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Research Article

HISTOMORPHOCHEMICAL CHARACTERIZATION OF MEIBOMIAN AND CILIARY GLANDS OF SHEEP (*OVIS ARIES*)

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ABSTRACT

The present study was conducted on five healthy young sheep of local mixed breed of either sex to study the histomorphology and histochemistry of the meibomian and ciliary glands of sheep. The meibomian glands were of compound tubuloalveolar type consisting of linear arrays of sebaceous glands in the tarsal plates of the eyelids; however the ciliary glands were of simple coiled tubular type. The lobules of the meibomian glands consisted of clusters of secretory glandular cells (meibocytes) of various sizes, resting on a basement membrane surrounded by a thin connective tissue sheath. The meibocytes have a vacuolar cytoplasm and their oval nuclei were situated at centre of the cell. The peripheral meibocytes were smaller, whereas central cells were larger and polyhedral in nature. The meibocytes were connected to a central duct, lined by stratified squamous epithelium, which directly opened at the muco-cutaneous junction of the eyelid. The secretory portion of the ciliary glands comprised of large irregular lumen lined with cuboidal epithelial cells having eosinophilic cytoplasm. The apical surface of these cells showed varying cytoplasmic protrusions; however, the ducts of these glands were lined by stratified cuboidal epithelium and were directly associated with hair follicles. Histochemical studies revealed the absence of neutral mucopolysaccharides, weakly acidic sulfated mucosubstances, sialomucins and hyaluronic acid in the secretory units of both meibomian and ciliary glands.

Keywords: Ciliary gland, Histology, Histochemistry, Meibomian gland, Sheep

The meibomian gland, also known as tarsal gland, is a holocrine type of exocrine gland, located at the rim of the upper and lower eyelids inside the tarsal plate, responsible for the supply of meibum, an oily substance that prevents evaporation of the eye's tear film. Dysfunctional meibomian glands often cause dry eyes, one of the most common eye conditions. The ciliary glands, also known as glands of Moll, function is unknown. Moll glands have apocrine secretion and leave their secretions inside the eyelash follicles (McGavin *et al*., 2001). The tear film comprises of three layers: the superficial lipid layer produced by the meibomian glands and Zeis glands, the middle aqueous layer derived from the major lacrimal glands and the accessory lacrimal glands of Krause and Wolfring, and the inner mucinous layer predominantly from the goblet cells of the conjunctiva (Argueso and Gipson, 2001). So far, a contribution to the tear film from the ciliary glands and from the surface epithelium is not mentioned in the literature, however; the glandular secretions of the porcine ciliary glands may play an essential role in the preservation of humidity and the protection against environmental pathogens not only in the eyelid skin, but also on the ocular surface (Yasui *et al*., 2006). In certain mammals, recent studies have demonstrated that the apocrine glandular secretions contain antimicrobial substances such as lysozyme and defensins (Yasui *et al*., 2006).

Although, the histology and histochemistry of the meibomian and ciliary glands has been studied in brown brocket deer (Ajmat *et al*., 2004), calf (Yuksel *et al*., 2005), pig (Yasui *et al*., 2006), African black ostrich (Kleckowska-Nawrot *et al*., 2016), there is a lack of literature on the light microscopic details of the meibomian and ciliary glands in young sheep and its comparison with other domestic animals. Keeping in view the importance of these glands, the present study describes the histological and histochemical findings of Meibomian and ciliary glands in young sheep.

MATERIALS AND METHODS

The upper and lower eyelids used in the present study were obtained from five healthy young sheep of local mixed breed of either sex. The heads were procured from local slaughter house immediately after decapitation and 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 μm) 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). In addition, selected sections were processed 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).

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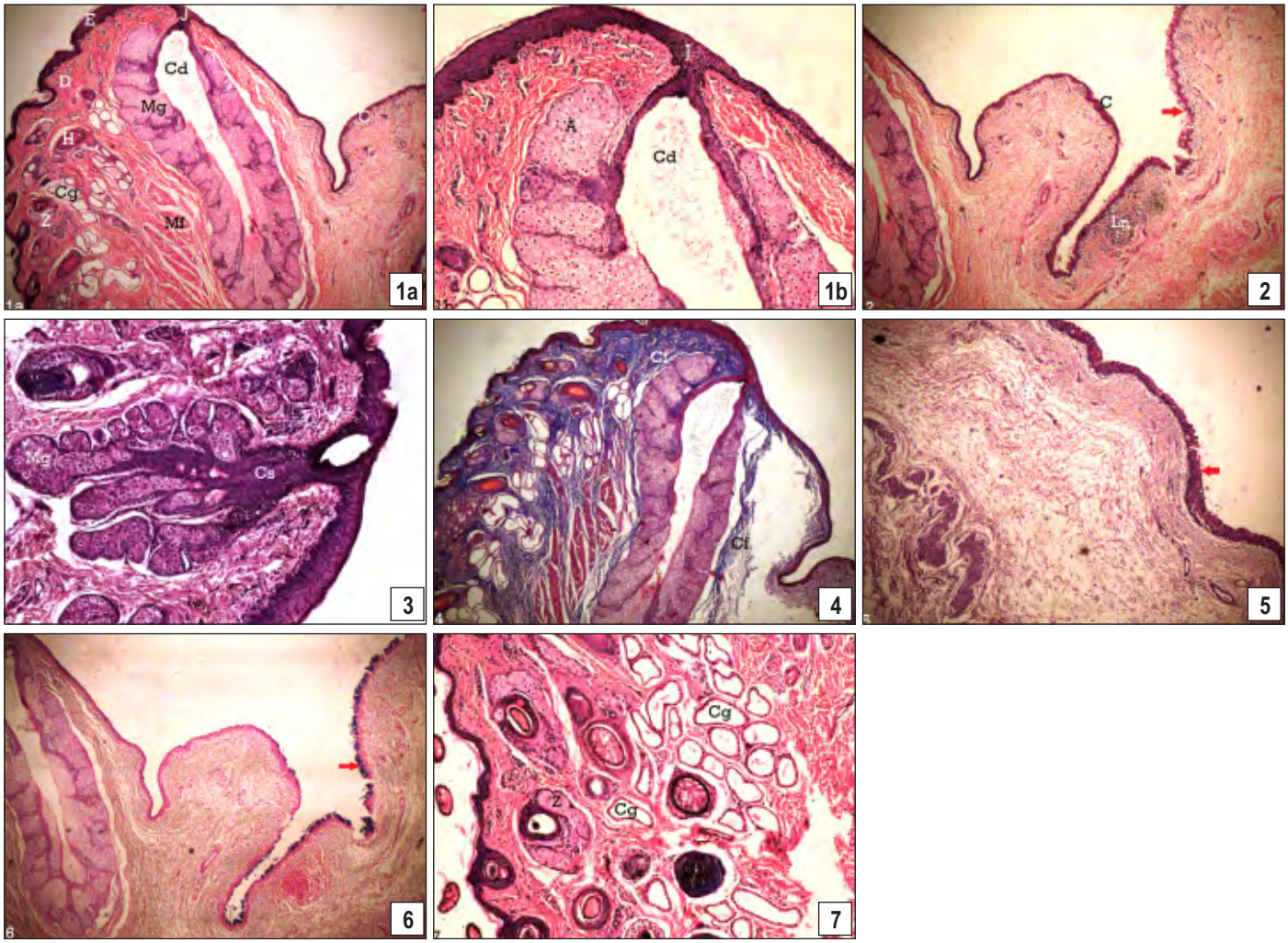
RESULTS AND DISCUSSION

Histologically, the eyelids consisted of four main layers, from exterior to interior, viz., skin (epidermis and dermis), muscular layer, tarsal layer and palpebral conjunctiva. The epidermal layer comprised of stratified squamous keratinized epithelium, and a dermis of loose collagenous connective tissue along with hair follicles. Associated with these hair follicles, were small sebaceous (Zeis) glands and modified apocrine sweat (Moll) glands (Fig. 1a). Similar findings were reported in mammals (McGavin *et al*., 2001). However, the porcine upper eyelid contained prominent apocrine ciliary glands, sebaceous glands and less-abundant tarsal glands (Yasui *et al*., 2006). The muscular layer was comprised of fascicles of striated muscle fibres of orbicularis oculi muscle. Next to this layer, was the tarsal plate, which contained large sebaceous, tarsal (meibomian) glands with alveoli emptying into long central ducts that opened at the free edge of the eyelids (Fig. 1b). While in avian eyelids sebaceous glands are absent but the epidermal epithelium as a whole produces the phospholipids and neutral fats usually produced by these glands (Stettenheim, 2000). The conjunctiva (palpebral) lined the inner surface of the eyelids, where the epithelium was gradually changed from stratified squamous to pseudostratified columnar having numerous goblet cells. Lymphatic nodules were located in the subconjunctival tissue towards the palpebral conjunctiva and these were termed as conjunctiva-associated lymphoid tissue (CALT) (Fig. 2). CALT seems to play an important role in the protection of ocular surface by initiating and regulating immune responses (Steven and Gebert, 2009). Saeed (2015) reported that the several lymphoid follicles were present on the palpebral surface of the eyelids of camel, specifically near the medial canthus. Kleckowska-Nawrot *et al*. (2016) also showed the presence of organized lymphoid follicles only in the lower eyelid of red kangaroo. Organized conjunctiva-associated lymphoid tissue, consisting of a well-developed lymphoid follicle in intimate contact with palpebral epithelium, was reported in mammals such as humans (Knop and Knop, 2000) and rabbits (Liu *et al*., 2005). In birds, the CALT and the Harderian gland form the head-associated lymphoid tissue (HALT) (van Ginkel *et al*., 2012). CALT was also described as solitary or aggregate lymphoid follicles in Angora goats, Japanese monkeys and domestic animals (Cain and Philips, 2008). Together with the orbital glands and the CALT they moisturize the eye and supply the cornea and conjunctival sacs with nutrients and antibodies to maintain their health (Jochems and Philips, 2015).

The meibomian glands of sheep were of compound tubuloalveolar type. These glands were modified sebaceous glands which reside within the upper and lower eyelids. These glands were more numerous and better developed in the upper eyelids than the lower one. These linear arrays of sebaceous glands resembled a grape-like clusters attached to a central stalk (Fig. 3). These large multilobulated glands were arranged in the tarsal plate, a thick plaque of dense fibrous connective tissue (Fig. 1a). The posterior palpebral margins in the red kangaroo and other mammals are lined with multiple tarsal glands which appear as yellow columns positioned parallel to one another and perpendicular to the eyelid margins (Nasrin *et al*., 2013). However, the camel eyelids showed an ill-developed tarsus at the center of the eyelid which is infiltrated by some bundles of connective tissue (Saeed, 2015). The lobules were composed of clusters of secretory glandular cells (meibocytes) of various sizes (Fig. 1b), resting on a basement membrane which was surrounded by a thin connective tissue sheath. The meibocytes have a light pink (acidophilic) cytoplasm, and the vacuoles were filled with a homogenous eosinophilic material. The connective tissue sheath was mainly made up of collagen fibres and blood vessels of varying sizes (Fig. 4). These findings were in fully agreement with the reports in guinea pig (Gasser *et al*., 2011) and camel (Saeed, 2015). The oval nuclei of meibocytes were situated at centre of the cell. The peripheral meibocytes were smaller, whereas central cells were larger and polyhedral in nature (Fig. 1b). Similar findings were reported in guinea pig (Gasser *et al*., 2011). The meibocytes were connected to a central excretory duct, lined by stratified squamous epithelium, which directly opened at the muco-cutaneous junction of the eyelid (Fig. 1b). The tarsal plate was covered externally by skeletal muscle fibers and internally by the palpebral conjunctiva having goblet cells. Kleckowska-Nawrot *et al*. (2016) also showed the presence of goblet cells in the palpebral and bulbar conjunctiva zones of both eyelids, but numerous goblet cells were identified in the palpebral conjunctiva zone of the lower eyelid of red kangaroo. Saeed (2015) reported that the palpebral conjunctiva of the dromedary camel was lined with epithelia that varied from stratified squamous to stratified columnar, however, goblet cells were present in all forms of epithelia except over the lymphoid follicles, where the epithelium was transformed to low stratified squamous.

Histochemically, a periodic acid-Schiff (PAS) negative reaction was observed in the secretory units of the gland indicating the absence of neutral mucopolysaccharides.



Figs. 1-7. **1a.** Photomicrograph of eyelid of sheep showing various histological layers (E- epidermis; D- dermis; H- hair follicles; Z- zeis glands; Cg- ciliary/Moll glands, Mf- muscle fibres of orbicularis oculi; Mg- meibomian/tarsal glands; Cd- central duct; J- muco-cutaneous junction; C- conjunctiva) (H & E x 40). **1b.** Photomicrograph showing large sized multilobular sebaceous (meibomian) glands of sheep eyelid with alveoli (A) emptying into long central duct (Cd) that opened at the muco-cutaneous junction (J) (H & E x 100). **2.** Photomicrograph of eyelid of sheep showing the palpebral conjunctiva (C) lined the inner surface of the eyelids. The epithelium gradually changed from stratified squamous to pseudostratified columnar having numerous goblet cells (arrow). Lymphatic nodules (Ln) were also seen in the subconjunctival tissue towards the palpebral conjunctiva (H & E x 40). **3.** Photomicrograph of eyelid of sheep showing the linear arrays of meibomian glands (Mg) arranged in grape-like clusters attached to a central stalk (Cs) (H & E x 100). **4.** Photomicrograph of eyelid of sheep showing the connective tissue sheath which was mainly made up of collagen fibres (Cf) and blood vessels of varying sizes (Crossman Trichrome x 40). **5.** Photomicrograph of eyelid of sheep showing the strong PAS positive reaction in the goblet cells (arrow) of the palpebral conjunctiva indicating the presence of more amounts of neutral mucopolysaccharides (PAS x 40). **6.** Photomicrograph of eyelid of sheep showing a negative PAS-AB reaction for both neutral and acid mucopolysaccharides in the secretory units; however, the goblet cells (arrow) showed the presence of these substances (PAS-AB x 40). **7.** Photomicrograph of eyelid of sheep showing the Zeis glands (Z) and modified apocrine (ciliary) glands (Cg). The secretory portion of ciliary glands comprised of large irregular lumen and lined with cuboidal epithelial cells having eosinophilic cytoplasm (H & E x 100).

However, a strong PAS positive reaction was seen in the goblet cells of the palpebral conjunctiva indicating the presence of more amounts of neutral mucopolysaccharides (Fig. 5). These results were in accordance with the findings in camel (Saeed, 2015). In gerbil, however, goblet cells were observed either as solitary or in clusters, but their distribution was detected throughout the entire conjunctiva (Voigt *et al*., 2012). These glandular units also showed negative reaction to weakly acidic sulfated mucosubstances, sialomucins and hyaluronic acid with Alcian blue (AB) at pH 2.5, while the unicellular mucous glands showed strong positive reaction for the same. PAS-AB technique revealed negative reaction for both neutral and acid mucopolysaccharides in the secretory units; however the goblet cells showed the presence of these substances (Fig. 6). Mayer's mucicarmine showed negative reaction which indicated the absence of acid mucopolysaccharides in the secretory cells, whereas the goblet cells showed the presence of same.

The ciliary glands of sheep were of simple coiled tubular type. These were modified apocrine sweat glands and were located at the base of the hair follicles, anterior to the Meibomian glands. These were comparatively well developed in the upper eyelids. Associated with these hair

follicles, were small sebaceous (Zeis) glands (Fig. 1a). The secretory portion was comprised of large irregular lumen and lined with cuboidal epithelial cells having eosinophilic cytoplasm (Fig. 7). These findings were in fully agreement with those reported in pig (Yasui *et al*., 2006). The apical surface of the cells showed varying cytoplasmic protrusions. These glands were separated from each other with a septa composed of connective tissue fibres and vascular tissue. The ducts of these glands were lined by stratified cuboidal epithelium and were connected with hair follicles. Histochemical studies revealed the absence of both acidic and neutral mucopolysaccharides in secretory cells by PAS-Alcian blue method. The reaction intensities of the secretory epithelium and luminal secretion were generally weak as reported by Yasui *et al*. (2006) in pig. The secretory units of the gland also showed the absence of neutral mucopolysaccharides by McManus' method. Also, these secretory cells showed negative reaction to Alcian blue (pH 2.5) and Mayer's mucicarmine methods.

It may be concluded that the dermal layer of the eyelids was comprised of loose collagenous connective tissue that contains hair follicles and associated unilobular sebaceous glands (Zeis glands), modified multilobular sebaceous glands (meibomian or tarsal glands) and apocrine sweat glands (Moll or ciliary glands). The large meibomian glands were arranged as separate, single strands in parallel arrangement in the tarsal plates of the both eyelids. The secretory cells (meibocytes) emptied into a large central duct, which directly opened at the muco-cutaneous junction of the eyelids. The ciliary glands were modified apocrine sweat glands and located at the base of the hair follicles. The secretory portion of the ciliary glands was comprised of large irregular lumen and lined with cuboidal epithelial cells having eosinophilic cytoplasm. Histochemical studies revealed the absence of neutral mucopolysaccharides, weakly acidic sulfated mucosubstances, sialomucins and hyaluronic acid in the secretory units of both Meibomian and ciliary glands.

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