Comparative morphology of the iris of donkey *(Equus asinus)* and buffalo *(Bos bubalis)*

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With 16 figures, 3 histograms, 5 tables. Received October 2011, accepted for publication January 2012

Abstract

This work was carried out on 22 eyeballs collected from two animal species namely donkey and buffalo (11 from each) to elucidate gross anatomical, light and scanning electron microscopical features of the iris in addition to some morphometrical characteristics. The iris constitutes a variable relative surface area (in relation to uvea) being the highest in the donkey. Breadth of the iris varies topographically. In buffaloes, the dorsal and ventral parts are wider than the medial and lateral ones with a subsequently oval pupil. In donkeys the breadth of the iris is nearly uniform encircling a semicircular pupil. The dorsal side of the pupillary border of the iris carries several variably-sized black masses (*Corpora nigra*). Anterior and posterior surfaces of the iris are studded by circular and longitudinal folds indicating the arrangement of constrictor and dilator pupillary muscles, respectively. The constrictor muscle occupies variable breadth of the iris indicating its myotic strength, while the thickness of the dilator indicates its medriatic efficiency.

Keywords

Morphology – iris – donkey - buffalo

Introduction

Functions of the vascular tunic of the eyeball include; regulating the amount of the light entering the eyeball through the pupil, distributing the light within the eyeball, producing the aqueous humor, changing the visual focus via the ciliary muscles, providing nutrition to the structures within the eye and increasing the photostimulation of the retina under low light levels (Davson, 1963, Bloom and Fawcett, 1970 and Samuelson, 1999).

The anatomy and histology of the vascular tunic of the eyeball have been extensively studied by several authors (Prince et al. 1960, Bellairs et al. 1975 and De Lahunta and Habel, 1986, Samuelson, 1991 and 1999, Slatter, 2001 and Aly, 2003).

Previous studies on the vascular tunic of the eyeball focused either on anatomical or structural aspects. However, detailed studies on this tunic relating morphometry, anatomy, scanning electron microscopy, light microscopy and immunohistochemistry are lacking in the available literature. The current study aims to elucidate the morphometrical, anatomical and structural characteristics of the vascular tunic of the bulb of the eye in two species of domestic animals differing in their mode of life and feeding habits. The study also aims to relate all the structural characteristics of the vascular tunic to their functional adaptations in the studied species.

Materials and Methods

The present work was carried out on 22 eyeballs, collected from each of the two animal species (11 eyeballs from each species); namely donkey (*Equus asinus*) and buffalo (*Bus bubalis*). In case of buffalo, the eyeballs are collected from Assiut slaughter houses while those of donkey were obtained from the dissecting room of the Department of Anatomy and Histology and the Teaching Hospital of the Faculty of Veterinary Medicine, Assiut University. All specimens were obtained from adult and clinically healthy animals of both sexes.

The eyeballs were carefully excised and dissected from the surrounding periorbital fat and extraocular muscles, then weighed in grams. The total volume of each eyeball was determined using fluid displacement (Table 1). The aqueous humor was aspired from each eyeball and its volume in (ml) was also determined by using measuring cylinder.
For gross anatomy, five eyeballs were used from each studied animal. The eyeballs were incised at the equator, the vascular tunic was then separated and its total volume as well as the volume of iris was measured using fluid displacement. Total surface area of the vascular tunic as well as the surface area of the iris were measured. In addition, the dimensions (vertical and horizontal diameters) of the pupil were measured. The materials were then preserved in 10% formaldehyde solution (formalin) and kept for further examination.

Using the obtained measurements, some relative calculations were

made and all were listed in tables (1-6) and displayed as histograms (1-3).

For scanning electron microscopy three eyeballs from each studied animal were used. Small pieces were taken from the iris, fixed in a mixture of paraformaldehyde solution (2.5%) and glutaraldehyde solution (2.5%) in phosphate buffer for 24 hours. The specimens were then washed in 0.1M phosphate buffer (7.3 pH), dehydrated in ascending graded ethanol, critical point - dried in liquid carbon dioxide, then coated with gold palladius in sputtering device. The specimens were then examined and photographed using JSM-5400 LV Scanning electron microscope operated at 20 KV in the EM Center of Assiut University.

For histological studies, three eyeballs from each studied animal were immediately obtained after slaughtering. For paraplast embedding, small pieces were taken from different parts of the iris, fixed in Bouin's solution for 24 hours. After proper fixation, the specimens were dehydrated in graded ethanol, cleared in methyle benzoate, embedded in paraplast and sectioned at 3-5 µm thick. The prepared sections were stained with haematoxylin and eosin for general histological description and Masson's trichrome stain for detection of muscles (Drury et al. 1967).

For Epon-araldite embedding, small pieces of the iris were fixed in paraformaldehyde - glutaralde-hyde solution in phosphate buffer (Karnovsky, 1965). Specimens were post-fixed in 1% osmium tetraoxide for one hour, washed in 0.1M phosphate buffer (7.3 pH), then dehydrated in graded ethanol and embedded in epon-araldite mixture (Mollenhauer, 1964). Semi thin sections (1µm thick), were cut, and stained with Toluidine blue (Richardson et al. 1960) and examined microscopically.

All sections were examined with light microscope and photographed. In addition, some morphometric measurements were made using an image analysis system (Leica Q500). These measurements included: height of iridal epithelia as well as breadth and thickness of the iridal muscles (constrictor and dilator, respectively). In addition, some relative calculations were made in order to facilitate comparison between the studied animals.

For the immunohistochemical studies, some of the paraffin sections were used to visualize iridal muscles (Avidin-Biotin-Complex Method) according to (Hsu et al. 1981).

Sections were deparaffinized in xylene for 20 minutes, rehydrated in a

graded series of alcohol and distilled water. After washing three times in phosphate buffer (pH 7.4) for 5 minutes each, endogenous peroxidase was inhibited with 1% H_2O_2 for 10 minutes. After intense washing in normal tap water for 10 minutes, the sections were washed in phosphate buffer (three times, 5 minutes each). The sections were covered with DAKO protein block serum-free for 10 minutes at room temperature. They were then incubated with the specific primary antibody (smooth muscle actin, 1:40) for 12 hours at 4ºC. After washing with PBS for 5 minutes, incubation with a secondary antibody (IgG biotin from rabbit, 1:300) for 30 minutes at room temperature was performed. All previous substrates were supplied by DAKO, Hamburg, Germany.

After washing in phosphate buffer saline (three times 5 minutes each) the sections were incubated with streptavidin-biotin horseradish peroxidase complex (DAKO, Hamburg, Germany) for 30 minutes at room temperature. The reaction was developed using diaminobenzidin solution for 10 minutes in room temperature. Sections were dehydrated by using graded series of alcohols, cleared with xylene, and covered with DPX.

The nomenclature used in this study is that adopted by Nomina Anatomica Veterinaria (1993).

Results

Anatomy:

The iris is the direct anterior continuation of the ciliary body. It is a delicate and adjustable diaphragm perforated centrally by the pupil. It is insinuated between the cornea and the lens partially dividing the anterior segment of the eye into anterior and posterior chambers. It constitutes a variable volume proportion (in relation to the eyeball) in the two studied species. The volume proportion in buffaloes (about 1.2%) is higher than that in donkeys (about 0.9%) as shown in table (2). The absolute and relative surface area of the iris in relation to the whole vascular tunic represents also different values between studied species (Tables 3, 4 and Fig 1). It constitutes the higher relative surface area in donkeys (about 12.17%) than in buffaloes (about 9.43%). The breadth of the iris varies topographically in the studied animals (Table 5). In buffaloes, the dorsal and ventral breadths are larger than the medial and lateral ones while in donkeys the breadth of the iris is nearly uniform circumferentially. Accordingly, the vertical and horizontal diameters of the pupil vary in

studied animals (Fig 2). It is transversely oval in buffaloes, but it is semicircular in donkeys.

The iris has two surfaces; anterior and posterior and two borders; pupillary and ciliary borders. The anterior surface is dark brown in color in both studied animals. It is characterized by the presence of two zones; central pupillary and peripheral ciliary demarcated by an annular elevation (collarette). The ciliary zone is marked by a series of fine radial striations. The pupillary zone, however, demonstrates a number of circular folds, it is very wide in donkeys and relatively wide in buffaloes (Figs 3, 5). The posterior surface of the iris is generally black in color and presents numerous fine radial lines except for a narrow band at the pupillary margin. This surface is partially covered peripherally by the anterior ends of the ciliary processes in all studied animals (Figs 4, 6). The pupillary margin encircles the pupil. In both studied species, this margin carries several black masses (*Corpora nigra* or *Granula iridica*) of variable size specially on the upper pupillary margin. These masses are larger in donkeys than buffaloes.

Scanning electron microscopy:

The iridal surface is studded by numerous radial and annular folds indicating the arrangement of the dilator and constrictor pupillary muscles, respectively. The annular folds are predominant on the anterior surface while the radial folds are predominant on the posterior one. The radial folds are variably thick even along the length of the same fold. They are separated by variably deep intervening grooves (Fig 7, 8). In both studied animals, these folds are thrown into numerous secondary transverse ridges. These ridges are prominant in donkeys (Fig 7) and weakly developed in buffaloes (Fig 8).

Light microscopy:

The iris is covered anteriorly by a thin epithelial layer and posteriorly by a thick pigmented epithelium. The stroma of the iris is composed of a network of fine collagen fibers, which host blood vessels, iridal muscles and numerous melanocytes (Figs 9-12). The anterior epithelium (Figs 13, 15) consists of flat or fusiform cells underlined by a thin layer of spindle-shaped melanocytes (anterior stromal sheath). The posterior epithelium is formed of highly folded layer of cuboidal to low columnar pigmented epithelial cells (Figs 14, 16). The cells of this epithelium are filled with coarse, dark brown melanin granules that obscure the nuclei and cytoplasmic details. In buffaloes, this epithelium demonstrates apically placed vacu-

olar aggregations (Fig 16). Toward the pupillary border of the iris, the posterior pigmented epithelium demonstrates cystic-like collections forming the *Corpora nigra*.

The iridal muscles are represented by dilator and constrictor pupillary muscles (Figs. 11, 12, 17, 18). The dilator muscle runs longitudinally below the posterior epithelium of the iris with which it is closely intimated. It occupies different proportional thickness of the iris in the examined animals (Table 6 and Fig. 19). It represents 1.5% in donkeys and 0.9% in buffaloes. The constrictor pupillary muscle is an annular band of smooth muscle bundles occupying nearly the same breadth (about 28%) in relation to that of the iris in both studied species (Table 6 and Fig. 19).

Discussion

The iris is the most anterior segment of the vascular tunic representing a delicate and adjustable diaphragm perforated centrally by the pupil. The breadth of the iris varies topographically in the studied animals. The dorsal and ventral breadths are larger than the medial and lateral ones in buffaloes, while in donkeys, the breadth of the iris is nearly uniform circumferentially. In donkey, however, the pupil has a longer horizontal axis than the vertical one. The horizontal pupil is believed to be of an adaptive advantage providing them with a wider field of vision (Rahi et al. 1980).

The pupillary border of the iris carries several black masses of variable size (*Corpora nigra* or *Granula iridica*) specially on the upper side. These masses are large in donkeys than buffaloes. Prince et al. (1960) mentioned that the equine *Corpora nigra* of the upper border are capable of meeting and fitting between those of the lower border and, thus, divide the pupil into medial and lateral apertures. Misk et al. (1998) reported that the presence of *Corpora nigra* gives the pupil of buffaloes a dumb-bell shaped appearance. Moreover, Samuelson, (1999) mentioned that the *Corpora nigra* augment the effectiveness of pupillary constriction i.e. miosis. The principal function of the iris is to regulate the amount of light entering the eyeball (Tortora and Anagnostakos, 1981). It seems that Corpora nigra may play a role in decreasing the amount of light entering the pupil in the two studied species which spend most of the day outdoors in fields.

Heavy pigmentation of the iris in its anterior and posterior epithelia as well as in the stroma has been postulated to prevent the passage of

the light into the interior of the eye except through the pupil (Junqueira et al. 1998). This function is achieved by contraction of the muscles of the iris, which are arranged annularly (constrictor) and radially (dilator). In species with transversely elongated pupils(like equines), the dilator muscle is poorly developed adjacent to the long axis of the pupil (Prince et al. 1960). In the same concern, (Patt and Patt, 1969) has assumed that animals whose habits expose them to a little variability in light intensity generally have limited iridal excursion- i.e., difference in pupil diameter between fully constricted and fully dilated iris. Maximal excursion is a characteristic of animals that are active both under conditions of dim light and full sunshine.

The present findings, of the scanning electron microscopy, reveal that the posterior surface of the iris is studded by longitudinal folds indicating the arrangement of the dilator pupillary muscle. These folds are thrown into numerous transverse ridges that have been attributed to the circumferential arrangement of the covering epithelial cells (Donovan et al. 1974). These transverse ridges could be also an indication to the strength of the dilator pupillary muscle. This postulation explains why these ridges are clear in donkeys, which have strong dilator muscles contrasting the picture in buffaloes.

In all studied animals, the anterior surface of the iris is covered by a single layer of flat epithelial cells, which has been considered as continuation of the corneal endothelium (Leeson and Leeson, 1970). In agreement with (Dellmann, 1971 and Diesem, 1975), the anterior epithelium is underlined by anterior stromal sheath in the form of a layer of fusiform or rod-shaped melanocytes contained in a network of fine reticular and collagen fibers, which give the color of the iris.

The posterior iridal epithelium is greatly folded and is closely intimated with the dilator pupillary muscle. (Ham, 1974) has mentioned that the iris is covered posteriorly by two layers of pigmented epithelial cells continuous with the two layers of the retinal epithelium, which line the ciliary body. In the same respect, (Dellmann, 1971 and Misk et al. 1998) have reported that the deep layer of the posterior epithelium is transformed into the dilator pupillary muscle.

The posterior iridal epithelium also demonstrates apically placed vacuolar aggregations, which seem to be extensions of the *Corpora nigra,*

which have the same structural appearance. (Samuelson, 1999) has considered the corpora nigra as extensions of the posterior pigmented epithelium.

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Table (1): Absolute volume (cm^3) of the eyeball, vascular tunic and iris.

Animal	Donkey	Buffalo
Eyeball	32.6 ± 3.27	34.2 ± 1.81
Vascular tunic	2.5 ± 0.38	2.3 ± 0.36
Iris	0.3 ± 0.06	0.6 ± 0.09

Table (2): Relative volume of some parts of the vascular tunic and iris in relation to its total volume

Animal	Donkey	Buffalo
Vascular tunic	7.63%	6.57%
Iris	0.90%	1.20%

Table (3): Surface area (cm^2) of the vascular tunic and iris in studied animals

Animal	Donkey	Buffalo
Vascular tunic	44.09 ± 3.5	42.07 ± 3.5
Iris	5.37 ± 0.3	3.97 ± 0.2

Table (4): Relative surface area of the iris in relation to total surface area of eyeball

Animal	Donkey	Buffalo
Iris	12.17%	9.43%

Table (5): Some measurements of the iris and pupil (mm) in different studied animals

Animal	Donkey	Buffalo
Breadth of the iris:		
Dorsally	5.6 ± 0.4	7.0 ± 0.5
Ventrally	5.0 ± 0.4	6.0 ± 0.3
Medially	4.5 ± 0.3	2.5 ± 0.2

Laterally	5.0 ± 0.2	3.0 ± 0.2
Dimensions of the pupil: Vertical diameter Horizontal diameter	 11.5 ± 0.9 13.0 ± 1.1	 6. 5 ± 0.5 13.5 ± 1.0

Table (6): Some morphometric aspects of the iridal muscles

Animal	Donkey	Buffalo
Thickness of dilator muscle / iris	1.5%	0.9%
Breadth of constrictor muscle / iris	28.6%	28.2%

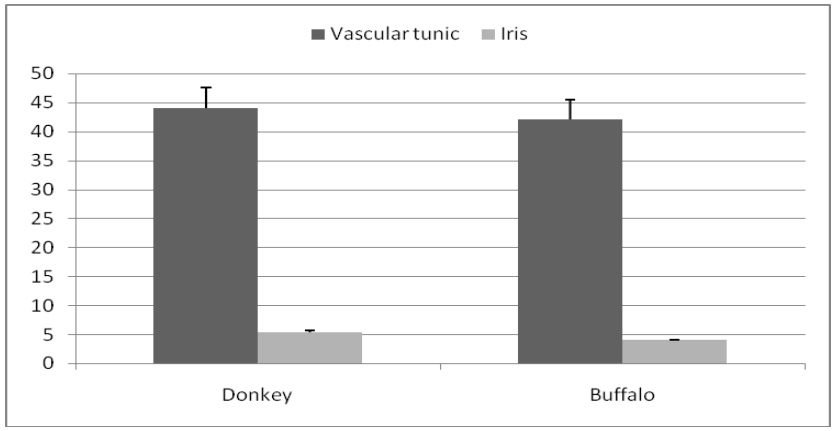


Fig (1): Relative surface area of different parts of the vascular tunic in relation to its total surface area of eyeball

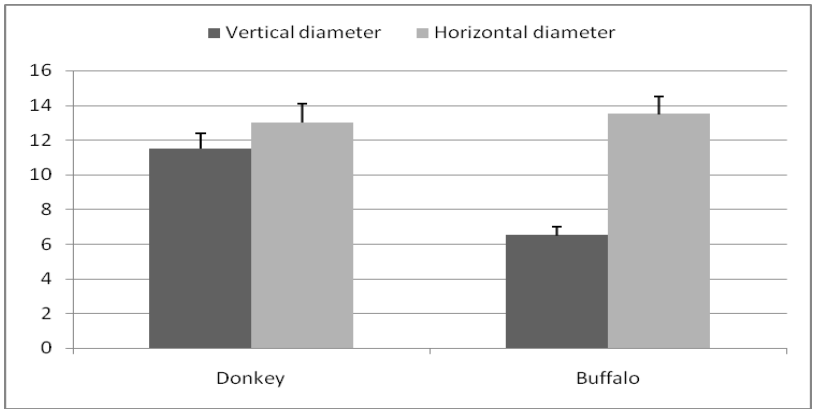
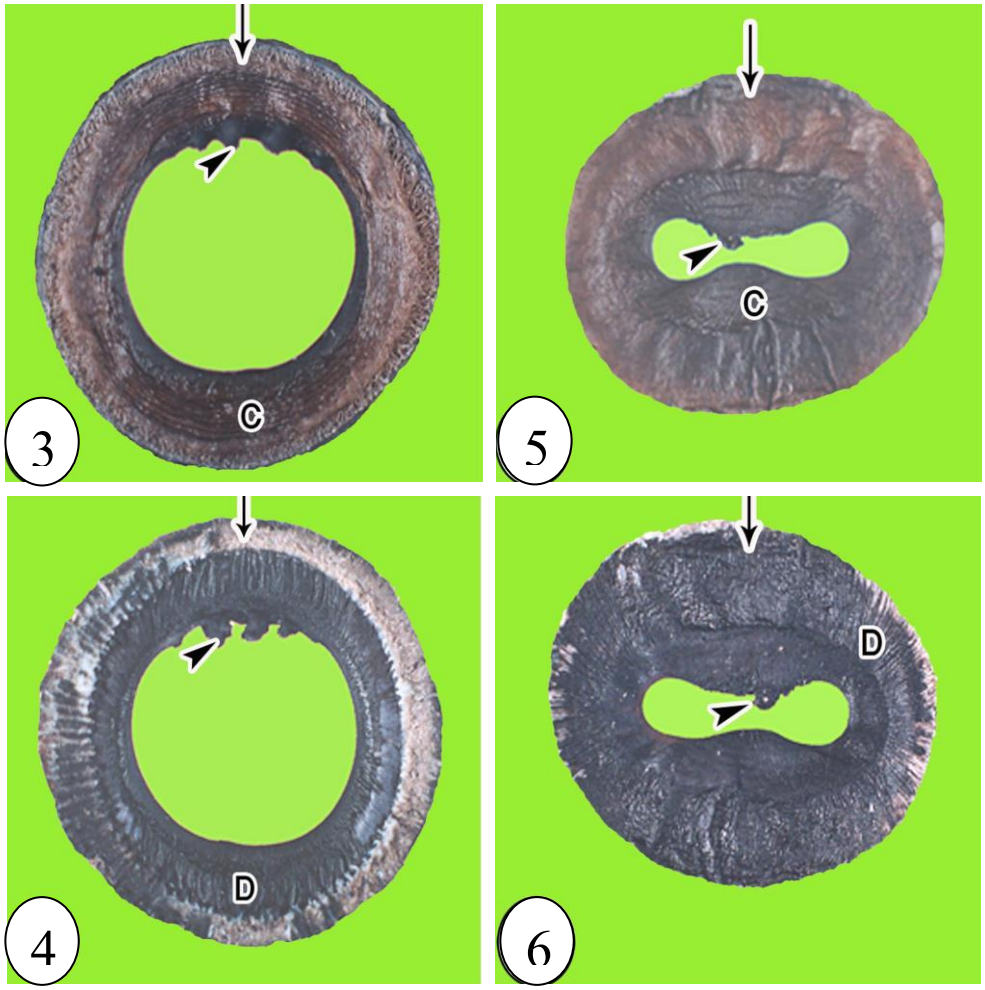
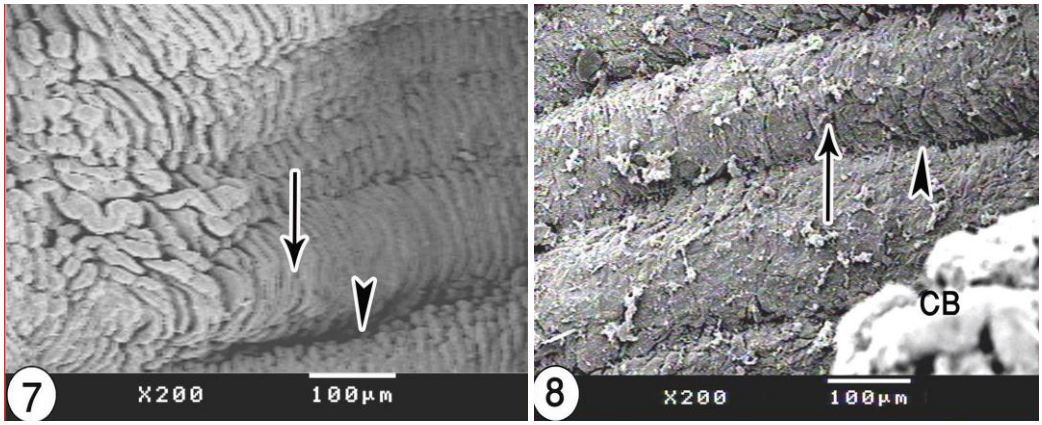


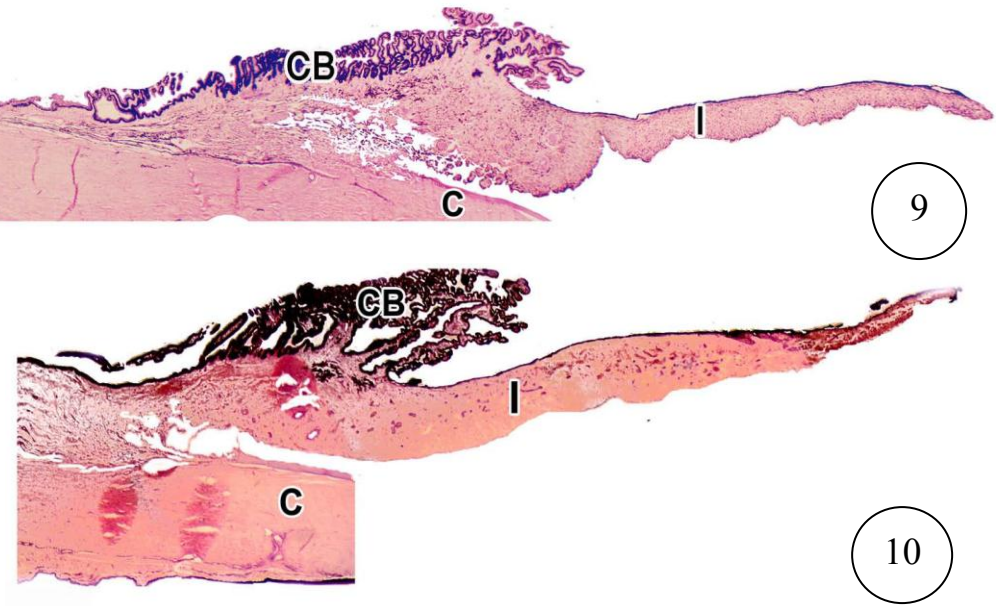
Fig (2): Dimensions of the pupil (mm) in donkey and buffalo



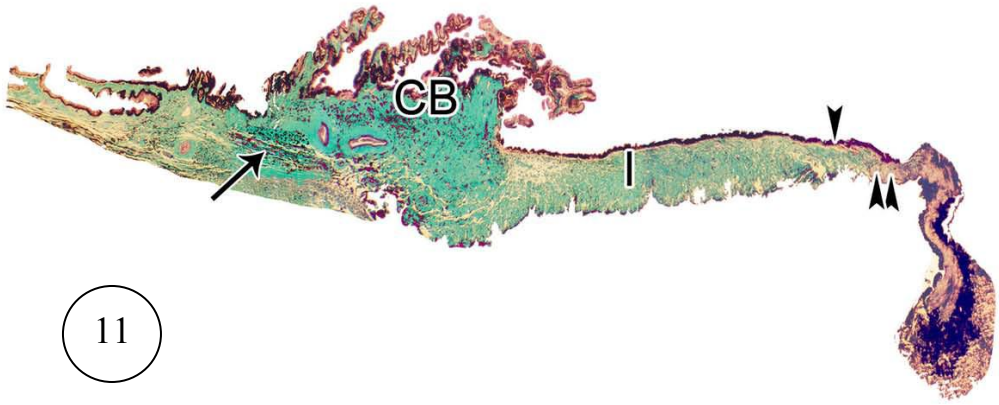
Figs (3- 6): The anterior and posterior surfaces of the iris
Figs (3, 4): The anterior and posterior surfaces of the iris of donkey.
Figs (5, 6): The anterior and posterior surfaces of the iris of buffalo.
The iris is dark brown. The anterior surface shows numerous low annular folds indicating the arrangement of constrictor pupillary muscle (C). The posterior surface demonstrates many radial striations indicating the arrangement of dilator pupillary muscle (D). Arrow points to the dorsal part of the iris. Note the *Corpora nigra* (arrowhead), which are well developed dorsally.

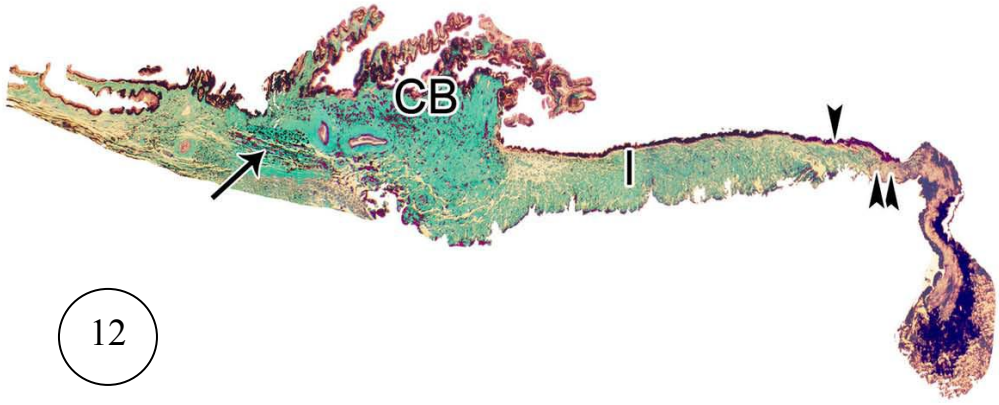


Figs (7- 8): Scanning electron micrographs on the posterior surface of the iris of donkey and buffalo respectively. It shows numerous radial folds (arrow) indicating the arrangement of the dilator pupillary muscle which are separated by variably deep intervening grooves (arrowhead). The ciliary epithelial cells are arranged with their long axis circumferentially oriented. (Fig 7 is of bad quality and not focused).

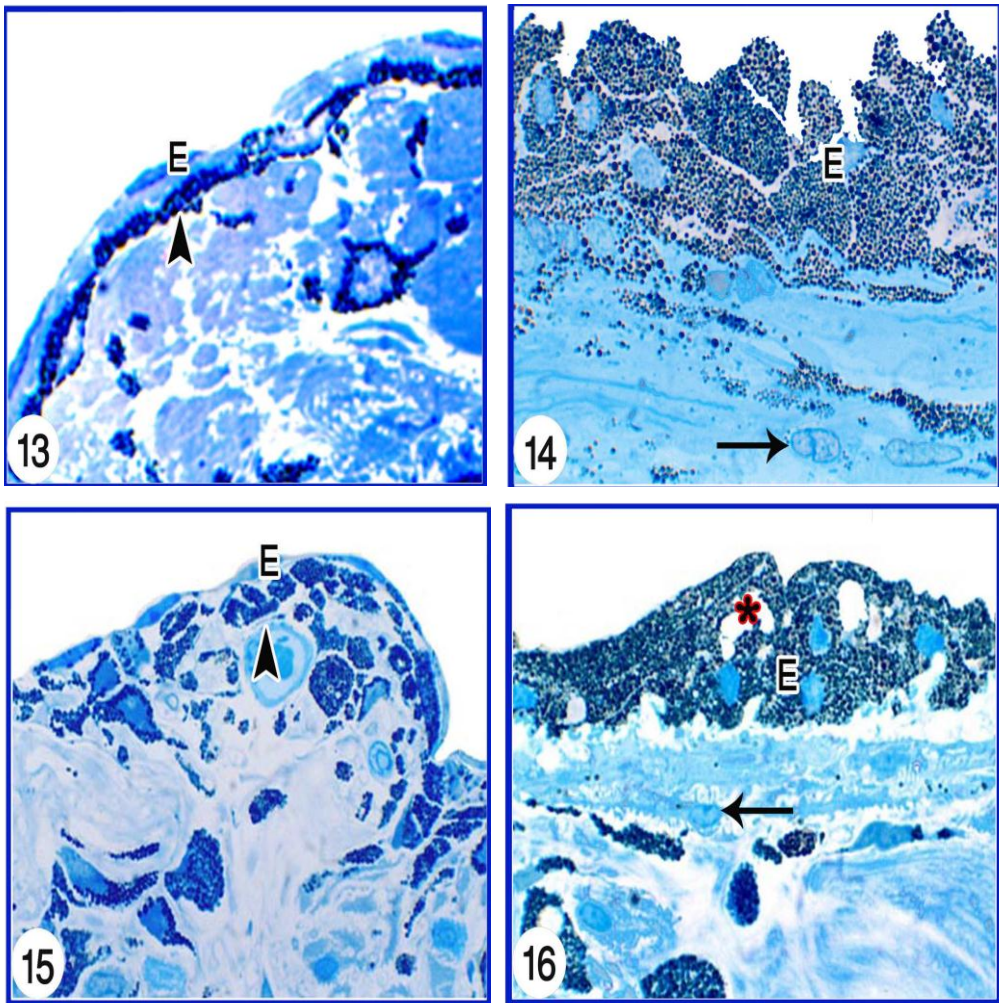


Figs (9 - 10): Paraffin sections showing overviews of the anterior uvea in donkeys and buffaloes, respectively. It consists of the iris (I), ciliary body (CB) and cornea (C). Note the iridocorneal angle (astrisk) where on the figures?, (Haematoxylin and Eosin, X25, insets: X50).

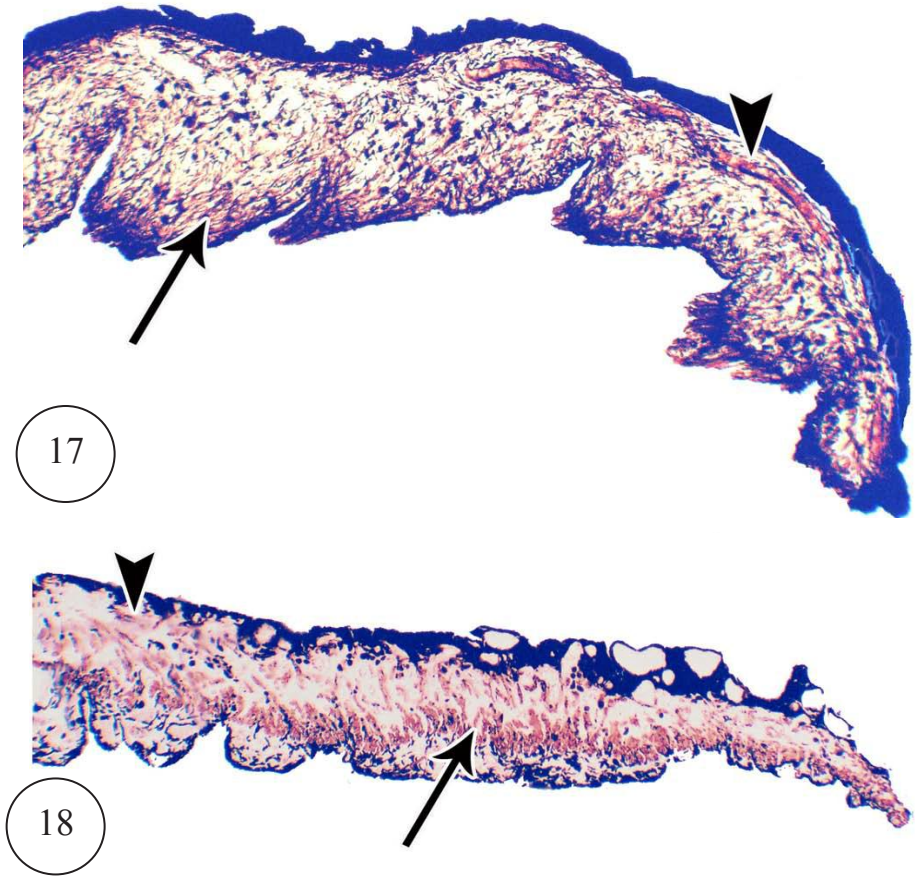




Figs (11- 12): Paraffin sections showing overviews of the anterior uvea in donkeys and buffaloes, respectively. Note the ciliary muscle (arrow) of the ciliary body (CB), dilator pupillary muscle (arrowhead) and constrictor pupillary muscle (double arrowheads) of the iris (I). (Crossmon's trichrome, X25).



Figs (13 -16): Semithin sections showing the anterior (Figs. 13, 15) and posterior (Figs. 14, 16) epithelium of donkeys and buffaloes, respectively. The anterior epithelium (E) is thin consisting of flat or fusiform cells underlined by a thin layer of spindle-shaped melanocytes (arrowhead). The posterior epithelium (E) is formed of greatly folded layer of cuboidal to low columnar pigmented cells. The dilator muscle runs longitudinally below the posterior epithelium of the iris with which it is closely intimated (arrow). In buffaloes, this epithelium demonstrates apically placed vacuolar aggregations (asterisks). Arrows point to the dilator pupillary muscle. (Toluidine blue, X1000)



Figs (17- 18): Paraffin sections showing the iris of donkeys and buffaloes, respectively. The immunolocalization of SMA demonstrates the arrangement of the constrictor pupillary (arrow) and the dilator pupillary (arrow head) muscles. (X50).

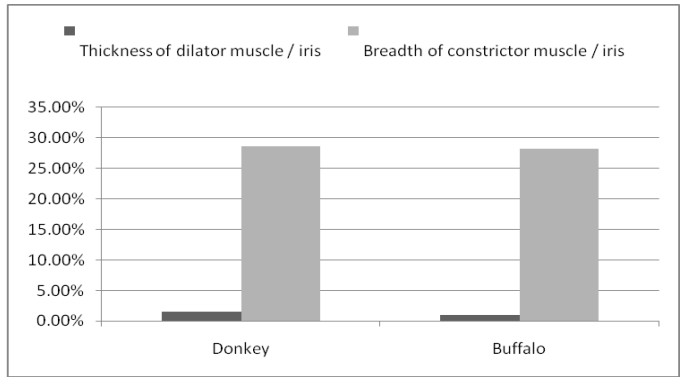


Fig (19): Some morphometric aspects of the iridal muscles in donkey and buffalo