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Histological Studies on the Non-Osseous component of the Hard Palate in Domestic Pig (*Sus scrofa domesticus*)

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ABSTRACT

The bony palate along with the mucosa covering its oral surface together constitutes the hard palate. The microanatomical studies of the non-osseous component of the hard palate showed the keratinized stratified squamous epithelium and propria-submucosa. The propria-submucosa was composed of dense irregular connective tissue containing collagen and reticular fibers, adipose tissue, smooth muscle cells, nerve fibers, blood vessels, capillary plexuses, lymphatic vessels and connective tissue cells like lymphocytes, fibroblasts and fibrocytes. The highest vascularity was observed at the papilla incisiva level, where the nasopalatine duct and plate-like hyaline cartilage surrounded by a perichondrium were also present. Notably, no glands were observed at any level of the non-osseous component of the hard palate in domestic pigs.

Key words: Non-osseous component of hard palate, papilla incisiva, propria submucosa.

INTRODUCTION

Pig is one of the earliest domesticated animals, having been domesticated over 7000 years ago (Rothschild and Ruvinsky, 1999), and it has a wide geographical distribution with nearly 500 breeds worldwide. Physiologically and anatomically, several organ systems of pigs, including the heart, circulatory system and alimentary tract, as well as dietary habits and dental arrangement, closely resemble those of humans (Bustad, 1966). As an initial step toward clinical application in tissue repair and oral surgery, the feasibility of a laser-based, suture-free wound closure technique was evaluated using the porcine palate (Birkelbach *et al.*, 2020). The existing literature on the histology of the non- osseous component of the hard palate in domestic pigs is limited. Therefore, the present study was undertaken to examine the microanatomical features of this region.

MATERIALS AND METHODS

For microanatomical studies, fresh specimens of the non-osseous component of the hard palate were collected from 12 adult domestic pigs immediately after slaughter.The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) videRef. No.281/go/ Rebi/S/2000/CPCSEA/CVSc/TPTY/001/ VeterinaryAnatomy/2023 dated 8.05.2023. Tissue samples from the non-osseous component of the hard palate were collected at various levels i.e., at first incisor, third incisor, canine, fourth premolar, and third molar teeth. The samples were fixed in 10% Neutral Buffered Formalin, washed thoroughly, dehydrated through graded alcohols and cleared in xylene (Bancroft and Gamble, 2008 and Singh and Sulochana, 1997). The tissues were then impregnated with paraffin wax (59-60°C) using a hot air oven and embedded in paraffin

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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 fibers, Gomori's method for reticular fibres, Bielschoswky method for nerve fibres (Singh and Sulochana, 1997, Bancroft and Gamble, 2008).

RESULTS AND DISCUSSION

Epithelium

In the present study, the non-osseous component of the hard palate consisted of epithelium and propria-submucosa (Fig.1). The tunica mucosa of the hard palate was lined by keratinized stratified squamous epithelium throughout its length, extending from the level of the first incisor tooth to the last molar tooth (Fig.1) as reported by Eurell and Frappier (2006) in domestic animals, Mahdy *et al.* (2018) in Egyptian goats, Madkour *et al.*(2021) in Rahmani sheep, Farrag *et al.* (2021) in buffaloes and Ari and Uslu (2019) in Anatolian bobcats. In contrast, the hard palate of rabbits was lined by parakeratinized stratified squamous epithelium (Mahdy and Mohammed, 2021). In the present study, the thickness of the epithelium remained uniform throughout the hard palate. Contrary to this observation, the oral surface was reported to be more keratinized than the aboral surface of the hard palate in buffaloes (Farrag *et al.*, 2021), while in rabbits (Mahdy and Mohammed, 2021) the epithelial thickness gradually decreased caudally. At the junction between the epithelium and connective tissue, inter papillary pegs of varying widths and heights were observed (Fig.1), which concurs with the findings of Halata *et al.* (1999) in goats, Halata and Baumann (1999) in rhesus monkeys, Mahdy *et al.* (2018) in Egyptian goats and Ari and Uslu (2019) in Anatolian bobcats. The opening of the incisive duct, observed within the fissure of papilla incisiva, was lined by non-keratinized stratified squamous epithelium (Figs.2,3), similar findings were also reported by Abood (2017) in indigenous buffalo.

In the present study the keratinized stratified squamous epithelium showed five different layers i.e., stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale, from superficial to deep layers. The deepest layer resting on the basement membrane was stratum basale which consisted of a single layer of columnar cells with vertically oriented nuclei and basophilic cytoplasm. The stratum spinosum consisted of five to six rows of polyhedral cells with round to oval, darkly stained nuclei and basophilic cytoplasm. The superficial cells of this layer appeared flattened with spindle shaped nuclei and less basophilic cytoplasm. The stratum granulosum contained of one or two layers of spindle shaped cells with elongated nuclei and granular eosinophilic cytoplasm. The stratum lucidum appeared as a translucent layer composed of flattened anucleated cells with indistinct boundaries. The stratum corneum, outermost layer of the epithelium consisted of flattened cells without nucleus and eosinophilic cytoplasm (Fig.4). These observations concur with the findings of Eurell and Frappier (2006) in domestic animals.

Propria-submucosa

The propria submucosa formed the thickest layer of the hard palate and extended into the interpapillary pegs of the epithelium. It consisted

of dense irregular connective tissue (Fig.6), formed by the collagen fibers, reticular fibers, adipose tissue, nerve fibers, blood vessels, lymphatic vessels and connective tissue cells like smooth muscle cells, lymphocytes, fibroblasts and fibrocytes (Figs.1,5,10). These findings were similar to the reports of Madkour *et al.* (2021) in Egyptian goats, Eurell and Frappier (2006) in the domestic animals and Mahdy and Mohammed (2021) in domestic rabbits. In the present study, smooth muscle cells were observed as isolated bundles located beneath the epithelial layer. A densely distributed capillary plexuses were also observed in this layer, which were in different sizes as reported (Fig.4) by Eurell and Frappier (2006) in the domestic animals. In the present study, the highest vascularity was observed in the connective tissue at the papilla incisiva level (Fig.11). Contrary to this, maximum vascularity was observed at the first premolar tooth level in Rahmani sheep (Madkour *et al.*, 2021). Collagen fibers were widely distributed throughout the propria submucosa and interpapillary pegs and were arranged in different directions (Figs.6,7). Elastic fibers were found only in the walls of blood vessels (Fig.8). Reticular fibers were observed at the basement membrane of the epithelium, and also in interpapillary pegs (Fig.9). These findings were similar to the reports by Mahdy *et al.* (2018) in Egyptian goats and Ari and Uslu (2019) in Anatolian bobcats. In the present study, no glands were observed at any level of the hard palate which concurs with the findings of Trautmann and Fiebiger (1952), Banks (1993), Eurell and Frappier (2006) and Bacha and Bacha (2012) in pigs. In contrast, Nickel *et al.* (1979) reported the presence of glands in the cranial region of the hard palate in pigs. According to Trautmann and Fiebiger (1952), Banks (1993), Eurell and Frappier (2006), the cranial region of the hard palate in most domestic animals lacks glands, while the caudal region contains numerous branched tubuloalveolar mucous or mixed glands. The nasopalatine duct and hyaline cartilage were observed within the propria-submucosa of the papilla incisiva. A plate like hyaline cartilage surrounded by a perichondrium, comprising an inner chondrogenic cellular layer and an outer fibrous layer, was present. The cartilage matrix was composed of numerous isogenous groups or cell nests (Fig.12). These findings were similar to the reports of Eurell and Frappier (2006) in domestic animals. A vast amount of irregularly distributed adipose tissue was observed in the propria-submucosa of the hard palate (Fig.5), which is in agreement with Mahdy *et al.* (2018) in Egyptian goats and Farrag *et al.* (2021) in buffaloes. In contrast, Copenhaver and Johnson (1958) and Kelly *et al.* (1984) reported that the anterior portion (fatty zone) of the hard palate contained abundant adipose tissue in humans.

The present study revealed that the non-osseous component of the hard palate in domestic pigs shows well-defined histo-morphological features. It is lined by a uniformly thick keratinized stratified squamous epithelium and supported by a dense, highly vascular connective tissue layer rich in collagen fibers, adipose tissue, nerves, and blood vessels. The absence of glands and the presence of the nasopalatine duct and hyaline cartilage in the papilla incisiva reflect species-specific structural adaptations. These features highlight the functional importance of the hard palate in mastication and oral protection and the findings may serve as baseline microanatomical data for experimental studies using porcine models.

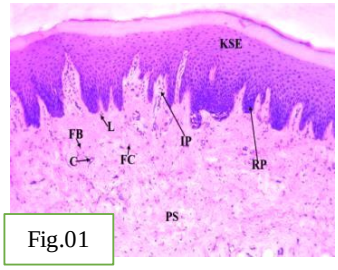
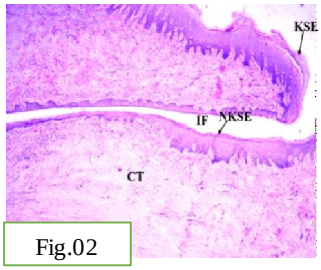


Fig.01



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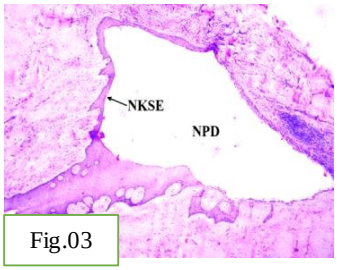

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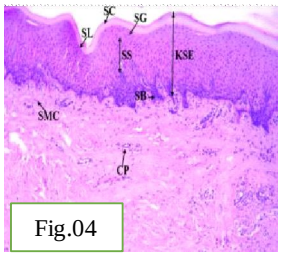

Fig.04

Fig.1 Photomicrograph of the hard palate of pig showing keratinized stratified squamous epithelium (KSE) and propria-submucosa (PS).C- Capillary, FC- Fibrocyte, FB- Fibroblast, IP- Inter papillary peg, L- Lymphocyte, RP- Rete pegs. Haematoxylin and eosin method x100 , **Fig.2** Photomicrograph of the hard palate of pig at papilla incisiva level showing the incisive fissure (IF). C- Connective tissue, KSE- Keratinized stratified squamous epithelium, NKSE- Non-keratinized stratified squamous epithelium. Haematoxylin and eosin method x100, **Fig.3** Photomicrograph of the hard palate of pig at papilla incisiva level showing the nasopalatine duct (NPD) lined by non-keratinized stratified squamous epithelium (NKSE). Haematoxylin and eosin method x100, **Fig.4** Photomicrograph of the lining epithelium (KSE) of the hard palate of pig showing stratum corneum (SC) stratum lucidum (SL) stratum granulosum (SG) stratum spinosum (SS) and stratum basale (SB). CP- Capillary plexus, SMC- Smooth muscle cells. Haematoxylin and eosin method x100

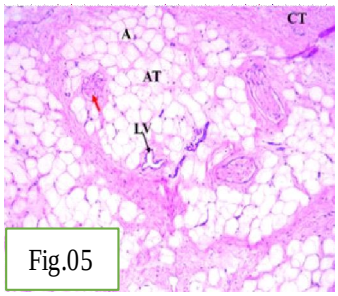

Fig.05

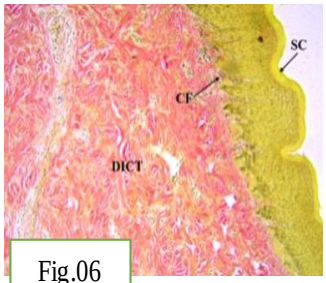

Fig.06

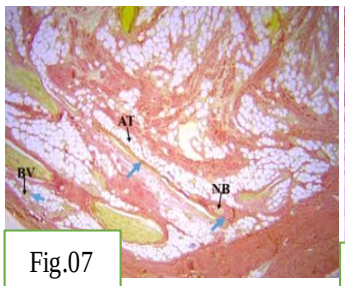

Fig.07

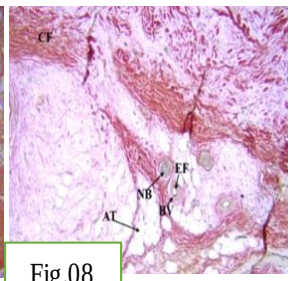

Fig.08

Fig.5 Photomicrograph of propria-submucosa of hard palate of pig showing the connective tissue (CT), adipose tissue (AT), lymph vessel (LV) and nerve bundles (Red arrow). A- Adipocyte. Haematoxylin and eosin method x100, **Fig.6** Photomicrograph of the propria-submucosa of the hard palate of pig showing the dense irregular connective tissue (DICT).CF- Collagen fibers in the Interpapillary pegs, SC- Stratum corneum of Epithelium. Van Gieson's method x100, **Fig.7** Photomicrograph of the propria submucosa of the hard palate of pig showing the collagen fibers (Blue arrows) surrounding the adipose tissue (AT), blood vessel (BV) and nerve bundle (NB). Van Gieson's method x40 , **Fig.8** Photomicrograph of the propria submucosa of the hard palate of pig at papilla incisiva level showing the elastic fibers (EF) in the walls of blood vessel (BV). AT- Adipose tissue, CF- Collagen fibers, NB- Nerve bundle. Verhoeff's method x100

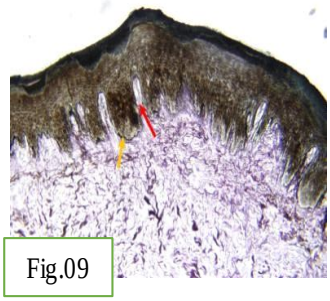

Fig.09

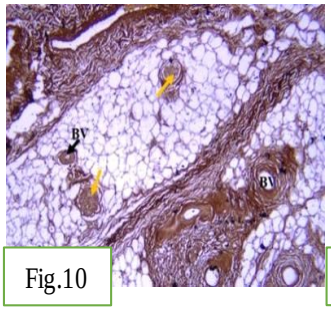

Fig.10

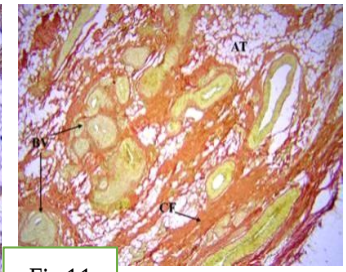

Fig.11

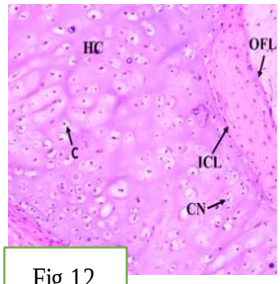

Fig.12

Fig.9 Photomicrograph of the hard palate of pig showing the Reticular fibers in the basement membrane of the epithelium (Yellow arrow) and in the interpapillary pegs (Red arrow). Gomori's Reticulum method x 40, **Fig.10** Photomicrograph of the propria-submucosa of the hard palate of pig showing the nerve bundles (Yellow arrow) and blood vessels (BV). Bielchowsky method x 100, **Fig.11** Photomicrograph of the propria submucosa of the hard palate of pig showing more vascularity (BV) at papilla incisiva level. AT- Adipose tissue, CF- Collagen fibers. Van Gieson's method x40, **Fig.12** Photomicrograph of the propria submucosa of the hard palate of pig at papilla incisiva level showing the hyaline cartilage (HC). C- Chondrocyte in lacunae, CN- Cell nest, ICL- Inner cellular layer, OFL- Outer fibrous layer. Haematoxylin and eosin method x400

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