

Histomorphological Study on cornea and sclera of the adult Surti Buffalo (*Bubalus bubalis*)

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ABSTRACT

The present study was conducted on 10 pairs of eye balls to study the histomorphological structures of cornea and sclera. The cornea was composed of four layers such as (i) Anterior epithelium (ii) Corneal stroma (iii) Descemet's membrane and (iv) Endothelium. The sclera was composed of three layers such as (i) episclera (ii) sclera proper (iii) lamina fusca.

The mean value of the total thickness of the cornea was $798.607 \pm 69.17 \mu\text{m}$ at the periphery and it was $789.13 \pm 64.20 \mu\text{m}$ at the center. The micrometrical mean values of the thickness of the sclera at the periphery was $445.96 \pm 23.05 \mu\text{m}$ and that of the center was $856.95 \pm 33.84 \mu\text{m}$.

KEYWORDS : surti buffalo, eye ball, retina, histomorphology

Introduction

The eye is an important sensory organ designed for vision. Since the well being and performance of the animals is directly related to proper vision, the animal with poor vision usually results in poor performance and also prone to accidents and dangerous violent activities. The cornea and the lens act as refractive media because of their transparency and curvature. Cornea, being central and outermost part of the eye ball, is frequently subjected to get injury causing ulcer, keratitis, opacity etc. resulting pain and discomfort to the animal. Hence, with these views in mind, this work was carried out and it is hoped that the study would go along way in helping the surgeons in refining ophthalmic surgery in the buffalo.

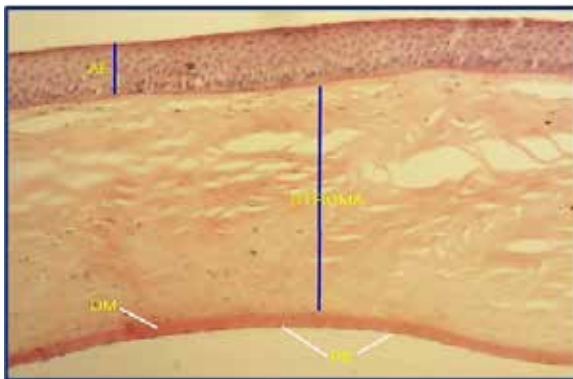


Fig.1: Photomicrograph of cornea of surti buffalo (150X, H&E) showing (AE) Anterior epithelium, Corneal stroma, (DM) Descemet's membrane and (PE) Posterior Endothelium.

Materials and Method

The samples were collected immediately after slaughtering of animals from local slaughter house, Anand. The eye balls were fixed in Davidson's fixative to prepare the paraffin sections. The histological sections were stained with Haematoxylin and Eosin Stain (Singh and Sulochana, 1996) for normal routine staining and Masson's trichrome stain (Luna, 1968) for special staining. The micrometrical measurements of the different parameters taken from the center and periphery of cornea and sclera were recorded with the help of graduated eye piece. The data was analyzed statistically (Snedecor and Cochran, 1967).

Results and Discussion**Histology of cornea**

In the present study, the cornea was observed to be composed of four layers (Fig.1). This finding is similar to that noted by Martin and Anderson (1981) in domestic animals excluding cattle and Ramkrishna et al. (1997) in Indian water buffalo.

The four layers from the outward to the inward were as below:

1. Anterior Epithelium
2. Corneal stroma / Substantia Propria
3. Descemet's Membrane and
4. Endothelium

Anterior epithelium

It was the outermost layer of the cornea and was composed of rows of epithelial cells. These cells were of stratified squamous non-keratinized type and the nuclei of these epithelial cells were of blackish blue coloured in H&E staining. The basal cells of the epithelial layers were resting on the basement membrane and were of columnar type while the cells towards the anterior surface were found to be progressively flattened or squamous type (Fig.2).

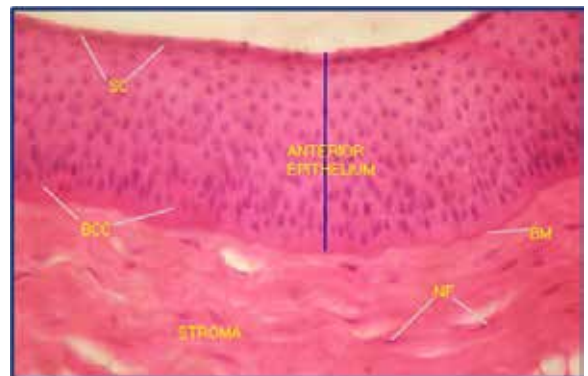


Fig.2: Photomicrograph of cornea of surti buffalo (300X, H&E) showing Anterior epithelium, Corneal stroma, (SC) Superficial squamous cells, (BCC) Basal columnar cells, (BM) Basement membrane and (NF) Nuclei of fibroblasts.

Corneal stroma or substantia propria

This layer constituted the major part of the cornea and was found to be composed of regularly arranged sheets or lamellae of the collagen fibers along with the fibroblast cells. The nuclei of fibroblast cells were easily recognized from their elongated shape along the fibers (Fig.2).

Descemet's membrane

This membrane was found to be interposed in between the stroma and the endothelium. It was uniformly thick, homogenous, eosinophilic membrane. It was composed of loosely arranged collagen fibers which were faintly stained (Fig.3).

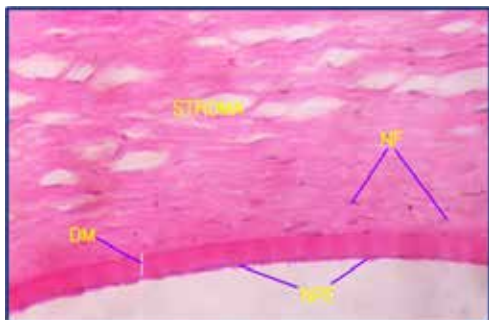


Fig.3: Photomicrograph of cornea of surti buffalo (300X, H&E) showing (NPE) Nuclei of Posterior endothelium, (DM) Descemet's membrane, (NF) Nuclei of fibroblast cell and some portion of Corneal stroma.

Endothelium

The endothelium was found to be the last and the most posterior layer of the cornea. It was composed of a single row of flattened cells with prominent elongated nuclei lying at the caudal border of Descemet's membrane parallel to the surface of cornea (Fig.3).

The present study showed that the general histological structures of cornea in different species of animals were similar to one another. However, in contrary to the present findings, the layer of the cornea i.e. "Bowman's membrane" reported by Smythe (1956) and Prince et al. (1960) in cattle, Ross and Edward (1985) in human beings, Martin and Anderson (1981) in cattle, Banubakode (1992) in cattle and Khaled (2003) in bovine eyeball was indistinct in the present study. Gelatt (2007) had mentioned the absence of Bowman's layer in most animals. Ramkrishna et al. (1997) also reported that the subepithelial basal lamina was not evident in Indian water buffalo.

Micrometry

The micrometrical observations of cornea were shown in Table 1.

Table 1: Statistical analysis of micrometrical observations of cornea and sclera							
Parameters	Periphery			Center			't' value
	Range	Mean±SE	C.V %	Range	Mean±SE	C.V %	
(A) Cornea							
Number of epithelial cell layers	7 to 12	9.42±0.11	4.31	7 to 12	9.29±0.11	4.11	0.41 ^{ab}
Thickness of epithelium (µm)	67.59 to 132.00	99.92±5.32	18.47	46.20 to 121.20	98.67±4.49	15.77	0.85 ^{ab}
Thickness of stroma (µm)	324.24 to 1080.80	667.32±67.27	34.92	330.21 to 996.66	657.59±63.05	33.21	0.91 ^{ab}
Thickness of Descemet's membrane (µm)	9.90 to 27.02	18.79±2.36	43.63	9.9 to 26.42	18.62±2.31	43.08	0.95 ^{ab}
Thickness of endothelium (µm)	3.30 to 6.60	5.08±0.37	25.71	3.3 to 6.60	4.95±0.35	24.61	0.79 ^{ab}
Total thickness (µm)	474.49 to 1215.90	798.98±69.17	29.99	378.28 to 1109.93	789.13±64.20	28.18	0.91 ^{ab}
(B) Sclera							
Total thickness (µm)	297.22 to 540.33	445.96±23.05	17.90	743.05 to 1080.80	856.95±28.68	9.57	2.005 ^{ab}

SE : Standard error, C.V% : Percentage of coefficient of variation, superscript (ab) : statistically non-significant at 5%, superscript (abc) : statistically significant at 5%

Thickness of the epithelium

The overall mean value of the thickness of the epithelium of both the center and periphery of cornea was 99.29±0.625 µm. The present observations are in agreement with the observations of Khaled (2003) in bovine (98.00 ± 1.50 µ). The observations of the thickness of epithelial layer of Prince et al. (1960) in exotic cattle (90 µm), Bloom and Fawcett (1962) in human beings (50 µm) and Banubakode (1992) in cattle (72.08±2.90 µm) were lower than those of the present observations.

Number of epithelial cell layers

The present observations are in corroboration with the observations of Banubakode (1992) in cattle (9.28±0.17 layers). However, the observations are found to be much lower than the observations of Diesem (1977) in bovines (14 to 18 rows) and Camber et al. (1987) in pigs (17 to 23 rows). The average number of epithelial layers was more in the periphery than at the center of cornea which is similar to the findings of Martin and Anderson (1981).

Thickness of corneal stroma

The present observations in surti buffaloes are found to be higher than the observations of Khaled (2003) in bovine, who reported the mean value of the thickness of corneal stroma as 580±40 µm.

Thickness of Descemet's membrane

The present observations are found to be higher than the observations of Prince et al. (1960) in cattle (10 to 25 µm) and Bloom and Fawcett (1962) in human beings (5 to 10 µm). However, the present observations are found to be lower than the observation of Khaled (2003) in bovine (30±1.0 µm).

Thickness of the endothelium

The present observations are lower than the observations of Prince et al. (1960) in cattle (6 µm), Diesem (1977) in bovines (6 µm) and Khaled (2003) in bovine (8±0.3 µm). Banubakode (1992) in cattle observed the thickness of endothelium as 2.22±0.067 µm which is around half of the present observations.

Total thickness of the cornea

The present observations are in agreement with the observations of Prince et al. (1960) in cattle (750 to 850 µm) and Bloom and Fawcett (1962) in human beings (800 to 900 µm). However, the present observations are found to be lower than the observations of Diesem (1977) in bovines (1500 to 2000 µm) and Banubakode (1992) in cattle (933.72 ± 15.35 µm), whereas, the observations are found to be higher than the observations of Camber et al. (1987) in pig (722 µm).

The present study clearly showed that variations are present in the thickness of epithelium, the number of cell layers in the epithelium, the thickness of stroma, the thickness of Descemet's membrane, the thickness of endothelium and the total thickness of cornea in different animals. These variations in the different observations of cornea may be due to the differences in the species, breed or some other factors.

In the present study, the thickness of the different layers of cornea was found to be varied from region to region. The mean value of the thickness of cornea was the thickest at around the limbus and the lowest in between the center and the limbus. However, the observations of the cornea showed non significant differences ($p>0.05$) between the center and the periphery.

SCLERA

Histology of sclera

The sclera was found to be composed of collagenous bundles running in various directions with fibroblasts and occasional melanocytes between them. It can be subdivided into three layers as follow (Fig.4) :

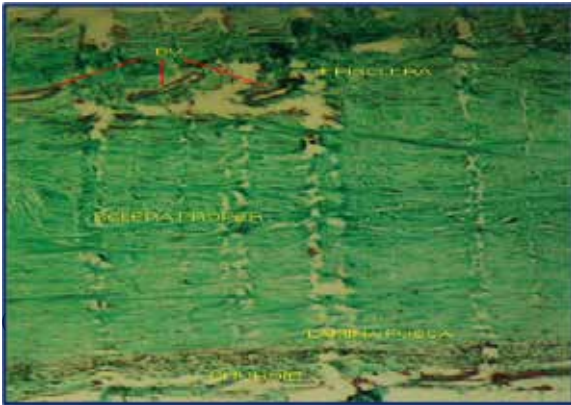


Fig.4: Photomicrograph of sclera of surti buffalo (30X, Masson's trichrome) showing episclera, sclera proper, lamina fusca, choroid and

- (1) The outermost layer or the episclera
- (2) The middle layer or the sclera proper
- (3) The innermost layer or lamina fusca

The episclera was found to be loose and vascular having fibrous connective tissue.

The sclera proper was composed of bundles of collagenous fibers which were oriented mainly parallel to the surface but with some interweaving. Elongated fibroblasts and melanocytes were found in some areas of the bundles of collagen fibers. Adjacent to the choroid, the innermost layer or lamina fusca or dark layer was present. This layer was composed of much smaller bundles of collagenous fibers with numerous melanocytes.

Similar observations were reported earlier by Dellmann (1993) in domestic animals, Ramkrishna et al. (1997) in Indian water buffalo and Khaled (2003) in bovine eye ball.

Micrometry

The micrometrical observations of sclera were shown in Table 1.

Khaled (2003) in bovine eye ball reported that the thickness of sclera was $1132 \pm 24.4 \mu\text{m}$, which is found to be much higher than the present observations. This variation in the thickness of sclera may be due to the differences in the species, breed or some other factors.

CONCLUSIONS

The histological structures of cornea was composed of four layers. These were (i) Anterior epithelial layer (ii) Corneal stroma (iii) Descemet's membrane (iv) Endothelial layer. The Bowman's membrane of cornea was not evident in the present study. The biometrical and micrometrical mean values of the thickness of cornea were non significantly higher at the periphery than that of the center. Histologically, the sclera can be subdivided into three layers such as (i) episclera (ii) sclera proper and (iii) lamina fusca. The present study showed that the general histological features of sclera of buffaloes are similar to the different domestic animals.

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