2b).

The muscularis mucosa of the lips of the suckling goat was found in the form of bands or stribs which were

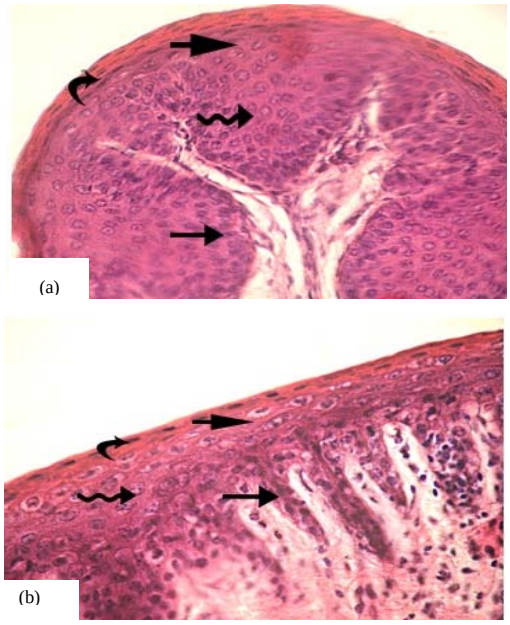

Fig. 2: a) Comparison of the different layers of the lamina epithelialis in the suckling kid and b) the adult goat's reticular groove which consisted of stratum basale "black arrow", stratum spinosum "zigzag arrow", stratum granulosum "arrow head" and stratum lucidum "closed arrow". Stain: H&E Obj. X40:Oc. X10

immersed in the lamina propria. These fascicles are the direct continuity of the muscularis mucosa of the esophagus and these bands spread into the papillae of the epithelium (Fig. 3a). In the case of the floor, the muscularis mucosa is composed of thin discontinuous transversely arranged smooth muscle fibers which appears along the whole length of the gastric groove (Fig. 3b).

The muscularis mucosa of the lips (Fig. 3c) and the floor (Fig. 3d) of the adult goat had the same characters as the suckling kid with thicker bands.

Tunica muscularis in the lips of the suckling goat consisted of a very thin inner circular layer and thick outer longitudinal smooth muscle fibers (Fig. 4a) but the floor consisted of a thick inner circular layer and outer longitudinal one (Fig. 4b) with wavy inner circular one.

Tunica muscularis in lips of adult goat was similar to the suckling kid with thicker bundles of smooth muscle fibers (Fig. 4c). Interestingly, the floor is extremely developed consisting of a well-developed inner circular layer and very thick outer longitudinal layer (Fig. 4d).

In the suckling goat, collagen (Fig. 5a), reticular (Fig. 5b) and elastic fibers (Fig. 5c) were distributed between muscle bundles. These fibers pass through

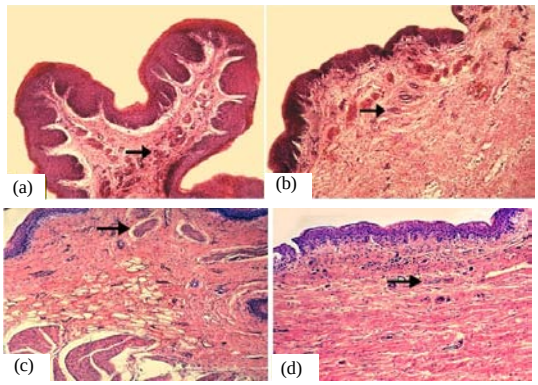

Fig. 3: a, b) Comparison of the muscularis mucosa "black arrow" in the lip and the floor of the suckling goat and c, d) the adult goat's reticular groove which composed of transversely arranged smooth muscle fibers. Stain: H&E (a, b and c): Obj. X5:Oc. X10 (d): Obj. X10:Oc. X10

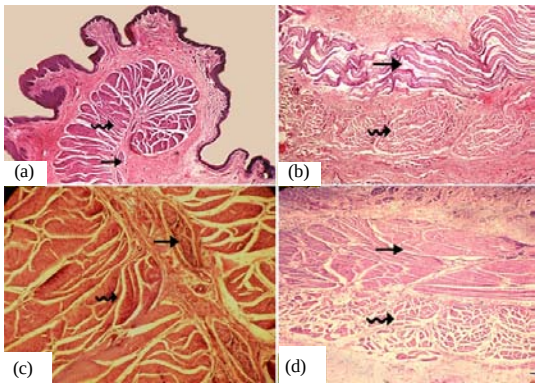

Fig. 4: a, b) Photomicrographs showing comparison of the tunica muscularis in the lip and the floor of the suckling goat and c, d) adult goat's reticular groove which composed of inner circular layer "black arrow" and outer longitudinal "zigzag arrow" smooth muscle fibers. Stain: H&E (a): Obj. X5:Oc. X10 (b, c and d): Obj. X10:Oc. X10

muscle fibers between myofibrils. The reticular grooves of the suckling lamb (Fig. 6a) and the adult sheep (Fig. 6b) differ from those of the suckling goat and the adult goat in the tongue shaped papillae present on the longitudinal folds.

Similar to the suckling goat, the reticular groove of the suckling lamb was covered by stratified squamous epithelium. In the case of the adult sheep, lamina epithelialis consisted of the same layers as the adult goat but the basement membrane had deeper folds and stratum

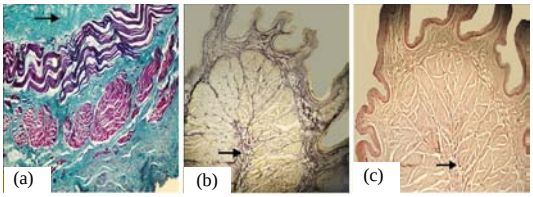

Fig. 5: a) The distribution of collagen fibers "black arrow" in the floor, b) reticular fibers "black arrow" in the lip and c) elastic fibers "black arrow" in the lip and of the suckling kid's reticular groove. Stains: (a) crossmon's trichrome; (b) silver impregnation and (c) Orcein; Obj. X5:Oc. X10

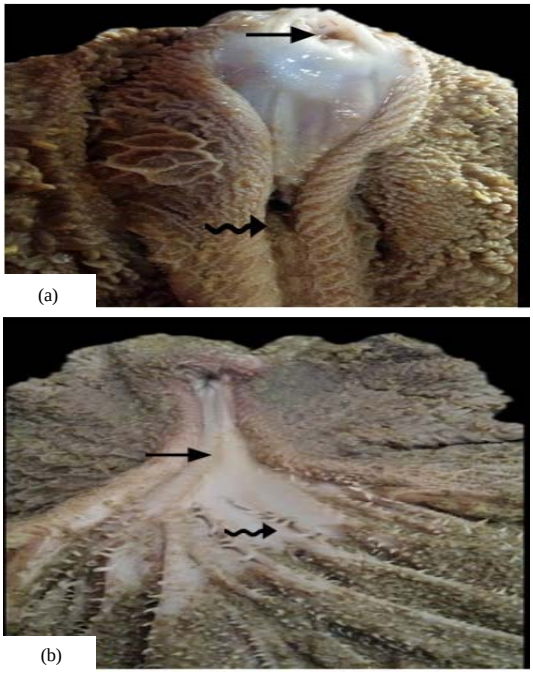

Fig. 6: Comparison of the reticular groove of suckling lamb: a) showing that it was extending from the cardiac orifice "black arrow" to reticulo-omasal orifice "zigzag arrow" limited by right lip and left lip and adult sheep's reticular groove and b) showing several longitudinal folds "black arrow" and several tongue shape papillae "zigzag arrow" were found in the floor

basale had highly condensed and darker in color. Muscularis mucosa and tunica muscularis of the lip and the floor of the suckling lamb and the adult sheep were similar to the suckling and the adult goat.

Very interestingly only in the adult sheep, mucous glands were found in the tunica submucosa at the level of the lips-floor junction (Fig. 7a) and also in the tunica muscularis among smooth muscle fibers of the outer

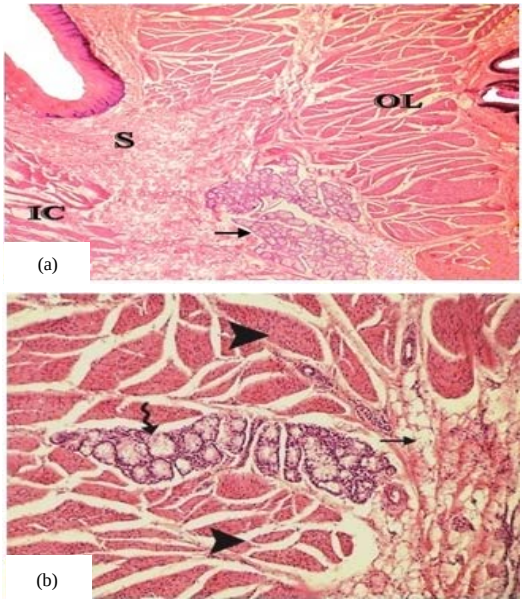

Fig. 7: The adult sheep's reticular groove showing that: a) the mucous glands "black arrow" were found at the tunica submucosa "S" at the level of the lip-floor junction between the outer longitudinal layer of the lip "OL" and the inner circular of the floor "IC" and b) it was "zigzag arrow" were found among the smooth muscle fibers "arrow head" with aggregation of the adipocytes "black arrow". Stain: H&E (a): Obj. X5: Oc. X10 (b):Obj. X10:Oc. X10

longitudinal layer of the lips (Fig. 7b). Mucous glands consisted of oval or round acini surrounded by some myoepithelial cells with spindle shape nucleus. These acini had some cells inside their lumens which were low cuboidal with pale acidophilic cytoplasm with an euchromatic nucleus and prominent nucleolus. These cells resemble the centroacinar cells of the pancreatic acini (Fig. 8a). These acini were positively reacted to PAS (Fig. 8b) and Alcian blue pH (2.5) showing several vacuoles inside the acinar cells. These acini were negatively reacted to BPB showing clear cytoplasm. There were no glands found in the suckling lamb, the suckling goat and the adult goat.

Quantitative aspects: The present measurements revealed some interesting facts which are explained in Fig. 9a-d. The individual layers of the reticular groove have clear and straightforward development from the proximal to the distal parts with prominent increase with age.

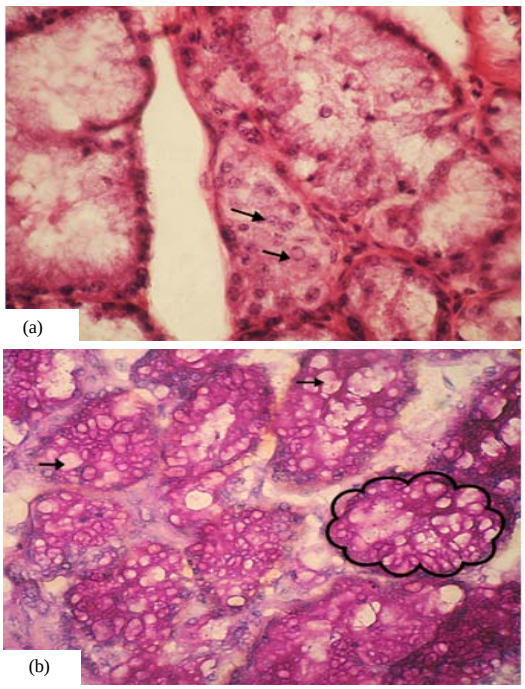

Fig. 8: The adult sheep's reticular groove showing that: a) The mucous glands had some cells inside their lumens which are low cuboidal "black arrow" and b) These acini "black circle" were positively reacted to PAS showing several vacuoles "black arrow" inside the acinar cells

DISCUSSION

The present study describes the histological structure of the reticular groove with particular attention to the comparison between the suckling and the adult goat and sheep. The ruminant's stomach consisted of four parts; rumen, reticulum, omasum and abomasum. These results go hand to hand with Kandeel *et al*. (2009) in the sheep and the goat. The gastric groove extended from the end of the esophagus into the beginning of abomasum with right and left lips limiting the floor of the groove. Similar data were reported by Garcia *et al*. (2005).

In the goat and the sheep, the gastric groove had longitudinal folds with thorn shape papillae. Similar results were recorded by Teixeria *et al*. (2009) in ruminant. In contrary to Wang *et al*. (2015) who mentioned that there no papillae were observed in Yak (Bos grunniens).

Teixeria *et al*. (2009) mentioned that the unguiculiform papillae in the ruminants act as a blocking structures to retain the larger food particles and prevent them from passing freely into the omasum. They act as a filter barrier to selectively keep back those large particles from the entry into the omasum and abomasum especially during the dilatation of the ROS where they prevent the obstruction of the distal gastric compartments.

The whole reticular groove was covered by stratified squamous epithelium which arranged in the form of several layers began firstly with wavy basement membrane, then a single layer of columnar cells with oval nuclei (called the stratum basale), followed by several layers of polyhedral cells with highly active rounded or spherical nuclei known as stratum spinosum and finally more flattened cells with flat nuclei (called stratum granulosum) which appeared in the form of threads. Similar results were reported by Ramkrishna and Gadre (2004) in domestic ruminants; Eurell and Frappier (2006) in ruminants; Lalatta-Costerbosa *et al*. (2011) in suckling lambs.

In the recent study, the epithelium was found with a vesicular nucleus in the suckling ruminant, then it is changed into the normal nucleus in the adult ruminant. Similar results were recorded by Tiwari and Jamdar (1970) who found that the epithelium with vesicular cells in the region of the stratum spinosum was found at birth. It changed to the normal adult type by 2 weeks of age in the indian water buffalo calf.

In the present study, we observe that the thickness of the whole epithelium increases with age, in contrary to the study of Tiwari and Jamdar (1970) on the Indian water buffalo calf where the thickness of the epithelium decreased with age but the thickness of the stratum corneum increased with age.

The muscularis mucosa of the suckling kid and lamb was composed of thin transversely arranged smooth muscle fibers in the floor and the lips which were immersed in the lamina propria. Similar results were recorded by Eurell and Frappier (2006) in ruminants.

The muscularis mucosa of the adult goat and sheep had the same features as the suckling one plus it was increased in its thickness. Similar results were recorded by Eurell and Frappier (2006) in the ruminants but the tunica submucosa was engorged with adipose tissue. Similar findings were reported by Riccillo *et al*. (2004) in the rat Honma *et al*. (2011) in the mouse liver where the adipose tissue was increased with advancing the age.

Tunica muscularis in the floor of the suckling kid and lamb consisted of a thick inner circular layer with undulant muscle fibers and outer longitudinal one. But in the case of adult goat and sheep, tunica muscularis in the floor is extremely developed consisting of well-developed inner circular layer and very thick outer longitudinal layer. Similar findings were reported by Lalatta-Costerbosa *et al*. (2011) in suckling sheep. Salama (1981) recorded that

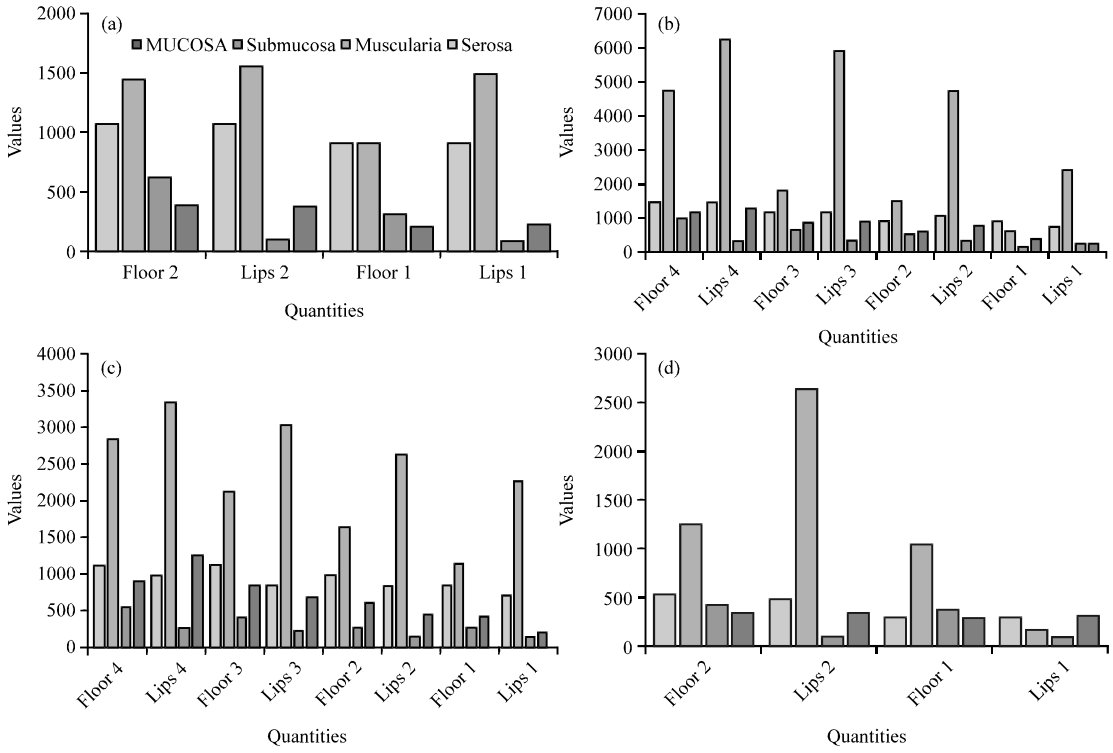

Fig. 9: a) Quantitative development of the esophageal groove in suckling kids; b) Quantitative development of the esophageal groove in adult goats; c) Quantitative development of the esophageal groove in suckling lambs and d) Quantitative development of the esophageal groove in adult sheep

smooth muscle fibers of tunica muscularis in younger camel's esophagus were spirally arranged. Tunica muscularis in the lips of the suckling lamb and kid consisted of very thin inner circular and thick outer longitudinal layer. But in the case of adult goat and sheep it was extremely developed consisting of a thin inner circular layer present at the center of the thickened outer longitudinal layer which surround the interior of the whole lips. Similar findings were reported by Lalatta-Costerbosa *et al*. (2011) in suckling lambs.

In the black bengal goat, Khan and Hossain (1996) and in pig Pirker *et al*. (2007) found that the smooth muscle fibers increased in length by 140% on the 90th day of age. The postnatal growth of the smooth muscle was due to hyperplasia of its muscle fibers. All the smooth muscle components showed a striking increase in muscle bulk between the neonate and adolescent stages.

CONCLUSION

In the present study, the thickness of the whole layers increased with age. In contrast, Tiwari and Jamdar (1970) found that the thickness of the epithelium decreased by age but the thickness of stratum corneum, muscularis mucosae, submucosa and the muscularis increased with age in the indian water buffalo calf. In the present study, we found that adult sheep reticular groove had some mucous glands. These glands were found in tunica submucosa at the level of the lip-floor junction. However, similar glands were observed in sheep and in the ruminants (Ramkrishna and Gadre, 2004). These glands were found also in tunica muscularis among the smooth muscle fibers of the outer longitudinal layer in the lips. Similar results were reported by Lalatta-Costerbosa *et al*. (2011) in the suckling lambs.

In the present study, all the existing glands were mucous glands. In contrast, Mutoh and Wakuri mentioned that serous and seromucous glands were found in younger age but mucous glands were found in the old age.

Mucous acini decreased in the number along the whole length of the gastric groove in contrary to the findings of Lalatta-Costerbosa *et al*. (2011) who stated that these acini became more numerous in the distal part. These glands consisted of some oval or round mucous acini. Similar findings were recorded by Mutoh and Wakuri in 1 statistical charts in Fig. 9. With advancing the age; there is a prominent increase in the length of the

gastric groove, both the lips and the floor increase in their size, the individual layers of the reticular groove have a clear and straightforward development from the proximal to the distal parts, prominent increase in their thickness with conspicuous amount of adipose tissue become apparent. Similar data were reported by Salama (1981) in the camel esophagus.

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