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Gross and Histo-Architectural Studies on the Vascular Tunic of Eyeball of Murrah Buffalo (*Bubalus bubalis*)

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ABSTRACT

The present study was conducted to elucidate the gross and histological details of the vascular tunic of eyeball of Murrah buffalo as the vascular tunic plays an important role in providing nutrition to the eye as well as additionally providing a peculiar metallic luster to the eye in dark. 20 pairs of eyeball from adult Murrah buffaloes, irrespective of sex, were collected, examined grossly, dissected and preserved in 10% NBF for further histomorphological studies. The present study revealed that the choroid was a deeply pigmented dark brown tunic lying between the sclera and the retina. The ciliary body was the largest component of anterior uvea. Tapetum was found in the dorsal part of fundus of the choroid. The iris was pigmented, contractile diaphragm present between cornea and the lens.

Key words: Buffalo, Gross, Histology, Choroid, Ciliary body, Iris.

INTRODUCTION

Eyes are the organs of vision which detect light and convert it into electro-chemical impulses. For animals, light perception is the trigger and the controlling sense for their behavior. The globe of eye consists essentially of three coats, the outer-most protective fibrous tunic is the sclera and cornea, the middle coat is mainly vascular consisting of the choroid, ciliary body and iris and the inner most layer is the retina, containing the essential nervous elements responsible for vision (Gelatt, 2007). Choroid also contains melanocytes that produce the melanin pigment that absorbs excess light and prevents reflection and scattering of light within the eyeball. Looking to the importance of this vascular tunic and paucity of literature in buffaloes, this work was designed to explore the gross and histological aspects of the vascular tunic of eyeball of buffalo.

MATERIALS AND METHODS

20 pairs of eyeballs from adult physically healthy Murrah buffaloes (*Bubalus bubalis*) of either sex were collected from the Tangra slaughter house, Kolkata, a government authorized abattoir of west Bengal. After measuring the gross parameters, the samples were preserved in 10% NBF. The samples were subjected to standard procedure of tissue processing and sectioning. The sections were stained with H & E for routine morphology and Masson's trichrome for the microscopic visualization of collagen fibers (Luna, 1968). H & E stained slides were used for micrometry by Leica Qwin Images Analyzer software in Leica DM 2000 Microscope.

RESULTS AND DISCUSSION

The vascular tunic of the eyeball of buffalo was a deeply pigmented tunic and was present between the outer fibrous layer and inner nervous layer. It was composed of three portions: anteriorly iris, posteriorly choroids and in between iris and choroid, ciliary body. The iris and ciliary body were termed the anterior uvea and the choroid formed the posterior uvea. This was in agreement with the findings of Ramkrishna *et al.* (1997) in Indian water buffalo and Barhaiya *et al.* (2014) in adult Marwari goat. The iris originated from the anterior portion of the ciliary body, and extended centrally to form a diaphragm in front of the lens. The uvea is concerned with the nutrition of the ocular tissues and also provides mechanisms for visual accommodation and reduction or exclusion of light in bovine (Samuelson, 1999).

Iris

It was the anterior most part of the vascular coat of eye ball. It was pigmented, contractile diaphragm and suspended in the aqueous humor between cornea and the lens. It was thin continuation of ciliary body and its free edge formed the boundary of the pupil (Fig.1). Similar observations were documented by Tortora and Anagnostakos (1981) in

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carnivores and Samuelson (1999) in bovines. The pupil was oval in horizontal plane. This was in agreement with the findings of Zayed *et al.* (2012) in buffalo.

Table 1: Mean diameters of the iris and pupil

	Horizontal diameter (mm)	Vertical diameter (mm)
Iris	26.89 ± 0.19	22.60 ± 0.06
Pupil	13.04 ± 0.15	5.03 ± 0.04

Table 2: Mean thickness of the different layers of the iris at equator (µm)

Pigmented epithelium (PE)	50.35 ± 0.84
Dialator muscle (DM)	22.96 ± 3.32
Stroma (ST)	251.49 ± 45.84
Sphincter muscle (SM)	76.90 ± 3.70
Rostral stromal sheath (RSS)	23.09 ± 1.01
Total iris thickness	**682.32 ± 12.37**

The horizontal and vertical diameters of the iris and pupil recorded in the present study (Table 1) was in agreement with the findings of Zayed *et al.* (2012) in buffalo but not with that of Samuelson (1999) in bovine who reported a much lower average diameter of pupil. The iris divided the anterior ocular compartment into anterior and posterior chambers which communicated through the pupil and was filled with aqueous humor. The anterior surface was dark brown in colour in most of the samples. The posterior surface was directed towards the lens and ciliary processes. This was in accordance with observations of Samuelson (1999) in bovines and Barhaiya *et al.* (2014) in goat.

Histologically, the iris was a thin delicate cell layer and consisted of pigmented layer, dilator muscles, stroma, sphincter muscles and rostral stromal sheath (Fig.2). The pigmented layer consisted of fibroblasts and pigmented cells, the melanocytes. This layer was rich in mucopolysaccharides. The dilator muscle and the sphincter muscle layers were composed of smooth muscle fibers. The sphincter muscles laid around the pupillary margin closely associated with pigmented layer on the posterior surface of the iris. The dilator muscle fiber contained some pigmented cells. The stroma was loosely arranged except around blood vessels and nerves, where it formed dense sheaths. It consisted of loose network of collagen fibers, smooth muscle fibers, blood vessels, melanocytes and fibroblasts. Various micrometrical observations of iris recorded have been tabulated in table 2. The present histological finding of iris was in accordance with Khaled (2003) in bovines and Zayed *et al.* (2012) in buffaloes.

Iridocorneal Angle

The iridocorneal angle was the structure associated with base of the iris, the anterior ciliary body and the inner aspect of sclero-corneal junction. It consisted of bundles of collagen fibers, fibroblast, pigmented cells, and trabecular mesh work (Fig.3). The pectinate ligament was located on the anterior part of the iridocorneal angle. It was strong band like structure extending from iridial base to the limbic zone. Trabecular tissue had two parts: the uveal part and the sclero-corneal part. The uveal meshwork was the inner part of the trabecular meshwork and was composed of numerous strands of trabeculae. The sclero-corneal meshwork was the external part of the trabecular meshwork and was characterized by small trabeculae with small inter trabecular spaces. The histological findings of iris were in accordance with those reported by Kassab *et al.* (2001) in buffalo. The morphology of the iridocorneal angle is clinically important as the aqueous humor leaves the anterior chamber of the eye through it (Gelatt, 2007).

Ciliary Body

The ciliary body was located at the base of iris and was the largest component of the anterior uvea. It comprised of ciliary rings, processes and ciliary muscles. The ciliary ring was a narrow posterior zone lying immediately anterior to the choroid. On its inner surface there were numerous fine ridges arranged in meridional direction. The ciliary processes were formed by the projection from the ciliary body into the posterior chamber. The ciliary processes were about 95 to 107 in numbers. Similar observation was reported by Dyce *et al.* (1987) in domestic animals, Samuelson (1999) in bovines and Shakir *et al.* (2013) in camel. The ciliary process play variable role in lenticular accommodation because these structures are intimately associated with a crystalline lens.

Histologically, the ciliary body consisted of six layers (Fig.4). Micrometrical observations on thickness of various layers of ciliary body have been tabulated in table no.3. The supraciliaris layer was the most peripheral layer of the ciliary body. It was

loosely structured, consisting of bundles of collagen fibers. The ciliary muscle comprised of three layers of smooth muscle fibers. The stroma of the ciliary body comprised of a large number of blood vessels and extended as dense network of capillaries into the ciliary processes. The epithelium was simple cuboidal with rounded nuclei. It was heavily loaded with round or oval melanin granules (Fig.4). The base of the cell was characterized by deep invaginations of the plasma membrane. The non-pigmented epithelial layer was the internal cellular lining of the ciliary body. Its cuboidal or low columnar cells contained oval nuclei. The surface membrane of this layer showed deep apical invaginations. The cells proceed forward on the posterior surface of the iris and were heavily pigmented. Each ciliary process consisted of a central core of connective tissue stroma and blood vessels covered by a double layer of epithelium: an inner pigmented cuboidal epithelium and an outer non-pigmented cuboidal epithelium (Fig.4). The present observation was in accordance with the reports of Samuelson (1999) in horse, Hees and Sinowatz (2000) in bovine, Shakir *et al.* (2013) in camel and Barhaiya *et al.* (2014) in Marwari goat.

Table 3: Mean thickness of the different layers of the ciliary body (µm)

Supraciliaris layer	15.03 ± 1.29
Ciliary muscle	89.53 ± 3.09
Stroma	866.68 ± 4.19
Bruch's membrane	7.03 ± 0.49
Pigmented epithelium	38.72 ± 1.31
Non-pigmented epithelium	28.09 ± 0.94

Choroid

The choroid was deeply pigmented tunic of dark brown, lying between the sclera and the retina. Anteriorly it was continuous with the ciliary body. Posteriorly it was pierced by the optic nerve. It was the site from where the ciliary vessels and nerve passed the choroid. Its outer surface was connected to the inner surface of the sclera. Internally, the choroid was intimately related to the pigment epithelium layer of the retina. Barhaiya *et al.* (2014) also reported that the choroid was anterior continuation of ciliary body and joined with the iris in case of goat. This was in agreement with our present observation.

Though the choroid was dark-brown in colour but a large area of its wall, above the point of entrance of the optic nerve had a peculiar metallic luster called as tapetum (Fig.5). This highly pigmented layer of the choroid absorbed light rays so that they were not reflected back out of the eyeball. Similar observation was also cited by Dellmann and Brown (1976) in domestic animals.

Table 4: Mean thickness of the different layers of the choroid at equator (µm)

Supra choroid layer	14.35 ± 0.97
Tapetum lucidum or stroma	19.04 ± 1.59
Chorio capillary layer	11.31 ± 1.41
Basal complex	14.56 ± 0.72
Vessel layer	5.8 ± 0.69
Total choroidal thickness	**66.53 ± 1.43**

Histologically, the choroid was sub divided into five layers viz; Supra choroid layer, Vessels layer, Stromal layer, Chorio-capillary layer and Basal complex (Fig.6). Micrometrical observations on thickness of various layers of choroid have been tabulated in table no.4. The most peripherally found supra choroid layer consisted of bundles of collagen fibers. The cell population of this layer consisted of fibroblasts and numerous flat melanocytes. Similar observations were cited by Samuelson (1999) in horse and Hees and Sinowatz (2000) in bovines. The vessel layer was situated just after suprachoroid layer. It consisted by intercrossing large and medium sized arteries and veins, separated by loose connective tissue stroma rich in chromatophores, similar to that of the suprachoroid. The vessel layer was containing strands of smooth muscle (Fig.6). The whole membrane was homogenous and revealing very clearly the passage of the capillaries. It was light reflecting layer. The tapetum was located mainly in the dorsal half of the fundus of the eye ball. Prince *et al.* (1960) in bovine and Samuelson (1999) in horse stated that the lamina of the tapetum was up to 8 µm thick, and the entire membrane varied in thickness from10 µm at the periphery to 50 µm at the center in domestic animals. The observation was quite similar with the present findings.

The chorio-capillary layer was containing a dense network of capillaries. It was immediately adjacent to the pigmented epithelial layer of the retina. The stromal layer consisted mainly of delicate collagenous and elastic networks, fibroblasts and occasional melanocytes. The basal

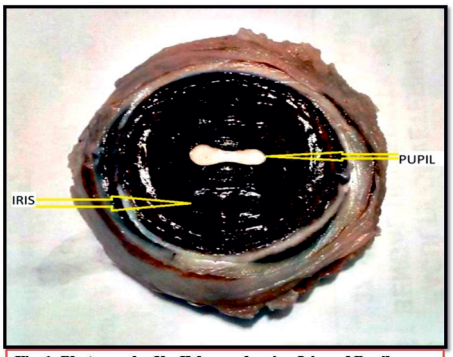

Fig. 1: Photograph of buffalo eye showing Iris and Pupil

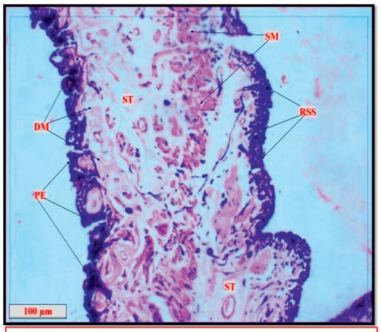

Fig. 2: Photomicrograph of Iris showing Pigmented Epithelium (PE), Dilator Muscle (DM), Stroma (ST), Sphincter Muscle (SM) and Rostral stromal Sheath (RSS). H&E 20x

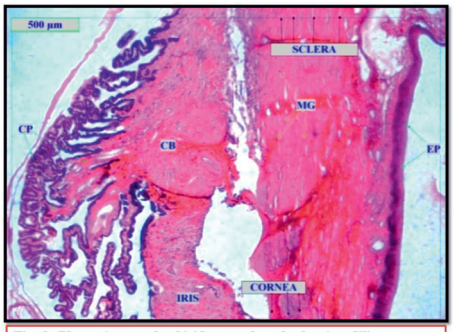

Fig. 3: Photomicrograph of iridocorneal angle showing Ciliary process (CP), Ciliary body (CB), Melanin granules (MG) and Epithelium (EP). H&E 4x

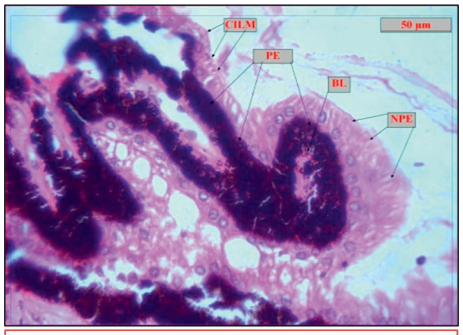

Fig. 4: Photomicrograph of ciliary body showing Ciliary Inner Limiting Membrane (CILM), Pigmented layer (PE), Basal Lamina (BL) and Non pigmented layer (NPE). H&E 40x

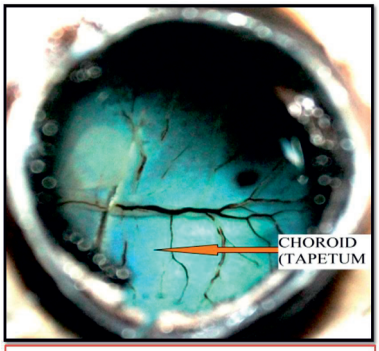

Fig. 5 : Photograph of buffalo eye showing Tapetum of Choroid

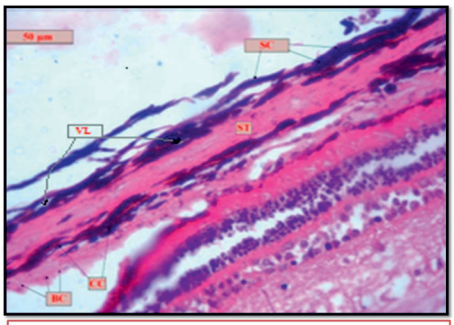

Fig. 6: Photomicrograph of choroid showing Supra Choroid (SC), Stroma (ST), Chorio-capillary layer (CC) layer, Basal Complex (BC) and Vessels layer (VL). H&E 40x

dense network of capillaries. It was immediately adjacent to the pigmented epithelial layer of the retina. The stromal layer consisted mainly of delicate collagenous and elastic networks, fibroblasts and occasional melanocytes. The basal complex was found in between the chorio-capillary layer and pigmented epithelium, which separated the choroid from retina (Fig.6). The present findings were similar to that of the reports of Prince *et al.* (1960) in cattle.

CONCLUSION

The present study revealed that the vascular tunic of eyeball of Murrah buffalo was a deeply pigmented tunic and was composed of anterior ciliary body and iris and posterior choroids,. The histological observations in the present study were similar as compared to other species but with varying micrometrical data. Numerous blood vessels in the choroid and ciliary body seem to provide the required nutritional supply to the retina. Choroid also contained melanocytes that might play a role in absorbing excess light thus preventing reflection and scattering of light within the eyeball. The tapetum was located mainly in the dorsal half of the fundus of the eye ball. The present gross and histological observations on the vascular tunic in Murrah buffalo might act as a basic data of eyeball in this breed of buffalo for future research.

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