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Research Article

HISTOLOGY, HISTOCHEMISTRY AND SCANNING ELECTRON MICROSCOPY OF THE THIRD EYELID OF DOMESTIC PIGS

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ABSTRACT

The present study was conducted on third eyelid of six adult domestic pigs (*Sus scrofa domesticus*). The apex of the palpebral surface and bulbar surface was lined by stratified squamous non-keratinized epithelium and transition of the epithelium was observed from stratified squamous to stratified cuboidal epithelium with numerous goblet cells which showed presence of the glycogen, weakly and strongly sulfated acidic mucopolysaccharides as well as mucins. Lamina propria on both sides of the cartilage was consisted of the loose to dense connective tissue that having collagen, reticular, and elastic fibers, fibrocytes, small blood vessels, arterioles and venules. Smooth muscle fibers were not observed. Conjunctiva associated lymphoid tissue was consisted of intraepithelial lymphoid cells, diffusely arranged lymphoid cells, and lymphoid follicles which were more towards the bulbar surface however only a few lymphoid follicle was seen towards the palpebral surface. Conjunctival epithelium over the lymphoid follicle became attenuated with noticeably absence of the goblet cells called the follicle associated epithelium.

Keywords: Third eyelid, Pig, Histology, Histochemistry, Scanning electron microscopy

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The third eyelid (nictitating membrane) is a large fold of the conjunctiva situated at the medial canthus of the eye and rest over the anterior surface of the globe. The nictating membrane persists in the form of plica semilunaris conjunctivae without apparent function in humans. It is supported by cartilaginous plate and covered by mucous membrane that serves to protect the cornea from injury, clear the debris and mucous, distributes the tears, produce part of the tear (Gelatt *et al.*, 2007). Local immunological protection was given by lymphoid tissue present in the loose connective tissue of the conjunctiva as well as immunoglobulins produced by the plasma cells that resides in the gland of third eyelid (Schlegel *et al.*, 2003). Conjunctiva constituted a barrier between the internal and external environment and constantly exposed to environmental pathogens which make it more prone to be affected by many pathological conditions as conjunctivitis and mechanical injuries. Any abnormality in the conjunctiva leads to disturbance in normal functioning of cornea as pre-ocular tear film and cornea is a major refractive interface. Nictitating membrane could be used as a potentially useful post-mortem diagnostic specimen for classical swine fever (Teifke *et al.*, 2005). There is a paucity of literature on the histology, histochemistry and scanning electron microscopic details of the third eyelid. Keeping the view of its significance in pathology, immunology and surgical procedure, the present study was conducted on third eyelid of pigs.

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MATERIALS AND METHODS

The present study was conducted on third eyelid of six healthy adult pigs of either sex. The tissues were collected immediately after slaughter and fixed in a 10% neutral buffered formalin solution for 48 hours. The tissues were subjected to routine tissue processing for light microscopic examination and prepared the paraffin blocks. The paraffin section of 5-6 m thickness were cut and stained by routine Harris' haematoxylin and eosin stain for general histomorphological examination, Gomori's stain for reticular fibres, Weigert's method for elastic fibres (Luna, 1968), Crossman's trichrome stain for collagen fibres (Crossman, 1937), mercury bromophenol blue method for proteins (Pearse, 1968). In addition, selected sections were processed for the histochemical demonstration of mucopolysaccharides using McManus' PAS method, Periodic-Acid-Schiff-Alcian blue (PAS-AB), Alcian blue (AB) at pH 2.5, Colloidal iron method and Mayer's mucicarmine method (Luna, 1968). For scanning transmission electron microscopy, fresh tissue of third eyelid were collected immediately after slaughter and fixed in Karnovsky's fluid for 8-12 hours after thorough washing with chilled 0.2 M phosphate buffer (pH 7.4). The tissues were again washed twice with 0.2 M phosphate buffer and rest of the procedure have been carried out at Sophisticated Analytical Instrumentation Facility, AIIMS, New Delhi. The processed tissues viewed under scanning electron microscope (Zeiss EVO-18).

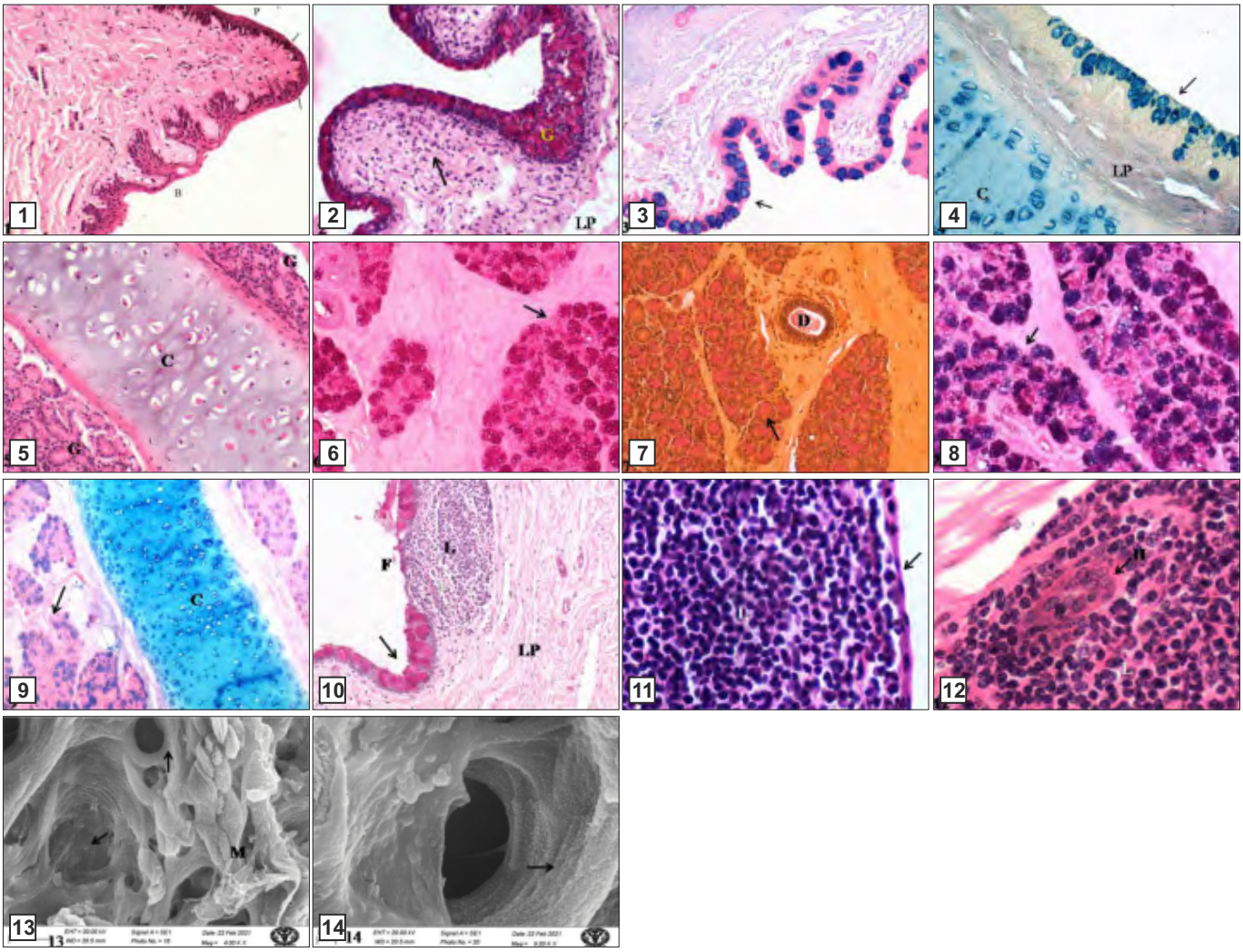
RESULTS AND DISCUSSION

A large fold of conjunctiva was formed at the medial canthus, supported by hyaline cartilage.It had two surfaces; the palpebral surface was convex and facing towards the inner lining of the eyelid whereas the bulbar surface was concave and rest on the globe. The free margin of the third eyelid was pigmented as described in bison (Nawrot *et al.*, 2015) and camel (Abuelhassan, 2007).The apex of the both surfaces was lined by stratified squamous non-keratinized papillated epithelium (Fig. 1). It was generally comprised of 8-10 rows of cell layers. The nuclei of basal cells were round to oval in shape and fine dusting of chromatin material was observed in the nucleoplasm with 1-2 nucleoli situated centrically or eccentrically. The cells of the stratum spinosum were generally oval in shape perpendicular to the epithelium although some nuclei were rounded and comparatively larger than those of the basal cells. They separated from each other by large inter cellular spaces. Their chromatin material was finely distributed. At some places vacuolated nuclei were also observed. Cytoplasm of these cells was granular and eosinophilic. Nuclei of superficial cells were flat, elongated and parallel to the surface of the epithelium. They were usually comparatively more basophilic than the other cells of epithelium. The nuclear material was densely packed and nucleoli were not observed in the nucleoplasm. Cytoplasm was granular and comparatively more eosinophilic. The transition of the epithelium was observed towards the base and changed from stratified squamous to stratified cuboidal epithelium with numerous goblet cells (Fig. 2) as observed in most of the domestic animals (Gelatt, 2007). Bulbar surface was smooth with no ridges and grooves whereas shallow mucosal folds were observed on the palpebral surface towards the base as described in domestic animals (Gelatt, 2007).

Varying size of goblet cells were observed and their concentration was also varied at different areas. These were usually densely arranged at mucosal folds towards the base (Fig. 2). Cytoplasm of the goblet cells was very scanty and less eosinophilic. Their nuclei were having densely packed chromatin material and pushed towards the base. The goblet cells showed the presence of the glycogen, weakly and strongly sulfated acidic mucopolysaccharides as well as mucins (Figs. 2, 3 & 4) as reported in cat (Schramm *et al.*, 1994).

The lamina propria on both sides of the cartilage consisted of the loose to dense connective tissue having fibrocytes, plasma cells, collagen, elastic and reticular fibers. The elastic fibres were distributed throughout the lamina propria however it was thick and densely arranged towards the base. A layer of elastic fibres was seen just beneath the epithelium. Cartilage was hyaline (Fig. 5) however few elastic fibres were also observed in the ground matrix which was similar as described in pig (Nawrot and Dziegiel, 2007; Schlegel *et al.*, 2001; Schramm *et al.*, 1994). It was hyaline in the dog (Gelatt, 2007), kangaroo (Nawrot *et al.*, 2016), camel (Abuelhassan, 2007) but in contrast, it was elastic in cat and horse (Schlegel *et al.*, 2001) and one-humped camel (Fahmy *et al.*, 1971). Cartilage showed a positive Alcianophilic reaction (Fig. 9) as reported in cats (Schramm *et al.*, 1994). Any smooth muscle fibres were not observed in the present study and their movement was passive and due to action of the extraocular muscles as described in pig (Nawrot and Dziegiel, 2007) whereas in cat, nictitating membrane having smooth musculature with elastic fibres which involved in their movement (Schramm *et al.*, 1994). The base of the cartilage was surrounded by a thick layer of fatty tissue in the horse (Schlegel *et al.*, 2001) which was not observed in the present study. A well-developed superficial gland of the third eyelid was observed surrounding the shaft of the cartilaginous plate (Fig. 5). The gland was tubuloacinar type that having seromucous acini with their duct system as described in buffalo (Verma *et al.*, 2019), cat (Cabral *et al.*, 2005), red kangaroo (Nawrot *et al.*, 2016), deer (Nawrot *et al.*, 2013). The gland covered by thin connective tissue capsule and parenchyma was having lobes and lobules separated from each other by septae that also having elastic fibres and carrying small blood vessels as described in buffalo calf (Verma *et al.*, 2019), red kangaroo (Nawrot *et al.*, 2016), camel (Abuelhassan, 2007) and bison (Nawrot *et al.*, 2015). Numerous adipocytes were observed either single or in form of aggregates in septae (Fig. 8) as reported in bison (Nawrot *et al.*, 2015). Acini of varying size were observed that was lined by pyramidal to columnar shape cells. Myoepithelial cells at the base of some acini were observed as described in bison (Nawrot *et al.*, 2015). The histochemical study showed that the superficial gland of the third eyelid was having mixed secretions (Fig. 6, 7 and 8) which were similar to findings of Nawrot *et al.* (2015), Verma *et al.* (2019). The glandular acini did not show any reaction to the mercury bromphenol blue method for proteins. Few goblet cells were also observed in the epithelial lining of the inter-lobular ducts.

Conjunctiva of the third eyelid was associated with lymphoid tissues in which diffusely arranged lymphoid cells were observed in the sub-epithelial loose connective tissue and these were more concentrated in the space between the mucosal folds (Fig. 2). Lymphocytic infiltrations were also observed in between the epithelial cells and contributed



Figs. 1-14. **(1)** Photomicrograph of the third eyelid showing the bulbar (B) and palpebral surface (P) lined by stratified squamous non-keratinized epithelium and pigmentation towards the margin (arrow). H. &E. X100; **(2)** Photomicrograph showing the fold of conjunctiva lined by stratified cuboidal epithelium, numerous goblet cells (G) possesses PAS positive secretory material, lamina propria (LP), diffusely arranged lymphoid cells (arrow). MacManus' PAS X 100; **(3)** Photomicrograph showing the presence of Alcianophilic acidic mucopolysaccharide in the goblet cells (arrow). Alcian Blue X100, **(4)** Photomicrograph showing the presence of more sulphated acidic mucopolysaccharide in the goblet cells (arrow), lamina propria (LP), cartilage (C). Colloidal Iron X 100; **(5)** Photomicrograph of the superficial gland of the third eyelid (G) located around the base of the cartilage (C) towards the base. H.&E. X100; **(6)** Photomicrograph of the third eyelid showing the presence of the neutral mucopolysaccharide (arrow) inthe seromucous acini of the superficial gland. MacManus' PAS X 200; **(7)** Photomicrograph of the superficial gland of the third eyelid showing presence of mucin in the acini (arrow) and lumen of the interlobular duct (D). Mayer 's mucicarmine X 100; **(8)** Photomicrograph of the seromucous acini of superficial gland of the third eyelid showing the presence of predominance of acidic mucopolysaccharide (arrow) PAS-AB X200; **(9)** Photomicrograph of the superficial gland of the third eyelid showing the presence of Alcianophilic acidic mucopolysaccharide (arrow). Alcian blue X100; **(10)** Photomicrograph of the conjunctiva showing the lymphoid follicle (L) in the lamina propria (LP), follicle associated epithelium (F) that devoid of goblet cells, goblet cells in conjunctival epithelium (arrow). MacManus' PAS X 100; **(11)** Photomicrograph of the follicle associated epithelium at higher magnification showing M cells (arrow) associated with the lymphoid tissue (L). H.&E. X400; **(12)** Photomicrograph of the lymphoid follicle at higher magnification showing High endothelial venules (arrow). H.&E. X400; **(13)** Scanning electron micrograph of the bulbar surface of the third eyelid showing holes of varying size (arrow) of the secretory stage of goblet cellsand M like cells (M). X4000; **(14)** Scanning electron micrograph showing the thick micro projections on inner wall of the holes (arrow). X9000

to form intraepithelial lymphoid tissue. Aggregated mass of lymphoid cells was observed in the form of lymphoid follicles which were more at the bulbar surface towards the base of the third eyelid (Fig. 10). However, only a few lymphoid follicles was seen at the palpebral surface as observed in cat (Giuliano and Finn, 2011). Lymphoid follicles were comprised of lymphocytes of different sizes, plasma cells, macrophages and fine blood capillaries. At some places, few lymphoid follicles showed the presence of corona with or without the germinal centre. High endothelial venules were seen in the parafollicular region which was lined by high cuboidal cells (Fig. 12). Conjunctival epithelium over the lymphoid follicle became attenuated and few sections showed only a single cell layer with the absence of the goblet cells called the follicle associated epithelium (Fig. 10). The flat to oval-

shaped nuclei were observed with a flat surface in the follicle associated epithelium which was related to the lymphocytes that resembled the M (microvillus) cells (Fig. 11). These observations were similar as observed in cats (Giuliano and Finn, 2011) and pigs (Moral *et al.*, 2020).

Scanning electron microscopic findings of the bulbar surface of third eyelid showed superficial cells of the stratified squamous epithelium that having microplicae of varying sizes and shapes but it was not thick and prominent as reported in domestic ruminants (Weyrauch, 1984). Serration on the palpebral was not observed in the present study however it was seen in ruminants (Weyrauch, 1984). The density of the goblet cells was increased towards the base. Numerous holes of varying size and depth were seen which represented the opening for the secretions of goblet cells (Figs. 13, 14). At some places, few cells were observed in the vicinity of the goblet cells which were look like M cells (Fig. 13). The inner lining of the holes was not smooth and showed the presence of very short and thin surface projections (Fig. 14).

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