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Histomorphochemical Observations on the Duct System of Minor Salivary Glands of Sheep (Ovis Aries)

KEYWORDS: histomorphochemical, duct system, minor salivary glands, sheep

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ABSTRACT *The tissues from the buccal and labial salivary glands of 25 healthy adult sheep of local mixed breed of either sex were collected and processed for paraffin and frozen sectioning techniques. Small intralobular ducts were lined by simple cuboidal type of epithelium whereas, large intralobular ducts were lined by simple cuboidal to simple columnar type of epithelium. Interacinar ducts were lined by simple to stratified cuboidal epithelium. In case of intralobular ducts, labial salivary glands have more ductal diameter as compared to buccal salivary glands whereas, luminal diameter was comparatively less in labial salivary glands. The ductal and luminal diameters of interlobular ducts of buccal salivary glands were more than labial salivary glands. All the epithelial cells of the ducts were negative to mucosubstances, glycogen, mucopolysaccharides, mucin and lipids studied during the present investigation except the goblet cells which contained acidic and neutral mucopolysaccharides and mucin*

INTRODUCTION

Saliva plays a great role in proper digestion in ruminants which is secreted from major and minor salivary glands. Saliva contains water, various enzymes, mucopolysaccharides and lubricating glycoproteins. These glands helps in restoration of normal ruminal pH and microbial protein synthesis to be used as dietary proteins. The salivary glands may be classified on the basis of their secretions as serous, mucous, or seromucous (mixed) glands. The distribution of these types varies from species to species (Konig and Liebich, 2004). Saliva is secreted into the oral cavity via a series of ducts in the ductal system. Dysfunction of salivary secretion (hyposalivation) causes xerostomia (dry mouth) and sequentially leads to severe dental caries as well as oral mucosal disorders (Featherstone, 2000). The salivary glands also secrete IgA and potassium, and sodium (Aspinall and Reilly, 2004). Some of the studies have been conducted on the secretory adenomeres of the minor salivary glands in sheep but still their duct system remained unexplained in detail. Hence the present investigation provides its detailed histomorphology and histochemistry.

MATERIALS AND METHODS

Samples

The tissues of buccal and labial salivary glands of 25 healthy adult sheep, of local mixed breed of either sex, were collected and processed for paraffin and frozen sectioning techniques.

Design of the study

The sections were stained with Harris' haematoxylin and eosin stain for histomorphological studies, Crossman's trichrome stain for collagen fibres, Gomori's stain for reticular fibres, Weigert's method for elastic fibres, Alcian blue for mucosubstances (pH 2.5), PAS-Alcian blue method for mucosubstances, Best's carmine method for glycogen, McManus' PAS method for glycogen, Diastase digestion method, Mayer's mucicarmine method for mucin, Sudan black B method for fats, Oil-red-O in propylene glycol method for fats (Luna, 1968), Colloidal iron stain for acid mucopolysaccharide (Thompson and Hunt, 1966), Nile blue method for neutral and acidic lipids (Drury and Wallington, 1967). Micrometry was done with the help of ocular micrometer.

RESULTS AND DISCUSSION

The intralobular ducts of dorsal buccal salivary gland were further subdivided into small and large intralobular ducts on the basis of their diameter, type of epithelium and thickness of lamina propria surrounding these ducts. The small intralobular ducts were smaller in size having average ductal and luminal diameter of 24.50±0.85 µm and 15.10±0.90 µm with a range value measuring 18.45 µm to 29.50 µm and 11.48 µm to 19.64 µm, respectively. These ducts were lined by simple cuboidal to low columnar type of epithelium. The epithelial height of the cells lining the ducts measured 5.74±0.29 µm with a range value of 2.74 µm to 8.39 µm. The cytoplasm of these cells was finely granular and eosinophilic. The nuclei were round to oval localized towards the centre of the cell. The large intralobular ducts in the mucous region were lined by simple cuboidal to low columnar type of epithelium. The average ductal and luminal diameter 43.11±1.06 µm and 25.12±1.01 µm with a range value measuring 36.51 µm to 52.75 µm and 18.67 µm to 31.83 µm, respectively. Their nuclei were round to oval localized towards the centre of the cell. The epithelial height of the cells lining the ducts measured 9.19±1.05 µm with a range value of 3.50 µm to 13.62 µm. The cytoplasm of these cells was finely granular and eosinophilic. The luminal surface of these ducts presented striated border and contains eosinophilic type of

Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

secretions.

The interlobular ducts of dorsal buccal salivary gland were localized at the level of interlobular septae. These ducts measured 88.11±1.03 µm with a range value of 69.91 µm to 95.60 µm and 61.18±1.18 µm with a range value of 48.82 µm to 72.30 µm in ductal and luminal diameter, respectively. These ducts were also lined by simple cuboidal to low columnar type of epithelium. The epithelium heights was relatively more as that of intralobular ducts and it ranged from 4.68 µm to 13.50 µm with an average height of 9.71± 0.91 µm. These ducts sometimes showed two cell layer epithelium at the point where the adjacent interlobular ducts united with each other. Lumen of these acini generally did not exhibited secretion where as the interlobular ducts in the same region showed the positive reaction. The nuclear characteristics were same as described earlier in ducts. In addition, few lightly stained nuclei was observed. These ducts were surrounded by loose irregular connective tissue, fibroblasts and myoepithelial cells (Fig. 1). Contrary to these findings, Parida and Das (1991) in domestic ruminants and Gupta (1995) in buffalo described that the duct system of the minor salivary glands included the intercellular canaliculi, intercalated, intralobular, interlobular and main excretory ducts. Unlike the major salivary glands, the intercellular canaliculi were associated with serous as well as mucous components (Shackleford and Klapper, 1962) in mammalian salivary glands. The excretory duct lined with stratified columnar epithelium at its beginning and stratified squamous near its terminations described in minor salivary glands of domestic ruminants (Parida and Das, 1991) was also found in the present investigation.

The intralobular ducts of middle buccal salivary gland were categorized into small and large sized ducts and were lined by simple cuboidal epithelium. The small intralobular ducts were smaller in size having average ductal and luminal diameter 25.15±2.53 µm and 14.10±1.15 µm with a range value measuring 18.53 µm to 31.90 µm and 8.82 µm to 21.50 µm, respectively. Both interlobular and intralobular ducts showed negative reaction by Colloidal Iron Method (Fig. 2). The nuclei were round to oval shaped and mainly localized in the centre. One or two nuclei were centric and eccentric in position and few nuclei were darkly stained. The epithelial height of the cells lining the ducts measured 3.15± 0.33 µm with a range value of 1.18 µm to 7.40 µm. The cytoplasm of the cells was strongly (+++) eosinophilic in nature. Few mast cells were also observed.

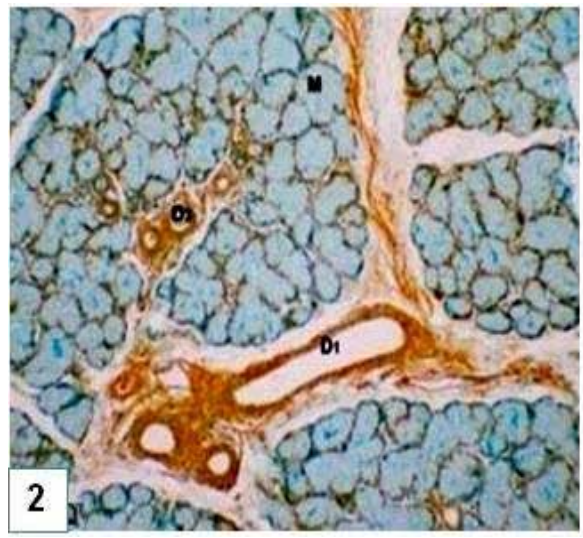


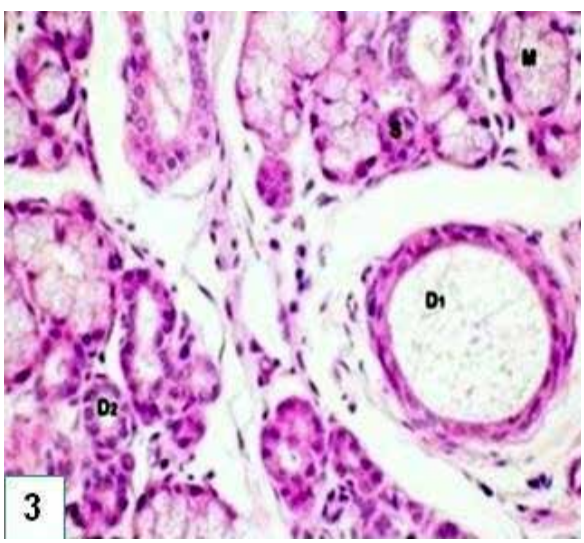
Fig. 1-2: Photomicrograph of 1) Dorsal buccal salivary gland showing the presence of large interlobular duct (D1) having goblet cells (arrow). Mucous acini (M) and small intralobular duct (D2) can also be seen (H. & E. Stain x 100). 2) Middle buccal salivary gland showing moderate reaction for colloidal iron in mucous acini (M) but negative in both interlobular (D1) and intralobular (D2) ducts (Colloidal Iron Method x 100).

The larger intralobular ducts having average ductal and luminal diameter 38.16±2.21 µm and 27.13±0.51 µm with a range value measuring 28.74 µm to 45.36 µm and 22.48 µm to 38.50 µm, respectively. These were also lined by simple cuboidal epithelium. The epithelial height of the cells measured 4.83±0.47 µm with a range value of 2.72 µm to 9.34 µm. The nuclei of these cells were round to oval having fine distribution of chromatin material being aggregated into smaller clumps throughout the nucleoplasts. Thus it presented slight vacuolated appearance to the nuclei. The cytoplasm of these cells was homogenous, finely granular and strongly eosinophilic (Fig. 3). The concentration of reticular and elastic fibres increased surrounded these ducts. In addition fine blood capillaries and few nerve fibres were also observed.

A large interlobular duct having goblet cells showed intense PAS positive reaction (Fig. 4). The interlobular ducts of middle buccal salivary gland were having very large size lumen and measured 91.66±0.81 µm with a range value of 73.60 µm to 97.53 µm and 59.39±1.10 µm with a range value of 38.70 µm to 65.82 µm in ductal and luminal diameter, respectively. These ducts were lined by varying type of epithelium. Generally these were lined by simple to stratified cuboidal epithelium which was of two cell layer thickness. At places the columnar epithelium cells were also seen which presented a pseudostratified appearance. The epithelial height of the cells measured 11.37±0.86 µm with a range value of 5.15 µm to 17.40 µm. These nuclei were round to oval along with few small sized differentiate nuclei. The intralobular ducts of ventral buccal salivary gland were of two types i.e., smaller and larger. Smaller ducts were having average ductal and luminal diameter 26.10±1.19 µm and 10.55±1.10 µm with a range value measuring 16.96 µm to 37.80 µm and 4.18 µm to 17.55 µm, respectively. These were lined by high cuboidal epithelium, the height of which measured from 2.80 µm to

Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

11.42 µm with an average of 7.12±0.63 µm. Their nuclei were round to oval placed in the centre of the cell. Their chromatin material was finely granular and distributed throughout the nucleoplast. One or two nucleoli was centric or eccentric in position. The cytoplasm of the cell was finely granular and strongly eosinophilic in nature. The eosinophilia was more at luminal and at the basal surface. These were surrounded by large number of myoepithelial cells, fibroblasts, connective tissue cells, reticular, elastic and collagen fibres and few fine blood capillaries. The larger ducts were having average ductal and luminal diameter 33.17±2.04 µm and 18.51±1.18 µm with a range value measuring 24.50 µm to 47.26 µm and 11.48 µm to 28.96 µm, respectively. These ducts were lined by simple columnar type of epithelium, the height of which measured from 3.49 µm to 14.52 µm with an average of 8.12±0.71 µm. The histological features were same as that of smaller one. The interlobular ducts of ventral buccal gland were having average ductal and luminal diameter 83.13±2.05 µm and 61.38±1.19 µm with a range value measuring 61.54 µm to 98.73 µm and 43.35 µm to 76.38 µm, respectively. These ducts were lined by stratified columnar epithelium with vacuolated cytoplasm showing goblet cell like appearance.



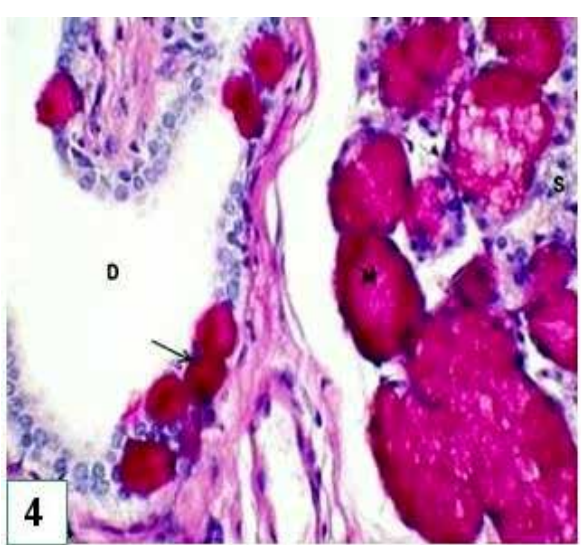


Fig. 3-4: Photomicrograph of 3) Labial salivary gland showing the presence of mucous (M) and serous (S) acini in the glandular parenchyma. Both interlobular (D1) and intralobular (D2) ducts can also be seen (H. & E. Stain x 400). 4) Dorsal buccal gland showing intensely PAS positive material in mucous acini (M) which was absent in serous acini (S). A large interlobular duct (D) having goblet cells (arrow) were PAS positive (McManus' Method x 400).

The epithelial height of the cells measured from 8.72 µm to 18.40 µm with an average of 13.16±0.78 µm. These ducts were surrounded by large number of myoepithelial cells. Young and Van Lennep (1978) reported the excretory units of ventral buccal glands in ruminants to be consisted of intercalated, intralobular and interlobular ducts which was partially in consonance with the present study. Similarly, Parida and Das (1991) also observed a gradual increase in the epithelial height of the cells lining the intercalated to excretory ducts of minor salivary glands in domestic ruminants. Stinson and Calhoun (1993) observed that in domestic animals the simple cuboidal epithelium lined the ducts with in the lobule whereas two layered cuboidal epithelium lined the larger interlobular ducts, which was in consonance with the findings of present study. Kay (1960) in cattle and sheep reported that the intralobular ducts of buccal glands were lined by flat epithelial cells. The intralobular ducts of labial glands were round to oval in shape. These ducts were having average ductal and luminal diameter 27.50±1.05 µm and 11.75±1.10 µm with a range value measuring 16.36 µm to 38.50 µm and 7.49 µm to 18.27 µm, respectively. These were lined by simple cuboidal epithelium. The epithelial height of the cells measured from 2.52 µm to 13.29 µm with an average of 5.87±0.65 µm. Nuclei were round to oval in shape. Nuclei were divided into lightly and darkly stained nuclei on the basis of distribution of chromatin and its affinity. Dellmann (1998) divided the ducts of minor salivary glands in domestic animals into intercalated, striated (salivary) and excretory ducts whereas Dhingra et al. (1978) and Gupta (1995) classified the duct system of labial glands of buffalo into intercalated, striated and interlobular ducts. Parida and Das (1991) recorded the presence of intercellular canaliculi, intercalated, striated, intralobular, interlobular and excretory ducts in the labial glands of domestic ruminants. But Magalhaes and Silva (1976) did not mentioned about intercellular canaliculi in labial glands of zebu, the presence of which was reported in mucous units of human labial glands (Young and Van Lennep, 1978). Cytoplasm was finely granular and eosinophilic in nature. The interlobular ducts of labial glands were larger in size. These ducts were having average ductal and luminal diameter 68.17±0.60 µm and 38.13±0.83 µm with a range value measuring 42.10 µm to 81.90 µm and 21.47 µm to 61.50 µm, respectively. These were lined by stratified cuboidal epithelium. The character of lining epithelium of different ducts gained support from the observations of Stinson and Calhoun (1993) in domestic animals and Gupta (1995) in buffalo. The epithelial height of the cells measured from 6.73 µm to 34.10 µm with an average of 15.16±0.71 µm. These ducts were surrounded by myoepithelial cells, collagen, elastic and reticular fibres. The cytoplasm of these cells was finely granular and eosinophilic in nature.

CONCLUSION

Small intralobular ducts were lined by simple cuboidal type of epithelium whereas, large intralobular ducts were lined by simple cuboidal to simple columnar type of epithelium. Interlobular ducts were lined by simple to stratified cuboidal epithelium. In case of intralobular ducts, labial salivary glands have more ductal diameter as compared to buccal salivary glands whereas, luminal diameter was comparatively less in labial salivary glands. The ductal and luminal

Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

diameters of interlobular ducts of buccal salivary glands were more than labial salivary glands. All the epithelial cells of the ducts were negative to mucosubstances, glycogen, mucopolysaccharides, mucin and lipids studied during the present investigation except the goblet cells which contained acidic and neutral mucopolysaccharides and mucin.

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