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Research Article

HISTOCHEMICAL STUDIES ON EYELIDS IN FETUSES OF INDIAN BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

The eyelids are composed of skin, subcutaneous, skeletal muscle (*orbicularis oculi*) submuscular areolar tissue, tarsal plates, non-striated muscle and conjunctiva. The chemical composition of secretions and its glands varies according to species specific activities *viz.*, the meibomian glands secret oily but the glands of third eyelid has mucous or serous or lipid like secretions. The upper, lower and third eyelids were dissected from both eyeball of six fetuses and fixed in 10% neutral Buffered formalin (n=6 for upper, lower and third eyelids, each) and cryopreserved for frozen section (n=6 for upper, lower and third eyelids, each). In upper and lower eyelids of buffalo fetuses, the strong PAS and AB activity was observed in the basement membrane of the alveoli of tarsal gland. The sebaceous glands associated with follicles showed positive alcian blue reaction and weak PAS activity. In the showed mixed reaction of PAS and AB indicating the presence of acid and neutral mucopolysaccharides in external root sheath whereas the glassy membrane was rich in the acid mucopolysaccharides as they were found positive for PAS reaction. The Mercury Bromophenol Blue staining showed that the connective tissue septae of the tarsal glands were rich in basic protein moieties whereas the glandular mass of these glands did not show any presence of the basic protein in the cells. With Sudan black B and Oil Red O, the eyelids of buffalo fetuses showed the presence of lipid moieties in the stratum corneum of epidermis, sebaceous and the tarsal glands.

Keywords: Eyelids, Histochemistry, Indian buffalo

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The development of eyelids involves a coordinated process of cell proliferation, changes in cell shape, migration and cell death as quoted by Tao *et al.* (2005) and these events are related to their functional significance. The blinking of eyelids helps in the distribution of lacrimal fluid and has a pumping effect on the lacrimal sac (Ahmed, 2009). The wall of the eyelids is composed of various tissue components from the external to globe side *viz*. skin, subcutaneous, striated skeletal muscle (palpebral part of the orbicularis oculi), submuscular areolar tissue, tarsal plates, non-striated muscle, and conjunctiva (Csillag, 2005). The chemical composition of secretions of the eyelids and its glands varies according to species specific activities. The histology of developing eyelids was described in bovine (Bister *et al.*, 1973), dog (Aguirre *et al.*, 1972), one humped camel (Zayed, 2004) and human (Hamilton & Mossman, 1972; Pearson, 1980). Although, some histochemical studies were also available in Egyptian buffalo (Ahmed, 2009) but the available literature revealed that there is paucity of information on the histochemistry of eyelids during prenatal life in Indian buffalo thus the present investigation was planned to the observe the distribution of various chemical moieties in fetal eyelids of Indian buffaloes.

MATERIALS AND METHODS

The present investigation was carried out on total 6 Indian buffalo (*Bubalus bubalis*; Linnaeus, 1758) fetuses ranged from 89.0 cm CVRL to 94.0 cm CVRL (274 days to 286 days of gestation). These fetuses were collected from the buffaloes presented either at Teaching Veterinary Clinical Complex of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) for release of dystocia or for necropsy at postmortem hall of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India. Formal permission from the Institutional Animal Ethical Committee (IAEC) was not required for the procurement of dead fetuses. The estimation of the gestational age was calculated as given by Soliman (1975).

$$Y = 73.544 + 2.256\,X \quad (CVRL > 20\ cm)$$

Where, Y is age in days and X is CVRL in centi-metres. After extirpation of eyeball, the upper, lower and third eyelids were dissected from both eyeball of all six fetuses and fixed in 10% neutral Buffered formalin (n=6 for upper, lower and third eyelids, each) and were cryopreserved (at -20º C) for frozen section (n=6 for upper, lower and third eyelids, each).

Sections of 6 µmthickness were stained with Periodic Acid Schiff's -Alcianblue for mucopolysaccharides (Luna, 1968) and mercury bromophenol blue for basic proteins (Pearse, 1972). The cryosection of 10 µm werestained with Sudan Black B and oil red-O for lipid and Baker's acid hematin for phospholipids (Drury & Wallington, 1980).

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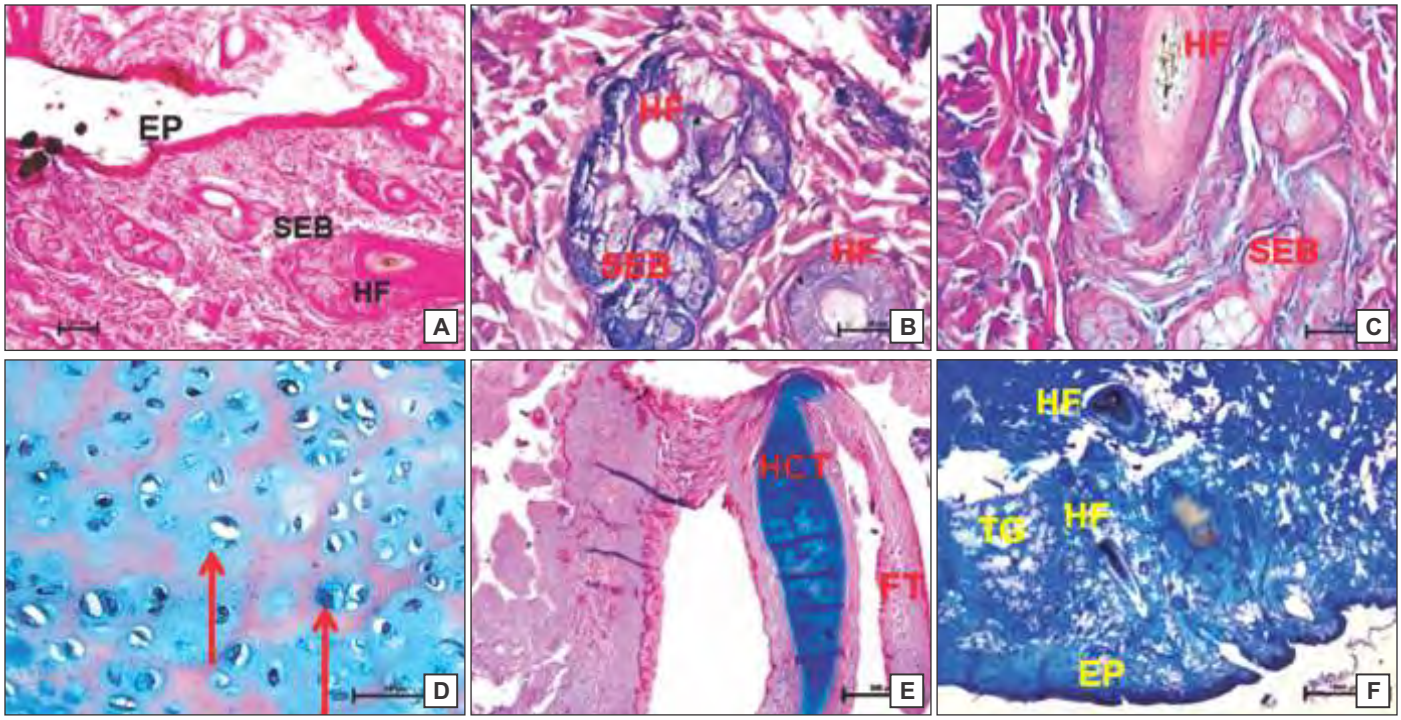

Fig. 1. **(A)** 94.0 cm CVRL fetus showing strong PAS activity in in epidermis (EP), eyelash follicle (HF) and in connective tissue sheath of sebaceous gland (SEB) in upper eyelid. PAS X 400. **(B)** 92.0 cm fetus showing mixed PAS-AB activity in eyelash follicle (HF), strong alcianophilic reaction in basal lamina of sebaceous gland (SEB) and moderate to strong PAS positive reaction in stroma of upper eyelid. PAS-AB X 100. **(C)** 89.0 cm CVRL fetus showing mixed reaction of PAS-AB in eyelash follicle (HF) and sebaceous gland (SEB)- (strong alcianophilic) in lower eyelid. PAS-AB X 400. **(D)** Cartilage of Third eyelid in 90.0 cm fetus showing intense reaction of alcian blue in chondrocytes, strong to moderate reaction in matrix surrounding the lacunae and weak PAS reaction in remaining matrix (PAS-AB X 100). **(E)** Third eyelid in 92.0 cm fetus showing strong alcianophilia in the cartilage and moderate to strong PAS reaction in other components (PAS-AB X 40). **(F)** Lower eyelid of 94.0 cm fetus showing spectrum of reaction for basic proteins in upper eyelid (Mercury Bromophenol method X 40)

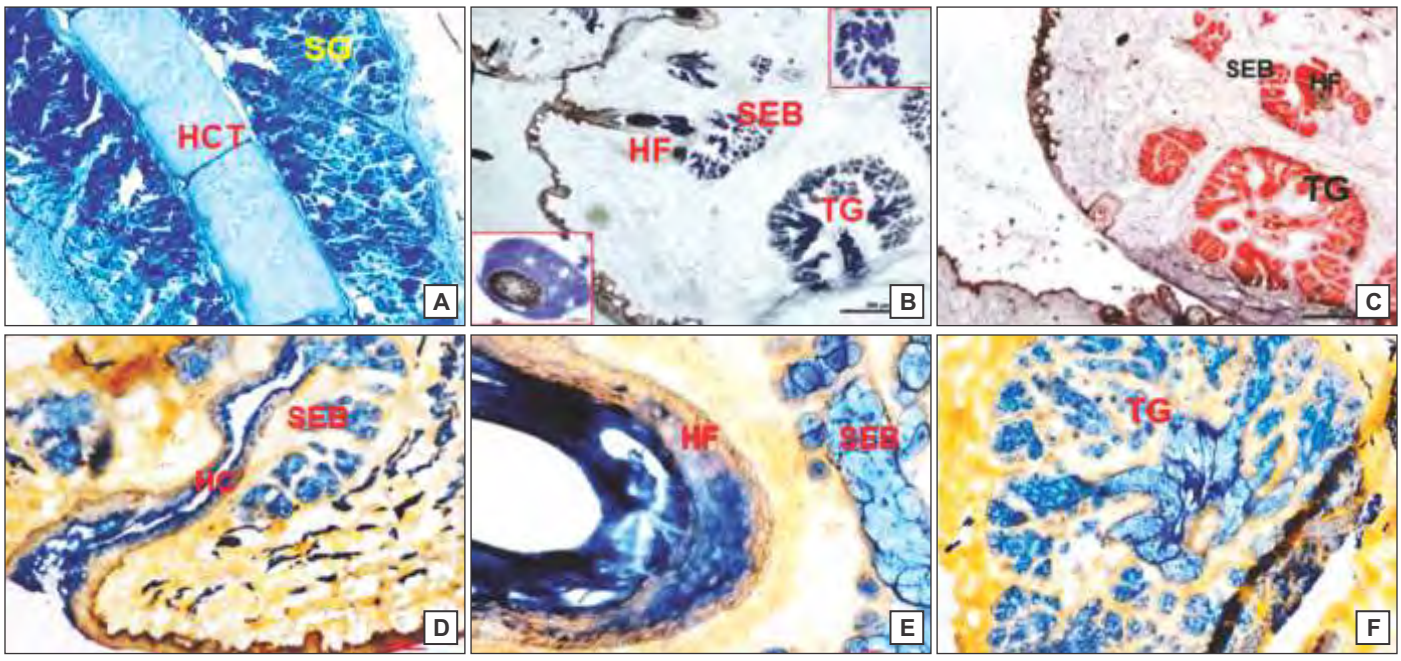

Fig. 2. **(A)** The cartilage of third eyelid in 89.0 cm fetus showing weak reaction for basic protein in ground matrix but strong reaction in the nuclei of chondrocytes (Mercury Bromophenol method X 40). **(B)** 89.0 cm CVRL fetus showing strong reaction for lipids in tarsal glands (TG), root sheaths of eyelash follicles (bottom inset: HF) and sebaceous glands (top inset: SEB) in lower eyelid. Sudan Black B method X 40. **(C)** 90.0 cm CVRL fetus showing strong reaction for lipids in stratum corneum of epidermis, tarsal glands (TG), root sheaths of eyelash follicles (HF) and sebaceous glands (SEB) in upper eyelid. Oil red O method X 40. **(D)** 94.0 cm CVRL fetus showing intense activity of phospholipids in eyelash canal (HC) and orbicularis oculi muscle but strong reaction in stratum tarsal glands, root sheaths of eyelash follicles and sebaceous glands (SEB) in upper eyelid. Baker's acid hematin method X 100. **(E)** 89.0 cm CVRL fetus showing intense activity of phospholipids in eyelash follicle (HF) but strong reaction in sebaceous glands (SEB) in lower eyelid. Baker's acid hematin method X 400. **(F)** 92.0 cm CVRL fetus showing strong reaction for phospholipids in alveoli of tarsal gland (TG) in upper eyelid. Baker's acid hematin method X 100.

RESULTS AND DISCUSSION

In upper and lower eyelids of buffalo fetuses, the strong PAS and AB activity was observed in the basement membrane of the alveoli in tarsal gland. The strong activity of mucous was also reported by Nawrot *et al.* (2023) in Lowland Tapir. However, Stoeckelhuber (2003) observed weak PAS positive reaction with strong alcianophilic reaction in the glands of human eyelids. The endothelium of blood vessels in subcutis and dermis of eyelids showed strong alcianophilic activity which may be due to more lipids/cholesterol related moieties in them. The eyelash follicles showed a strong PAS activity in external root sheath whereas the glassy membrane depicted a strong alcianophilic activity. The sebaceous glands associated with these follicles showed alcian blue positive moieties and weak PAS activity. The alcian blue has affinity with the acidic mucous which plays important role in lipid and cholesterol metabolism.

In the third eyelid of buffalo fetuses, Baba *et al.* (1992) conducted studies on nictitating apparatus of goat and sheep and they concluded that the epithelium of nictitating membrane in both the species manifested positive reactions for acid muco-polysaccharides. Basement membrane exhibited mild PAS positive reaction.The plate of hyaline cartilage was embedded in the superficial gland which was hyaline in nature. However, the third eyelid had elastic cartilage in camel (Abuelhassan, 2007). The chondrocytes showed intense reaction for alcian blue but the ground matrix surrounding the lacunae depicted strong to moderate reaction for alcian blue however, remaining matrix was weak positive for PAS (Fig. 1D & 1E). The presence of elastic cartilage in camels makes the third eyelid functional in camel whereas the hyaline cartilage gave the rigid morphology to the third eyelid in buffalo.

In upper and lower eyelids of buffalo fetuses, the Mercury Bromophenol Blue staining showed that the connective tissue septae of the tarsal glands were rich in basic protein moieties whereas the glandular mass of these glands did not show any presence of the basic protein in the cells. The eyelash follicles and the developing eyelash showed that the reaction for the basic proteins was present in entire eyelash follicle except the cortex. The cortex contains melanin which is amino acid polymer with acidic nature.The presence of polypeptides was also claimed by Wollina (1992) in human eyelash follicles which might be the intermediate stages of keratin synthesis. The glassy membrane of the eyelash follicle also showed a strong reaction for basic proteins. The orbicularis oculi muscles were also demonstrated the presence of basic proteins. The connective tissue of dermis and the endothelium of the blood vessels of the dermis of upper eyelid were also found positive for the presence of basic proteins (Fig. 1F).

In the third eyelid of buffalo fetuses, the eyelash follicles had a strong reaction for the basic proteins but the cortex was found rich in melanin. The tarsal glands of the third eyelid had strong reaction for the basic proteins in the connective tissue trabeculae but negative in its cellular contents. The epidermis of third eyelid depicted a light reaction for the presence of basic proteins. The cartilage showed a weak Mercury Bromophenol blue reaction in the ground matrix whereas the reaction was strong in the nuclei of chondrocytes (Fig. 2A).

The eyelids of buffalo fetuses showed the presence of lipid moieties in the stratum corneum of epidermis, sebaceous and the tarsal glands. The eyelash canal also showed strong activity of lipids. These lipid moieties provide the lubrication to the eyelids during movements of the eyeball. External root sheath of the eyelash follicle also demonstrated the presence of lipids towards the root of eyelash. A mild reaction was also present in the medulla and the cuticle of the eyelash follicle by Sudan Black B and Oil red O methods (Fig. 2B & 2C). The presence of lipid moieties was also observed by Stoeckelhuber (2003) in human eyelash follicles. The presence of lipid moieties can also be confirmed with the reports of Tomar and Bansal (2023) where they noticed the activity of non-specific esterase in buffalo fetuses.

The acid hematin staining of the eyelids in buffalo fetuses showed an intense to strong reaction in the glandular tissue and the ducts of the tarsal gland. The sebaceous glands also gave strong positive reaction for phospholipids. Eyelash follicle demonstrated the presence of abundant phospholipids in their external root sheath. The muscles of eyelids demonstrated a strong activity of phospholipids moieties (Fig. 2D, 2E & 2F).

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