

HISTOMORPHOCHEMICAL STUDY OF EYELID AND ASSOCIATED GLANDS OF PIG

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ABSTRACT

The present study was conducted on six healthy young pigs of local mixed breed of either sex to study the histomorphology and histochemistry of the eyelid and associated glands of pig. Tarsal or Meibomian glands were present in both upper and lower eye lids. Tarsal glands were located in tarsal plate and plate was shorter in lower eye lid as compared to upper. Meibomian glands were compound tubuloalveolar, holocrine modified sebaceous glands. The glandular acini were separated from each other by connective tissue especially collagen and elastic fibers along with blood vessels. These glands were more in number and size in upper eye lid as compared to lower eye lid. Ciliary glands or gland of Moll were simple coiled tubular modified sweat glands which were apocrine in nature. Ciliary glands were more in number as compared to tarsal glands. Zeis glands were present around the margins of eye lids. Accessory lacrimal gland or gland of Wolfring was observed in upper eye lid. Histochemical studies of all the glands showed very weak presence of neutral mucopolysaccharides, weakly acidic sulfated mucosubstances, sialomucins and hyaluronic acid. The goblet cells of palpebral conjunctiva showed positive reaction for mucopolysaccharides and other mucosubstances.

Keywords: Ciliary gland, Histology, Histochemistry, Meibomian gland, Pig, Zeis glands

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The eyeball is protected and its surface moisture is maintained by eyelids. The upper and lower eyelids of mammals are comprised of external (Skin) and internal surface (palpebral conjunctiva). The eyelashes along with Meibomian glands (tarsal glands), glands of Moll or ciliary glands (specialized apocrine glands) and glands of Zeis (sebaceous glands) constitute the histo-architecture of eyelids. The tear film comprises of three layers: the lipid layer produced by the Meibomian glands and Zeis glands, the aqueous layer derived from the major lacrimal glands and the accessory lacrimal gland (glands of Krause and Wolfring), and the mucinous layer predominantly from the goblet cells of the conjunctiva. The secretion of tarsal gland is responsible for spreading the tear on the cornea and prevents evaporation of the eye's tear film (Al-Rikabi, 2015). Dysfunctional gland often causes dry eyes, one of the most common eye conditions. The glandular secretions of the porcine ciliary glands may play an essential role in the protection against environmental pathogens not only in the eyelid skin, but also on the ocular surface (Yasui *et al.*, 2006). The histology and histochemistry of the Meibomian and ciliary glands so far has been studied in cattle (Ahmed, 2012), Philippine water buffalo (Maala *et al.*, 2009), dromedary camel (Al-Ramadan, 2015), calf (Yuksel *et al.*, 2005), sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021) and South African painted dog (Paszta *et al.*, 2021). Keeping in view the pathophysiological significance of these glands, the present study was undertaken to elucidate the histological and histochemical findings of Meibomian glands, Wolfring's or Ciaccio's glands and ciliary glands in pig.

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MATERIALS AND METHODS

The present study was conducted on 06 healthy young pigs of either sex of 6-8 months of age, of local mixed breed. The heads were procured from local slaughter house immediately after decapitation and the tissues were collected from the upper and lower eyelids. The tissues were fixed in a 10% neutral buffered formalin solution for 48 hours, subjected to routine tissue processing for light microscopic examination and embedded in paraffin blocks. The paraffin sections (5-6 m) were made through the upper and lower eyelids of left as well as right eyes and stained with routine Harris' hematoxylin and eosin stain for general histomorphological examination, Gomori's stain for reticular fibres, Weigert's method for elastic fibres (Luna, 1968) and Crossman's trichrome stain for collagen fibres (Crossman, 1937). For histochemical demonstration of mucopolysaccharides using McManus' method, Periodic Acid Schiff-Alcian blue method, Alcian blue (pH 2.5) method, Colloidal iron method and Mayer's mucicarmine method (Luna, 1968).

RESULTS AND DISCUSSION

The upper and lower eyelids of the pig were comprised of anterior (Skin) and posterior surface. The skin surface of the upper as well as lower eye lids were covered by a thin layer of stratified squamous epithelium with keratinization (Fig. 1). These results were in accordance with the findings in sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021), South African painted dog (Paszta *et al.*, 2021), local Iraqi breed goats (Al-Rikabi, 2015). The epithelium was 6-7 cell layered. The thickness

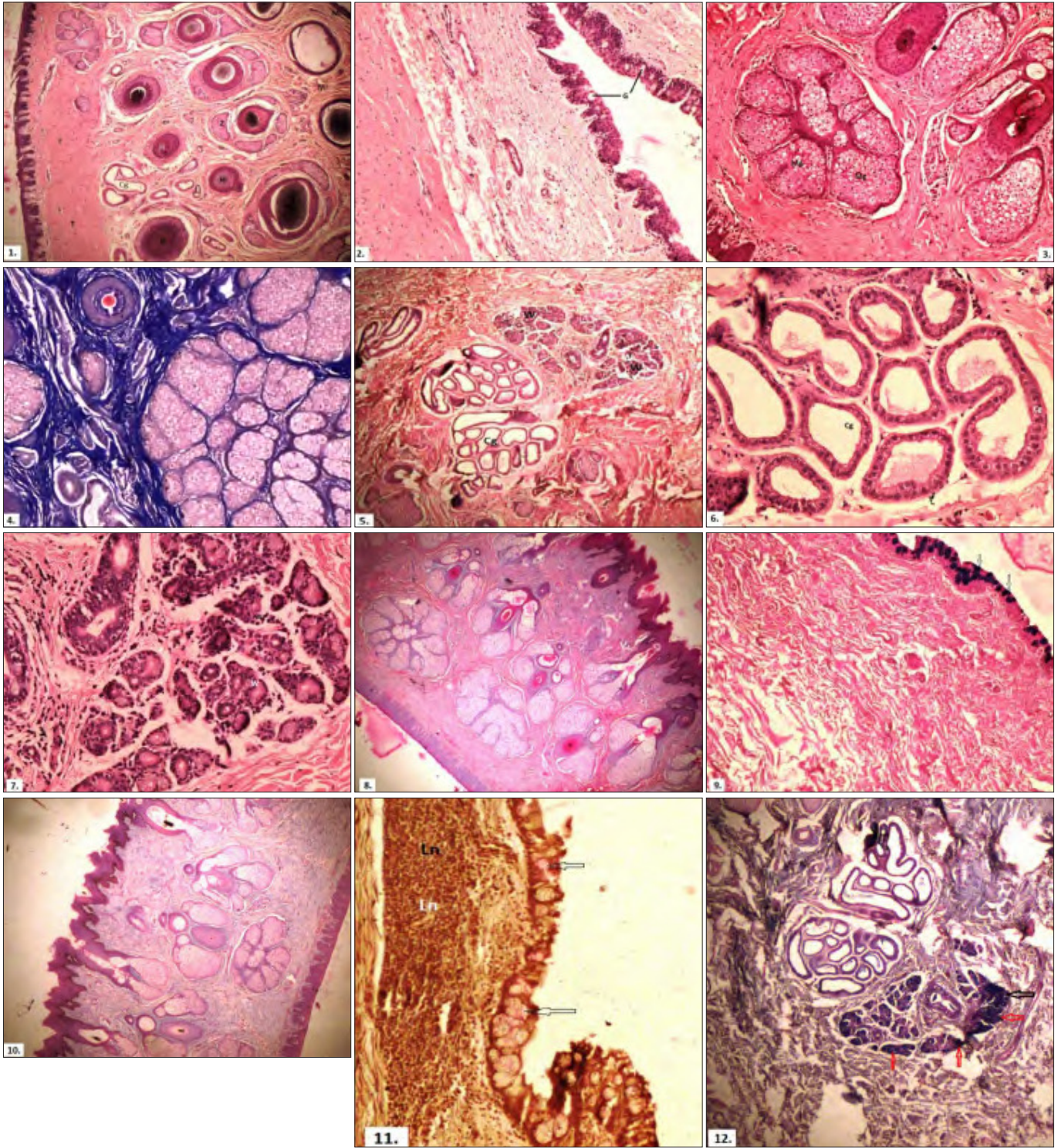
of epithelium varied at places. The upper margin of both the eyelids was having slightly more thick epithelium as compared to the lower margins. The undulating dermal papillae were present on both margins (Fig. 1) but the dermal papillae were uniform towards lower margin of both eye lids whereas, the dermal papillae of upper margin showed irregular appearance. The posterior aspect of eye lids was having non keratinized squamous epithelium. Towards basal surface of the epithelium melanocytes were observed as observed by Paszta *et al.* (2021) in South African painted dog. The epithelium was changed to pseudostratified columnar epithelium with large goblet cells towards the conjunctiva (Fig. 2). Similar findings were reported in sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021) and local Iraqi breed goats (Al-Rikabi, 2015). Whereas stratified squamous epithelium was observed at the palpebral edges in horse (Bourges-Abella *et al.*, 2007). The dense irregular connective tissue was found directly beneath the epidermis (Fig. 1). These results were in accordance with the findings in South African painted dog (Paszta *et al.*, 2021). The connective tissue was consisted of collagen fibres with numerous fibroblasts, diffuse lymphocytes and bundles of skeletal muscles fibres of orbicularis oculi muscle were present within the connective tissue of both eyelids as observed by Parkash *et al.* (2021) in goat. The eyelashes with hair follicles were in dermis in both eye lids (Fig. 1). The hair follicles were more towards the upper eye lid as compared to lower eye lids. Whereas, eyelashes were absent in the lower eyelids in the African wild dog (Paszta *et al.*, 2021). The bulbs of upper eyelash with sweat glands surrounded the hair follicles were present. The sebaceous glands (Zeis glands) and muscle fibres of orbicularis oculi muscles were observed at the anterior margins of both the eyelids (Fig. 1). The Zeis glands were present surrounded the bulb of hair follicle. Similar findings were reported in in sheep (Singh *et al.*, 2020).

The tarsal plate was observed which was made up of dense connective tissue (Fig. 1) consisted of rows of Meibomian glands or tarsal glands as reported earlier by Paszta *et al.*, 2021 in South African painted dog. These were modified sebaceous glands which were holocrine in nature. Similar findings were reported in porcine (Crespo-Moral *et al.*, 2020). The glandular acini of tarsal glands were compound tubuloalveolar in nature. These findings were in fully agreement with the reports in sheep (Singh *et al.*, 2020). The glandular cells (meibocytes) were having centrally placed round nuclei and eosinophilic cytoplasm (Fig. 3). The acini were separated from each other by connective tissue especially collagen (Fig. 4) and elastic fibers which were arranged densely in various directions.

The tarsal plate was short in lower eye lid and number and size of the tarsal glands were observed to be slightly less as compared to the upper eye lid. These results were in accordance with the findings in sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021) and South African painted dog (Paszta *et al.*, 2021). The blood vessels were also observed in the connective tissue.

The ciliary glands or glands of Moll were also observed which were modified sweat glands and apocrine in mode of secretion (Fig. 5). These were simple coiled tubular glands and lined with simple cuboidal epithelium with large irregular lumen (Fig. 6). These findings were in fully agreement with the reports in sheep (Singh *et al.*, 2020), South African painted dog (Paszta *et al.*, 2021). The myoepithelial cells surrounded the secretory acini of glands. Similar findings were reported in pig (Yasui *et al.*, 2006). The epithelium of the excretory ducts of ciliary gland was bistratified. In present study ciliary glands were more in number as compared to the tarsal glands. These results were in accordance with the findings in pig (Yasui *et al.*, 2006). Towards the upper eye lid scattered population of accessory lacrimal glands were also observed which were also known as glands of Wolfring's or Ciaccio's glands (Figs. 5, 7).

Histochemically the PAS-AB reaction was very weak in the Meibomian glands and ciliary glands although epithelium showed some activity for mucopolysaccharides (Fig. 8). These findings were in fully agreement with the reports dromedary camel (Al-Ramadan, 2015). The goblet cells in pseudostratified columnar epithelium of conjunctiva showed strong reaction for presence of predominantly acidic mucopolysaccharides (Fig. 9). The PAS activity was also observed very weak in both Meibomian and ciliary glands which indicated the negligible amount of glycogen (Fig. 10). However, a periodic acid-Schiff (PAS) negative reaction was observed in the secretory units of the Meibomian and ciliary glands as reported in sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021) and Philippine water buffalo (Maala *et al.*, 2009). The alcian blue activity was also very weak in all glandular population of the upper eyelid which indicated the very weak presence of weakly acidic sulfated mucosubstances, hyaluronic acid and sailomucins by Alcian blue method. These results were in accordance with the findings in pig (Yasui *et al.*, 2006). The activity of Mayer's mucicarmine also showed negative reaction towards all glandular cells of Meibomian and ciliary glands as observed earlier by Ahmed (2012) in cattle. However, the goblet cells in the conjunctiva and third eyelid region showed strong reaction (Fig. 11). These results were in accordance with the findings in dromedary camel (Al-



Figs. 1-12. (1) Photomicrograph of eyelid of pig showing various histological layers (E = epidermis; H=hair follicles; Z=Zeis glands; Cg= ciliary/Moll glands, Mf = muscle fibres of orbicularis oculi; Mg= Meibomian/tarsal glands; T= Tarsal plate). H & E×40; (2) Photomicrograph showing the palpebral conjunctiva (C) lined with pseudostratified columnar epithelium having numerous goblet cells (G) on the inner surface of the eyelids. (H & E x 100), (3) Higher magnification showing the Meibomian glands (Mg) arranged in grape-like clusters attached to a central stalk. H & E×200; (4) Photomicrograph showing the connective tissue mainly made up of collagen fibres (blue colour). Crossman Trichrome ×200; (5) Photomicrograph showing the modified apocrine (ciliary) glands (Cg) and glands of Wolfring's or Ciaccio's glands (W). H & E x40; (6) Higher magnification showing the secretory portion of ciliary glands (Cg) comprised of large irregular lumen and lined with cuboidal epithelial cells. H & E x200. (7) Higher magnification showing the secretory portion of glands of Wolfring's or Ciaccio's glands (W). H & E x200; (8) Photomicrograph of eyelid of pig showing a very weak PAS-AB reaction for both neutral and acid mucopolysaccharides in the secretory units of the Meibomian glands and ciliary glands; however epithelium showed activity for mucopolysaccharides. PAS-AB ×40; (9) Photomicrograph showing a very strong positive reaction for mucopolysaccharides in the goblet cells (arrow) towards the palpebral conjunctiva. PAS-AB ×100; (10) Photomicrograph of eyelid of pig showing the weak PAS reaction in the Meibomian glands and ciliary glands indicating the presence of negligible amounts of glycogen. PAS x40; (11) Photomicrograph showing a positive reaction for mucosubstances in the goblet cells (arrow) towards the palpebral conjunctiva. Mayer's Mucicarmine ×100; (12) Photomicrograph showing a very strong PAS-AB positive reaction for mucopolysaccharides in the accessory lacrimal glands (arrow) (glands of Wolfring's or Ciaccio's glands). PAS-AB ×40.

Ramadan, 2015), sheep (Singh *et al.*, 2020), goat (Parkash *et al.*, 2021) and South African painted dog (Paszta *et al.*, 2021). The accessory lacrimal glands though showed strong activity towards PAS-AB (Fig. 12), PAS and Alcian blue stains.

Organized lymphoid follicles were observed towards palpebral conjunctiva of both upper and lower eyelids (Fig. 11) which formed the Conjunctiva-associated lymphoid tissue (CALT). Goblet cells were scarce or even absent in the areas of the lymphoid follicle. Similar findings were reported in porcine (Crespo-Moral *et al.*, 2020), sheep (Singh *et al.*, 2020) and goat (Parkash *et al.*, 2021). However, CALT was in the form of several lymphoid follicles on the palpebral surface of the eyelids of dromedary camel (Al-Ramadan, 2015). Whereas the presence of a single conjunctival lymph nodule aggregate was seen in the orbital zone of the lymphoid region in the lower eyelids in South African painted dog (Paszta *et al.*, 2021).

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