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# Microanatomical studies on age related changes in the macula retina of buffaloes (*Bubalus bubalis*)

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#### Abstract

The retina macula appeared as strip-like and pale in colour located dorsal to the optic disc in buffaloes. Retina macula contained large RPE cells with tightly packed melanin pigment, a relatively larger number of cone cells, cells of inner nuclear, inner plexiform and nerve fibre layers showed less cystoid degeneration when compared to the peripheral retina. The total thickness ( $\mu$ m) of retinal macula in groups I, II and III was 229.05±3.56, 230.55±3.28 and 231.72±2.87 respectively. There were few degenerating changes observed in the macula when compared to the peripheral retina with the advancement of age in buffaloes.

Keywords: Histology, ageing changes, retina macula, buffaloes

# Introduction

In the Indian rural economy the buffalo plays an important role and contributes about 60% of total milk production. In India buffaloes are preferred over cattle because of their distinctive qualities such as better feed conversion efficiency, more resistance to diseases and higher milk fat percentage than cows (Bandhopadhyay *et al.*, 2003) <sup>[1]</sup>. The age-related changes of the retina in animals primarily lead to a loss of visual sensitivity and color discrimination as well as reduction of the visual area. Impairment of visual functions in aged animals has long been considered the consequence of the opacity of the optic media (Cavallotti *et al.*, 2001) <sup>[4]</sup>. Hence, the present study was carried out to establish basic data about ageing changes in the retina macula of the buffalo eyes.

# **Materials and Methods**

The present study was conducted on the eyeballs of 63 buffaloes at the Department of Veterinary Anatomy, College of Veterinary Science, Proddatur. The eyeballs of buffaloes were collected from the slaughterhouses located around Proddatur, irrespective of breed and sex of the animal. The buffalo's age was estimated by dentition. Total samples were categorized into 3 groups based on their age i.e., group I (1-5 yrs), group II (6-10 yrs) and group III (11 yrs and above). The eyeballs were dissected out from the orbital cavity immediately after slaughter and for initial fixation; Davidson's fixative fluid was injected into the anterior and posterior compartments of eyeballs. Then the samples were kept in the same fixative for 72 hours in the Davidson's fixative fluid (Latendresse *et al.*, 2002) <sup>[10]</sup>. For histological studies, the paraffin sections were subjected to Hematoxylin and Eosin staining for routine histological study and Fontana–Masson stain for melanin pigment. Further, micrometry was also done to study the thickness of the different layers of the retinal macula. These observations were subjected to statistical analysis (Snedecor and Cochran, 1994) <sup>[12]</sup> by using SPSS software.

# **Results and Discussion**

In the present study the macula retina or fovea in the retina of buffaloes was appeared as strip like and a pale coloured band located dorsal to the optic disc in temporal end (Fig. 1). Similarly, Konig and Liebich (2009)<sup>[9]</sup> also noted strip like retina macula in horse, pig, dog and ruminants.

Whereas, Dellmann and Eurell (2006) <sup>[6]</sup> opined that macula was a round or oval area located dorsolateral to the optic disc in domestic animals contrary to the present findings. Yu *et al.*, (2020) <sup>[13]</sup> reported that the fovea is located roughly 4.0 mm temporal and 0.8 mm inferior to the centre of the optic disc in the human retina.



Fig 1: Gross photograph of eye ball of buffaloe showing strip like retina macula (arrows)

The retinal pigment epithelial cells of macula were larger and more pigmented compared to peripheral retina in buffaloes (Fig. 2). The cells were cuboidal in shape in group I and enlarged and their height was slightly decreased in group II and III buffaloes. Melanin content in the cells of retina macula was dense in group I (Fig. 2), moderate in group II (Fig. 3) and sparse in group III buffaloes (Fig. 4). No literature is available pertaining to the above observations in domestic animals.

The mean thickness (µm) of retinal pigment epithelium of group I, II and III buffaloes was  $6\pm0.2$ ,  $7.56\pm0.23$  and  $6.11\pm0.18$  respectively (Table.1). There was a significant (p<0.01) decrease in thickness of retinal pigment epithelium of macula of group III buffaloes when compared to group II. But there was no significant difference between group I and III buffaloes in thickness of retinal pigment epithelium of macula in buffaloes. Whereas, Friedmann *et al.* (1968) <sup>[7]</sup> have reported higher and narrower RPE in retinal macula of aged human being contrary to present finding in buffaloes.

The rods and cones in the photoreceptor layer of macula were arranged densely in all age group of animals when compared to peripheral retina. The cones were comparatively more in number than the peripheral retina, but number of rods were more than the cones in the macula of all age groups of buffaloes in the present study. The number of cones was progressively increased from group I to group III buffaloes. The number and density of nuclear profiles in outer nuclear layer was decreased from group I (Fig. 2) to group II (Fig. 5) and to group III buffaloes (Fig. 4). These reports were in correlation with Curcio and Drucker (1993)<sup>[5]</sup> and Gao and Hollyfield (1992)<sup>[8]</sup> who mentioned that the density of rods in the macula decreased to 30% between 34 and 90 years of age, while the number of cones remains stable in humans. The mean thickness (µm) of photoreceptors and outer nuclear layers in retina macula of group I, II and III was 69.1±2.19, 60.94±0.92 and 66±0.97 respectively (Table.1). There was a significant (p<0.01) decrease in thickness of photoreceptors and outer nuclear layer of macula in group II buffaloes when compared to group I and III. Similar observations were also made by Bloom and Fawcett (1970)<sup>[2]</sup> in retinal macula of human beings. Similarly, Curcio et al. (1993)<sup>[5]</sup> and Bonnel et al. (2003)<sup>[3]</sup> in primates, noted reduction of nuclei in the outer nuclear layer of the macula with increase in age.

The outer plexiform layer of macula was comparatively thin when compared to peripheral retina in group I (Fig. 2), group II (Fig. 5) and group III buffaloes (Fig. 4). The mean thickness ( $\mu$ m) of outer plexiform layer of macula in retina of group I, II and III was 8.72±0.28, 8.61±0.25and 7.67±0.14 respectively (Table.1). The above findings suggested that there was a significant (p<0.05) decrease in thickness of outer plexiform layer of group III animals. But there was no significant difference between the group I and group II animals. No literature is available pertaining to the above observations in domestic animals.

The density of the nuclei of horizontal, bipolar and amacrine cells of inner nuclear layer of macula retina was greatly increased, besides that numerous capillary plexuses and astrocytes were also observed in the inner nuclear layer of retina macula than the peripheral retina in group I (Fig. 2), group II (Fig. 5) and group III buffaloes (Fig. 4). It is in conformity with the findings of Dellmann and Eurell (2006)<sup>[6]</sup> in domestic animals. The mean thickness (µm) of inner nuclear layer of retina macula of group I, II and III was 36.28±1.78, 35.11±0.82, and 32.11±0.56 respectively (Table.1). These findings suggested that, there was a significant (p < 0.05) decrease in thickness of inner nuclear layer of group III buffaloes when compared to group I and II. Further, there was a significant difference between group I and II buffaloes, and also between group II and III animals. The above observations confirmed that there is a change in thickness of inner nuclear and inner plexiform layer of macula retina of buffaloes with advancement of age in buffaloes.

The inner plexiform layer was comparatively thicker, highly vascular and consisted of numerous astrocytes in macula than the peripheral retina in group I (Fig. 2), group II (Fig. 5) and group III buffaloes (Fig. 4). The mean thickness ( $\mu$ m) of inner plexiform layer of retina macula of group I, II and III was 40.22±2.49, 56.05±1.38 and 51.11±1.77 respectively (Table.1). There was a significant (p<0.01) increase in thickness of inner plexiform layer of macula of group II buffaloes when compared to group I and III.

The macula retina of buffaloes consisted of both  $\alpha$  and  $\beta$  ganglion cells in all groups of animals, but their number was slightly decreased with advancement of age compared to peripheral retina. The nerve fiber layer contained the axons of ganglion cells and they were surrounded by Muller cell processes and occasionally by astrocytes. The mean thickness of ganglion cell, nerve fibre layer and inner limiting membrane was not altered with advancement of age in buffaloes unlike peripheral retina. Dellmann and Eurell (2006)<sup>[6]</sup> also opined that the retina macula is different from the remainder of retina as observed in the present study

The size of the blood vessels was increased in nerve fiber layer of retina macula from group I to III animals. Further, the inner limiting membrane was thick and uninterrupted in group I (Fig. 2), group II (Fig. 5) and also in group III buffaloes (Fig. 4).

The mean thickness ( $\mu$ m) of ganglion cell, nerve fiber layer and inner limiting membrane of macula of group I, II and III was 68.72±2.7, 62.22±2.14 and 68.72±1.58 respectively. There was a significant (p<0.01) decrease in thickness of ganglion cell, nerve fiber layer and inner limiting membrane of group II buffaloes when compared to group I and III (Table.1).

The total thickness ( $\mu$ m) of retinal macula in groups I, II and III were 229.05±3.56, 230.55±3.28 and 231.72±2.87 respectively (Table.1 and Fig. 6). There was no significant (p<0.01) increase in thickness of retina macula when compared to group I to II and III and there was no significant difference among three groups of buffaloes.

**Table 1:** Mean thickness  $(\mu m)$  of different layers of retina macula in<br/>buffaloes of different age groups.

	Retina	Group I	Group II	Group III
	Retinal pigment epithelium	6.0±0.2 <sup>b</sup>	7.56±0.23 <sup>a</sup>	$6.11 \pm 0.18^{b}$
	Photoreceptor & Outer nuclear layer	69.11±2.19ª	60.94±0.92 <sup>b</sup>	66±0.97ª
	Outer plexiform layer	$8.72 \pm 0.28^{a}$	8.61±0.25 <sup>a</sup>	$7.67 \pm 0.4^{b}$
	Inner nuclear layer	$36.28 \pm 1.78^{a}$	35.11±0.82 <sup>ab</sup>	32.11±0.56 <sup>t</sup>
	Inner plexiform layer	$40.22 \pm 2.49^{b}$	$56.05{\pm}1.38^{a}$	51.11±1.77 <sup>a</sup>
	Ganglion cell+ Nerve fiber layer+ Inner limiting membrane	68.72±2.7	62.22±2.14	68.72±1.58
	Total thickness	229.05±3.56	230.5±3.28	231.72±2.87

Mean values with different superscripts in rows differ significantly (p<0.05 and 0.01)

One way ANOVA, SE -Standard Error.



Fig 2: Photomicrograph of retina macula of buffaloes showing Bruch's membrane(BM), Retinal Pigment Epithelium (RPE), Rod cells (RC), Cone cells (CC), Muller cells (MC), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL), Horizontal cells (HC), Bipolar cells (BC), Amacrine cells (AC), Inner Plexiform Layer (IPL), Astrocytes (AS), α- Ganglion cells (AGC), β-Ganglion cells (BGC), Nerve Fiber Layer (NFL) and Inner Limiting Membrane (ILM). Haematoxylin and Eosin X 400



Fig 3: Photomicrograph of retina macula of group II buffaloes showing moderate melanin pigment in retinal pigment epithelium (Arrow). Fonatana -Masson X 40



Fig 4: Photomicrograph of retina macula of group III buffaloes showing Bruch's Membrane (BM), Retinal Pigment Epithelium (RPE), Rod cells (RC), Cone cells (CC), Muller cells (MC), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL), Horizontal cells (HC), Bipolar cells (BC), Amacrine cells (AC), Inner Plexiform Layer (IPL), Astrocytes (AS), α- Ganglion cells (AGC), β-Ganglion cells (BGC), Nerve fiber layer (NFL) and Inner Limiting Membrane (ILM). Haematoxylin and Eosin X 100



Fig 5: Photomicrograph of retina macula of group II buffaloes showing Bruch's membrane (BM), Retinal pigment epithelium (RPE), Rod cells (RC), Cone cells (CC), Muller cells (MC), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL), Horizontal cells (HC), Bipolar cells (BC), Amacrine cells (AC), Inner Plexiform Layer (IPL), Astrocytes (AS), α- Ganglion cells (AGC), β-Ganglion cells (BGC), Nerve Fiber Layer (NFL) and Inner Limiting Membrane (ILM). Haematoxylin and Eosin X 100



**Fig 6:** Mean total thickness (μm) of retina macula in buffaloes of different age groups

#### Conclusion

In conclusion, the present study offers detailed insights into the macula retina of buffaloes, revealing distinctive structural characteristics and age-related changes. Unlike previous assertions, the macula in buffaloes appeared as a strip-like, pale-colored band located dorsally to the optic disc, differing from other domestic animals. The retinal pigment epithelial cells exhibited varying melanin content and size, with a significant decrease in thickness observed in older buffaloes. Notably, the density of rods and cones in the photoreceptor layer differed in the macula compared to the peripheral retina, indicating age-related variations. The inner nuclear and plexiform layers showed significant alterations in thickness with age, along with changes in ganglion cell density. Despite these age-related variations, the total thickness of the retinal macula remained relatively consistent across age groups. These findings provide valuable insights into the structural dynamics of the macula retina in buffaloes, shedding light on potential age-related adaptations and contributing to our understanding of comparative retinal anatomy in domestic animals. Further research is warranted to elucidate the functional implications of these structural changes and their relevance to buffalo ocular health.

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