



Histomorphological studies on the soft palate of domestic pig (*Sus scrofa domestica*)[#]

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Abstract

The present study was undertaken on the soft palate of the SVVU T-17 breed of domestic pig at different levels i.e., proximal and distal portions and at the level of anterior and posterior pillars. The microanatomical studies of soft palate showed epithelium, propria-submucosa and tunica muscularis. The oral surface of the soft palate was lined by non-keratinized stratified squamous epithelium and the rostral half of the nasal surface was lined by the pseudostratified columnar ciliated epithelium while its caudal half was lined by non-keratinized stratified squamous epithelium. The propria-submucosa of the soft palate consisted of collagen, elastic and reticular fibres, connective tissue cells i.e., fibroblasts, fibrocytes, various sizes of lymphocytes, eosinophils, mast cells and plasma cells. In addition, adipose tissue, blood vessels, nerves, lymphoid tissue and seromucous glands were observed. Most of the tunica muscularis consisted of skeletal muscles and a very few smooth muscle fibres.

Keywords: Soft palate, oral surface, nasal surface, domestic pig

Pig is a highly prolific animal and is widely used as a model for various biological experiments (Kumar *et al.*, 2017). The histological structure of the palatal mucosa in pigs closely resembles that of humans. Hence, pigs provide a useful experimental model for studying biological mechanisms involved in scar-less wound healing, which may aid in the development of novel strategies to prevent scar formation (Wong *et al.*, 2009). Anatomical incongruity of the soft palate plays a crucial role in conditions such as intermittent soft palate displacement in farm animals and obstructive sleep apnea in humans (Batah *et al.*, 2020). The literature available on histology of soft palate in domestic pigs is scanty. Hence, the present study was undertaken to elucidate the histomorphology of the soft palate in domestic pig.

Materials and methods

For microanatomical studies, fresh specimens of the soft palate from 12 adult domestic pigs of the SVVU-T-17 breed were collected immediately after slaughter from the All India Coordinated Research Project (AICRP) on Pigs, SVVU, Tirupati. The research protocol was approved by Institutional Animal Ethics Committee (IAEC) vide ref. No. 281/

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go/Rebi/S/2000/CPCSEA/CVSc/TPTY/001/Veterinary Anatomy/2023 dated 08.05.2023. Tissue samples were collected from the proximal and distal portions of the soft palate and at the levels of anterior and posterior pillars. The samples were fixed in 10% Neutral Buffered Formalin, washed thoroughly, dehydrated through graded alcohols and cleared in xylene (Bancroft & Gamble, 2008 and Singh & Sulochana, 1997). The tissues were then impregnated with paraffin wax (59-60°C) using a hot air oven and embedded in paraffin blocks. Sections of 5 µm thickness were cut using a Leica semi-automatic microtome (Leica RM2125RTS) and mounted on clean, adhesive smeared glass slides. The sections were stained with Haematoxylin and Eosin method for routine histological examination, Crossman's trichrome for connective tissue, Verhoeff's method for elastic fibres, Van Gieson's method for collagen fibres, Gomori's method for reticular fibres and Bielschowsky method for nerve fibres (Singh & Sulochana, 1997, Bancroft & Gamble, 2008). The stained sections were examined under a light microscope to study the normal histomorphological features of the soft palate.

Results and discussion

Epithelium

The microanatomical studies of soft palate showed epithelium, propria-submucosa (Fig. 1) and tunica muscularis (Fig. 2). The oral surface of the soft palate was lined by non-keratinized stratified squamous epithelium (Fig. 1). On the nasal surface, the rostral half showed pseudostratified columnar ciliated epithelium (Fig. 4), while the caudal half was lined by non-keratinized stratified squamous epithelium, continuous with the oral epithelium (Figs. 3,5). These observations concur with the findings of Mercer (1900), Arrighi et al. (2011), Pichetto et al. (2015) in dogs; Maximow & Bloom (1957), Kelly et al. (1984), Berger et al. (2002) in humans; Cleaton-Jones (1975) and Klein et al. (1979) in monkeys; Bacha & Bacha (2012) in cattle; Kumar & Singh (2014) in sheep; and Batah et al. (2020) in cats. In contrast, the oropharyngeal surface has been reported to be keratinized in albino rats (Cleaton-Jones, 1971) and in rabbits and guinea pigs (Obead et al., 2022). Caudal to the posterior pillar and at the free border of the soft palate, the nasal epithelium was consistently non-keratinized stratified squamous with numerous folds, while rostral to the posterior pillar it remained pseudostratified ciliated epithelium, continuous with the nasal cavity epithelium, as reported by Maximow & Bloom (1957) in humans.

In this study, the non-keratinized stratified squamous epithelium showed irregular finger-like papillae and consisted of stratum basale, stratum spinosum and stratum granulosum, which is in agreement with the descriptions of Eurell & Frappier (2006) in domestic animals, Kumar & Timoney (2006) in horses and Ranjit

& Singh (2016) in young pigs. Long rete pegs were more prominent on the oral surface than on the nasal surface (Fig. 6), supporting the observations of Maximow & Bloom (1957) in domestic animals.

On the nasal surface rostral to the posterior pillars and at the level of anterior pillar, rete pegs were absent. The epithelium was pseudostratified columnar ciliated and comprised basal, columnar and goblet cells (Fig. 7), consistent with the findings of Kumar & Singh (2014) in sheep and Eurell & Frappier (2006) in domestic animals.

Propria-submucosa

The propria-submucosa found on both surfaces of the soft palate enclosed the tunica muscularis centrally. It was composed of dense irregular connective tissue containing collagen (Fig. 9), elastic (Fig. 10) and reticular fibres (Figs. 11,12); connective tissue cells (Fig. 8) i.e., fibroblasts, fibrocytes, various sizes of lymphocytes, eosinophils, mast cells and plasma cells; adipose tissue (Figs. 9, 20); blood vessels; nerves (Fig. 13); lymphoid tissue and glands at all levels of the soft palate (Figs. 3,4). These are in accordance with the findings of Kumar & Singh (2014) in sheep and Richardson et al. (2006) in equines.

In the present study, the subepithelial connective tissue consisted predominantly of densely arranged collagen fibres with few reticular fibres and also contained lymphatic aggregations. The connective tissue extended into the interpapillary pegs of the epithelium mainly consisted of connective tissue cells (Fig. 6), collagen (Fig. 1) and reticular fibres (Figs. 12) as reported by Richardson et al. (2006) in equines and Arrighi et al. (2011) in dogs. Collagen fibres were predominant and arranged as irregular bundles between the glands, muscle bundles and adipose tissue (Figs. 9,14). In contrast to this finding, the collagen fibres were oriented parallelly and placed between the glandular acini as thin septa in the propria-submucosa of the soft palate in albino rats (Cleaton-Jones, 1971).

The glands of the soft palate were compound tubulo-alveolar and seromucous in nature and were observed in the propria-submucosa on both surfaces of the soft palate (Fig. 19). This finding is in conformity with the observations of Banks (1993), Aughey & Frye (2001) and Eurell & Frappier (2006) in domestic animals. In contrary to the above finding, numerous mucous glands were found in the anterior half of the oral surface of the soft palate, but no glands were seen at the free border of the soft palate in monkeys (Cleaton-Jones, 1975), while in rabbits the oropharyngeal part was rich in mucous glands and the nasopharyngeal part lacked these glands (Mercer, 1900 and Obead et al., 2022). In the present study, glandular tissue remained fairly constant on both surfaces and along the whole length of the soft palate. Contrary to this finding, glands were reported to be more predominant in the

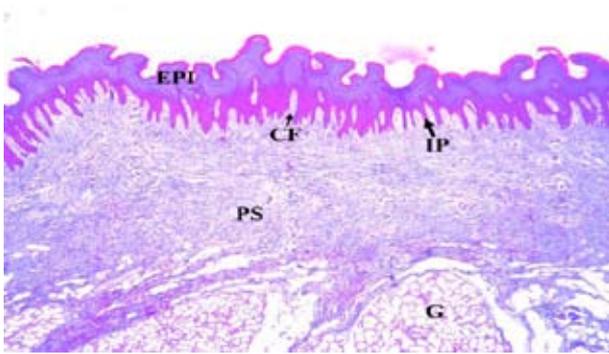


Fig. 1. Photomicrograph of the oral surface of the soft palate of pig at anterior pillar level showing the non-keratinized stratified squamous epithelium (NKSE) and propria-submucosa (PS). CF-Collagen fibres, G- Glands, IP- Inter papillary pegs. Crossman's Trichrome method x40

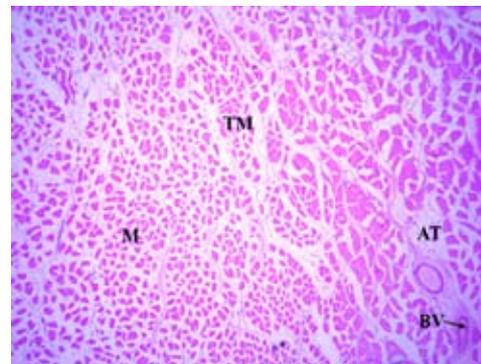


Fig. 2. Photomicrograph showing the tunica muscularis (TM) of the soft palate of pig. AT- Adipose tissue, BV- Blood vessel, M- Skeletal muscle. Haematoxylin and Eosin method x 40

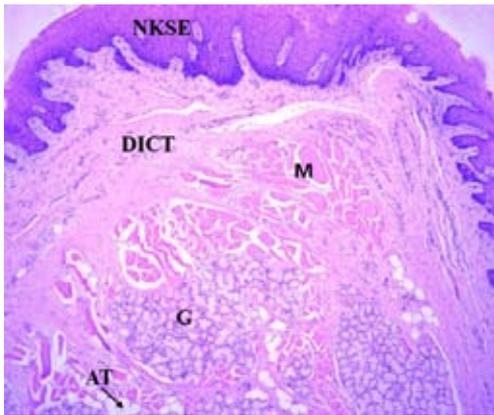


Fig. 3. Photomicrograph of the propria-submucosa of the soft palate of pig at free border showing adipose tissue (AT), glands (G) and muscle bundles (M). NKSE- Non-keratinized stratified squamous epithelium, DICT- Dense irregular connective tissue. Haematoxylin and Eosin method x 40

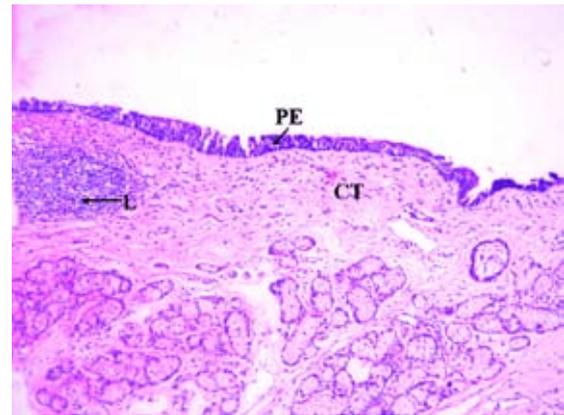


Fig. 4. Photomicrograph of nasal surface of the soft palate of pig at anterior pillar level showing the lymphatic aggregations (L) in the subepithelial connective tissue. CT- Connective tissue, PE- Pseudostratified ciliated columnar epithelium. Haematoxylin and Eosin method x 40

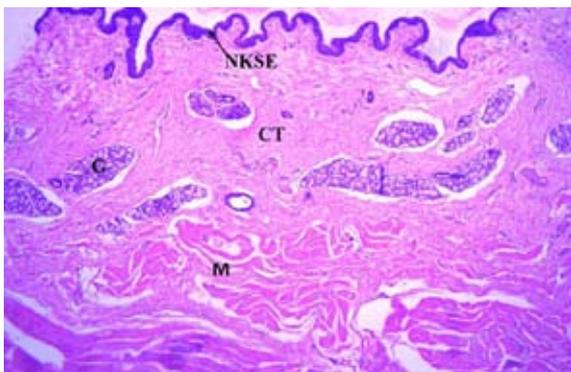


Fig. 5. Photomicrograph of the propria-submucosa of the nasal surface of the soft palate of pig at the posterior pillar level showing the glands (G), connective tissue (CT) and muscle bundle (M). NKSE- Non-keratinized stratified squamous epithelium. Haematoxylin and Eosin method x40

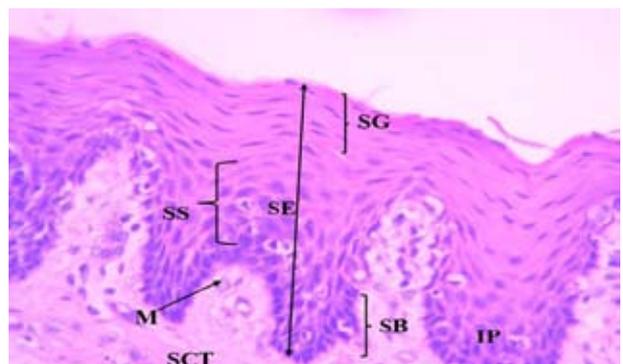


Fig. 6. Photomicrograph of non-keratinized stratified squamous epithelium (NKSE) of the soft palate of pig showing the stratum granulosum (SG), stratum spinosum (SS) and stratum basale (SB). IP- Inter papillary pegs with connective tissue cells, M- Mast cells, SCT- Sub epithelial connective tissue. Haematoxylin and Eosin method x 400

rostral than the caudal part in equines (Richardson et al., 2006), while the glands were fewer in number on the nasal surface than on the oral surface of the soft palate in cats (Batah et al., 2020) and in domestic animals (Trautmann & Fiebiger, 1952).

Most of the glands were predominantly mucous in nature, with a few serous acini and serous demilunes. The thick, viscous mucus secreted by mucous glands provides a protective layer over the lining of hollow organs exposed to the external environment, as reported by Eurell and Frappier (2006) in domestic animals. In contrast,

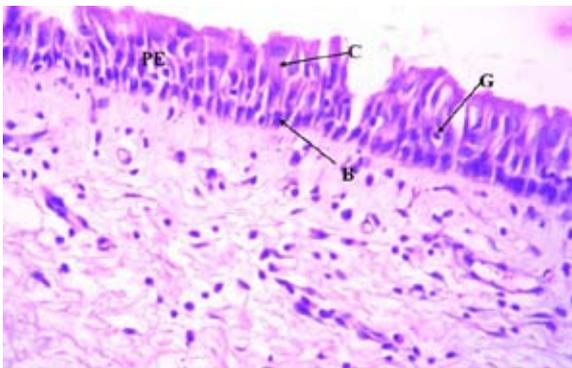


Fig. 7. Photomicrograph of the soft palate of pig at anterior pillar level showing the cells of pseudostratified ciliated columnar epithelium (PE). B- Basal cell, C- Ciliated columnar cell, G- Goblet cell.

Haematoxylin and Eosin method x400

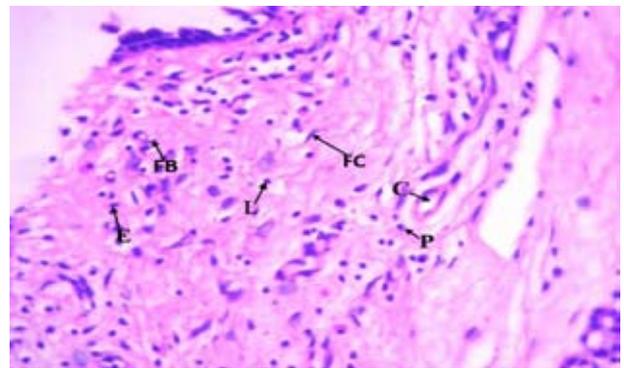


Fig. 8. Photomicrograph of the propria-submucosa of the soft palate of pig showing the lymphocyte (L), eosinophil (E), fibrocyte (FC), fibroblast (FB) and plasma cell (P). C- Capillary

Haematoxylin and Eosin method x400

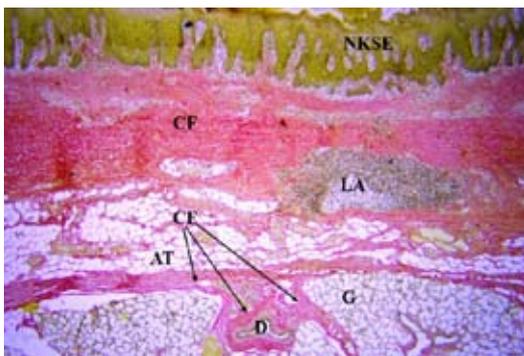


Fig. 9. Photomicrograph of the propria-submucosa of the oral surface of the soft palate of pig at anterior pillar level showing the collagen fibres (CF) around the glands (G), glandular duct (D) and adipose tissue (AT). NKSE- Non-keratinized stratified squamous epithelium, LA- Lymphatic aggregation.

Vangieson's method x 40

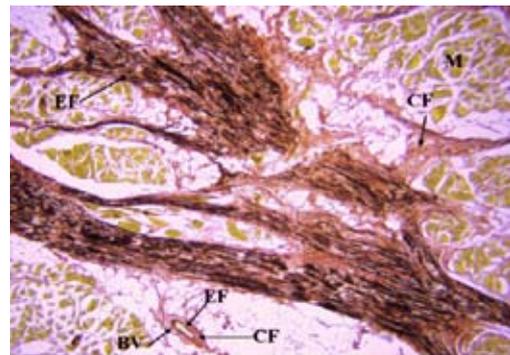


Fig. 10. Photomicrograph of the soft palate of pig showing densely arranged elastic fibres (EF) in between the muscle bundles (M). CF- Collagen fibres, BV- Blood vessel.

Verhoeff's method x40

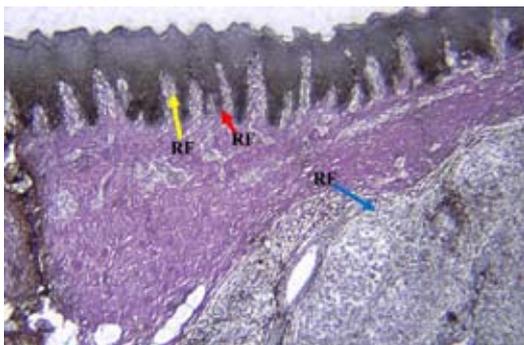


Fig. 11. Photomicrograph of the oral surface of the soft palate of pig showing reticular fibres (RF) in the basement membrane of epithelium (Red arrow), in the interpapillary pegs (Yellow arrow) and in the lymphatic tissue (Blue arrow).

Gomori's Reticulum method x 100

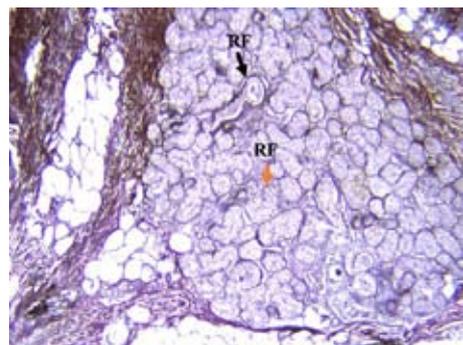


Fig.12.Photomicrograph showing the reticular fibres (RF) in the basement membrane of secretory acini (Orange arrow) and in the interlobar connective tissue of glands (black arrow) of the soft palate of pig.

Gomori's Reticulum method x 100

the majority of palatine glands were serous with a few mixed glands in dogs (Arrighi et al., 2011 and Pichetto et al., 2015). In humans, the oral surface of the soft palate contains only mucous glands, while the nasal surface contains both serous and mucous glands (Kelly et al., 1984, Fawcett, 1986, Ham, 1987 and Berger et al., 2002), whereas in monkeys, the entire soft palate predominantly

contains mucous glands, except in the posterior third of the nasal surface of the soft palate where mixed glands were observed (Cleaton-Jones, 1975). Different lobes of the glands were separated by bundles of skeletal muscle fibres, abundant collagen fibres, few elastic and reticular fibres, blood vessels and adipose tissue (Fig.19). These

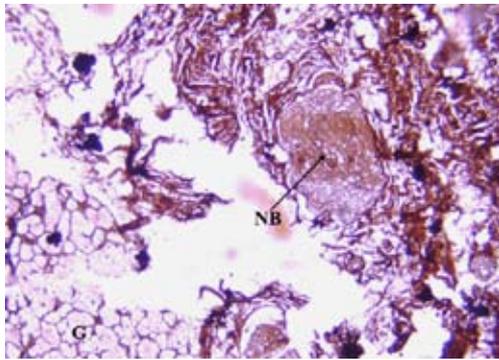


Fig. 13. Photomicrograph of the propria-submucosa at the fixed border of the soft palate of pig showing nerve bundles (NB). G- Glands

Bielchowsky method x 100

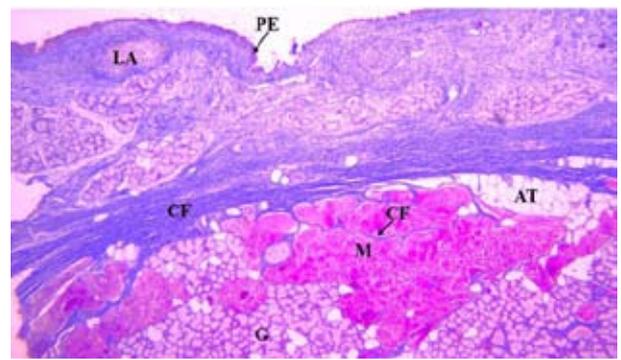


Fig. 14. Photomicrograph of the propria-submucosa of the nasal surface of the soft palate of pig at the anterior pillar level showing collagen fibres (CF) in between the muscle bundles (M). AT- Adipose tissue, PE- Pseudostratified ciliated columnar epithelium, G- Glands, LA- Lymphatic aggregations (LA).

Crossman's Trichrome method x 40

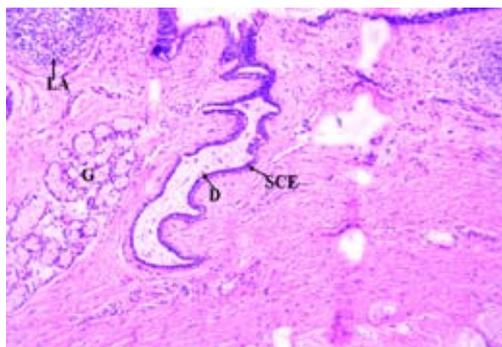


Fig. 15. Photomicrograph of the nasal surface of the soft palate of pig at the anterior pillar level showing opening of the interlobar duct (D) onto the epithelial surface. G- Gland, LA- Lymphatic aggregation, SCE- Stratified cuboidal epithelium.

Haematoxylin and Eosin method x40

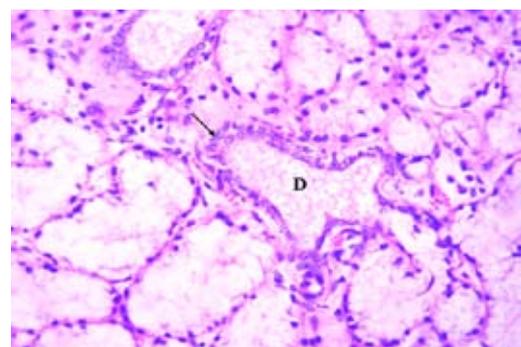


Fig. 16. Photomicrograph showing the intralobar duct (D) lined by stratified cuboidal epithelium (Arrow) in the glands of the soft palate of pig.

Haematoxylin and Eosin method x400

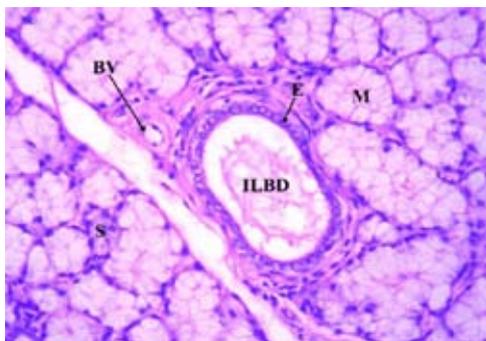


Fig. 17. Photomicrograph showing the seromucous glands with serous acini (S) and mucous acini (M) in the soft palate of pig. E- simple cuboidal epithelium BV- Blood vessel ILBD- Interlobular duct.

Haematoxylin and Eosin method x 400

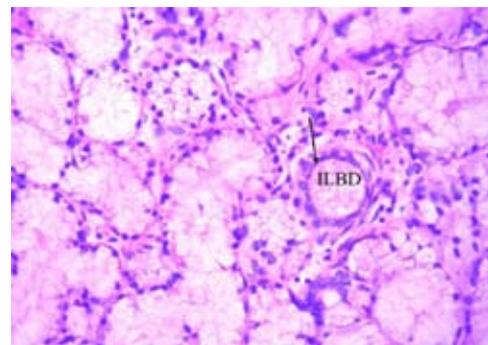


Fig. 18. Photomicrograph showing the intralobular duct (ILBD) lined by simple cuboidal epithelium (Arrow) in the glands of the soft palate of pig.

Haematoxylin and Eosin method x400

observations, concurs with the reports of Kumar & Singh (2014) in sheep and Batah et al. (2020) in cats.

The secretory ducts i.e., interlobar (Fig.15) and intralobar ducts (Fig.16) of glands in soft palate were lined by stratified cuboidal epithelium as reported by Kumar & Singh (2014) in sheep, Batah et al. (2020) in cats and Mercer (1900) and Obead et al. (2022) in rabbits. In contrary to this finding, the main ducts were lined by

cuboidal to columnar epithelium in dogs (Arrighi et al., 2011). The glands were incompletely divided into lobules by connective tissue septa, as reported by Cleaton-Jones (1975) in monkeys and Richardson et al. (2006) in equines, whereas distinct lobulation was observed in rabbits (Mahdy & Mohammed, 2021). The interlobular (Fig.17) and intralobular ducts (Fig.18) were lined by simple cuboidal epithelium, which concurs with the findings of Klein et al. (1979) in monkeys and Batah et al. (2020) in cats, whereas

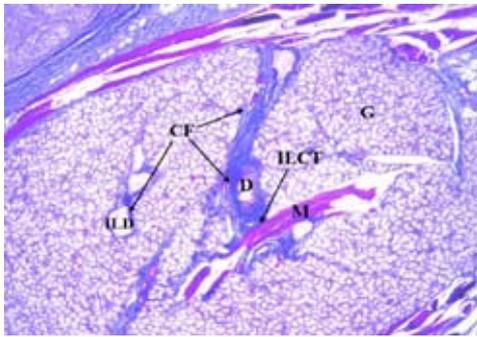


Fig. 19. Photomicrograph showing Collagen fibres (CF) and Muscle fibres (M) in the interlobular connective tissue (ILCT) of the glands (G) of the soft palate of pig. D- Interlobular duct, ILCT- Interlobular connective tissue.

Crossman's Trichrome method x40

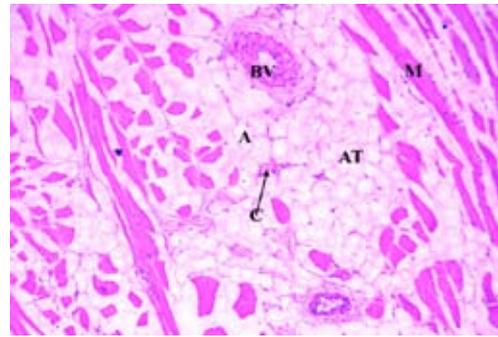


Fig. 20. Photomicrograph showing the adipose tissue (AT) in between irregularly arranged muscle fibres (M) of tunica muscularis of the soft palate of pig. A- Adipocyte, BV- Blood vessel, C- Capillary.

Haematoxylin and Eosin method x 100

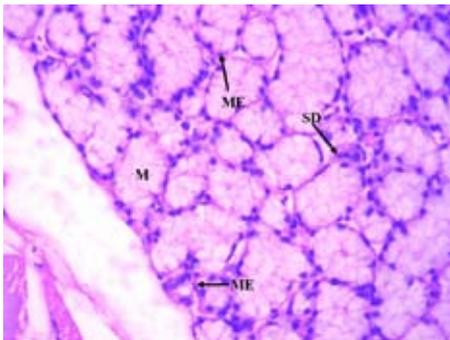


Fig. 21. Photomicrograph showing the mucous acini (M) and serous demilunes (SD) in the glands of the soft palate of pig. ME- Myoepithelial cell.

Haematoxylin and Eosin method x400

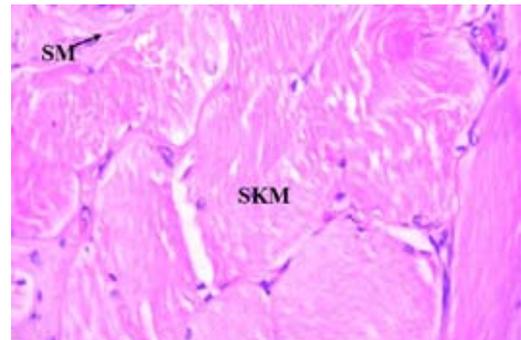


Fig. 22. Photomicrograph showing the skeletal muscle fibres (SKM) and smooth muscle fibres (SM) of tunica muscularis of the soft palate of pig.

Haematoxylin and Eosin method x 400

in rabbits the interlobular glandular duct was lined by simple squamous epithelium (Mercer, 1900 and Obead et al., 2022). Individual secretory acini were separated by a thin connective tissue network, which concurs with the findings of Obead et al. (2022) in guinea pigs.

The mucous acini were lined by columnar or polygonal cells resting on a basement membrane, with oval or flattened, darkly stained nuclei compressed against the base of the cells. Serous acini were smaller and lined by pyramidal cells with centrally located spherical nuclei. Mucous acini possessed larger lumen, while the serous acini had a smaller lumen (Fig.17). Some of the serous cells were present along with the mucous acini as crescent shaped demilunes. Flat, star shaped myoepithelial cells were observed between the secretory acinar cells and the basement membrane (Fig.21). These observations concur with the findings of Eurell and Frappier (2006) in domestic animals, Arrighi et al. (2011) in dogs and Kumar & Singh (2014) in sheep. In the present study subepithelial portion of the proprio-submucosa on the nasal surface showed glands in the form of isolated acini as reported by Arrighi et al. (2011) and Pichetto et al. (2015) in dogs.

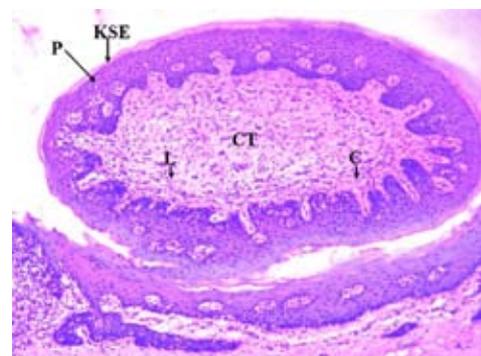


Fig. 23. Photomicrograph showing the keratinized stratified squamous epithelium (KSE) of the papilla (P) of the soft palate of pig. CT- Connective tissue, C- Capillary, L- Lymphocyte.

Haematoxylin and Eosin method x100

Tunica muscularis

The tunica muscularis formed the central core of the soft palate, which is consistent with the observations of Eurell & Frappier (2006) in domestic animals. This layer was thick in the middle and became thin at both ends, with no muscle tissue at the fixed (rostral) end, similar to the findings of Richardson et al. (2006) in equines. In the present study, the muscle bundles were irregularly

arranged (Fig. 20), whereas in adult cats the muscle fibres were longitudinal at the free end and oblique at the fixed end (Mercer, 1900). The tunica muscularis consisted predominantly of skeletal muscle fibres along with a few smooth muscle fibres (Fig. 22). According to Cleaton-Jones (1975), striated muscle was found only at the uvula region and posterior half of the soft palate in monkeys, while in albino rats a circular layer of striated muscle was found only at the nasopharyngeal hiatus (Cleaton-Jones, 1971).

Papillae

In the present study, the midline of the oral surface of the soft palate showed several oval islands or papillae lined by keratinized stratified squamous epithelium, consistent with the findings of Klein & Schroeder (1979) and Cleaton-Jones (1975) in monkeys. These papillae facilitate the passage of food into the oral cavity, as noted by Eurell & Frappier (2006) in domestic animals. The connective tissue core of the papillae contained lymphocytes, blood vessels and few collagen fibres, but no taste buds were observed (Fig. 23). In contrast, taste buds have been reported in the papillae of monkeys (Klein & Schroeder, 1979; Cleaton-Jones, 1975) and albino rats (Cleaton-Jones, 1971). The epithelial lining of the papillae resembled the oral epithelium of the soft palate, except for the presence of an additional keratin layer without any nucleus.

Conclusion

The histomorphological organization of the soft palate in the domestic pig reflects its functional role in swallowing and respiration. Regional variation in epithelial lining provides protection on the oral surface and facilitates respiratory function on the nasal surface. The well-developed propria-submucosa with abundant mucous glands contributes to lubrication and protection of the mucosa. The centrally placed tunica muscularis enables effective movement and flexibility of the soft palate. Keratinized papillae on the oral surface assist in directing the food bolus. These structural adaptations support efficient palatal function in the domestic pig.

Conflict of interest

The authors declare that they have no conflict of interest.

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