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Original Research Article

ABSENCE OR PRESENCE OF *TAPETUM LUCIDUM*: MACRO AND MICROSCOPIC INVESTIGATIONS IN DONKEY (*EQUUS ASINUS*), CAT (*FELIS DOMESTICA*) AND ONE-HUMPED CAMEL (*CAMELUS DROMEDARIUS*)

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Abstract: For explanation eye-shine phenomenon, we used both eyes of five healthy adult donkeys (*Equus asinus*), cats (*Felis domestica*) and one-humped camels (*Camelus dromedarius*). The eyes of a live animal of the three species were photographed under existing light and with a flash. The donkey's pupils appeared black centrally situated and horizontal in direction at daylight. Under flash, the light condensed centrally and the color changed to green or blue**.** The cat's pupils were oval vertically slit-like and colored yellow to red orange under flash. The camel's pupils characterized by presence of a small centrally spot of light with flash. *Tapetum* of the donkey was horizontal triangular in shape under the weak light colored indigo with dark blue spots changed into semicircular appearance under strong light. In the cat, *Tapetum* appeared semicircular in outline with yellow color under weak and strong light. The fundus of camel appeared divided into dark proximal half and lighter distal one under the weak light. Under flash, the two halves appeared transparent white. Microscopically, *Tapetum lucidum* of the donkey was fibrous in its texture while in cat, it was cellular. In camel, there was a brush's membrane and no *Tapetum lucidum*. In donkey, the thickness of the tapetal tissue and the degree of pigmentation in the retinal epithelium differed according to the region of *Tapetum*. The thick tapetal tissue and the unpigmented retinal epithelium combination created the greater reflectance of light. So the absence or presence of *Tapetum*, the tapetal tissue thickness, the degree of pigmentation in the retinal epithelium and the degree of illumination controlled the eye-shine phenomenon.

Key words: eye-shine; *Tapetum fibrosum*; *Tapetum cellulosum*; brush's membrane; choriocapillaries

Introduction

Eye-shine is phenomenon noticed in a wide range of animal species and is attributed to the presence of the *Tapetum lucidum* (1,2). The vision in a low illumination is getting better by the occurrence of an intraocular light- reflective structure; *Tapetum lucidum*, which giving the photoreceptors another possibility for absorbing the reflected beam (3-5). The high sensitivity to the reflected light from the ground depends on the position of *Tapetum lucidum* (6).

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Regarding the site of the reflective materials, *Tapeta lucida* can be categorized into retinal or choroidal tapetum. Retinal tapeta lucida are familiar in fish and some reptiles, while in mammals are seldom occur (1,7,8). The choroidal mammalian tapetum is present inbetween the thin choriocapillary lamina underneath the pigmented layer of the retina and the choroid (1). The choroidal tapetum is classified into fibrous in the horse, cow, sheep, elephant, sirenians and cetaceans and cellular one in carnivores (2,3).

The visual acuity of the horse is good, about half that of humans and double that of domestic cats (9,10) however, that of dromedary camel is higher than cats and rabbits, less than sheep, giraffes, rhesus monkeys and horse, and almost resemble African elephant and buffalos. The eye of the camel has no choroidal tapetum (11).

Tapetum lucidum is previously studied in various animal species but only a few very short reports describing the fibrous tapetum of the horse (3,12) and there is no researches are related to the donkey. So, the main objective of this study is to compare three species, one has no *Tapetum* (camel) and two have different types of it (donkey and cat), describing their macroscopical shapes after exposure to different levels of light, the organization of the tapetal tissue, and the degree of pigmentation of the retinal epithelium along the ocular fundus.

Material and methods

In this study, we used both eyes of five healthy adult donkeys (*Equus asinus*), cats (*Felis domestica*) and one-humped camels (*Camelus dromedarius*) of both sexes and variable ages. The eyes of one-humped camel were obtained from the slaughterhouse of Belbas; the donkeys were collected from various sites all over Sharkia Governorate, while the cats were bought from Pet Shop in Zagazig city, Sharkia Governorate, Egypt.

Anatomical examination

The eyes of a live animal of the three species were photographed using a digital camera with resolution (16.1 megapixels, Sony DSC-W690, 36v and 10x optical zoom) under existing light and with a flash. The donkeys were sedated with Xylazine[2] Hcl at 0.5 mg/kg then narcotized with chloral hydrate[3] 10% at 5 mg/ 50kg i.v while cats were sedated and anesthetized with 1 mg/kg of i.m. xylazine then followed by 5 mg/kg of i.m. ketamine (13). The exsanguinations of donkeys and cats were made through the common carotid artery. Animals were handled in this work following the guidelines of the Institutional Animal Care and the Research Ethics Committee of the Zagazig University, Egypt.

For fixed eyes; both eyes of three animals of the three species were used for gross morphological examination. The eyes were enucleated then were preserved in a mixture of 10% formalin, 3% glycerine and 1% thymol fixative solution to be cut carefully when the eyeball became sufficiently tough without deformation. The eyes were cut at the equator and the vitreous body was removed. The ocular fundus of the posterior half of the eyecup was photographed under ordinary light and with a flash. After removal of the retina, the fundus was rephotographed. The anatomical nomenclature used was based on Nomina Anatomica Veterinaria (14) whenever possible.

Histological examination

The posterior halves of the eyecup of two animals of each species were cut after fixation in 10% neutral buffered formalin for 24 hours into small pieces according to the color, the region of the fundus and the relation to the optic disc as the follow (Figure 1):

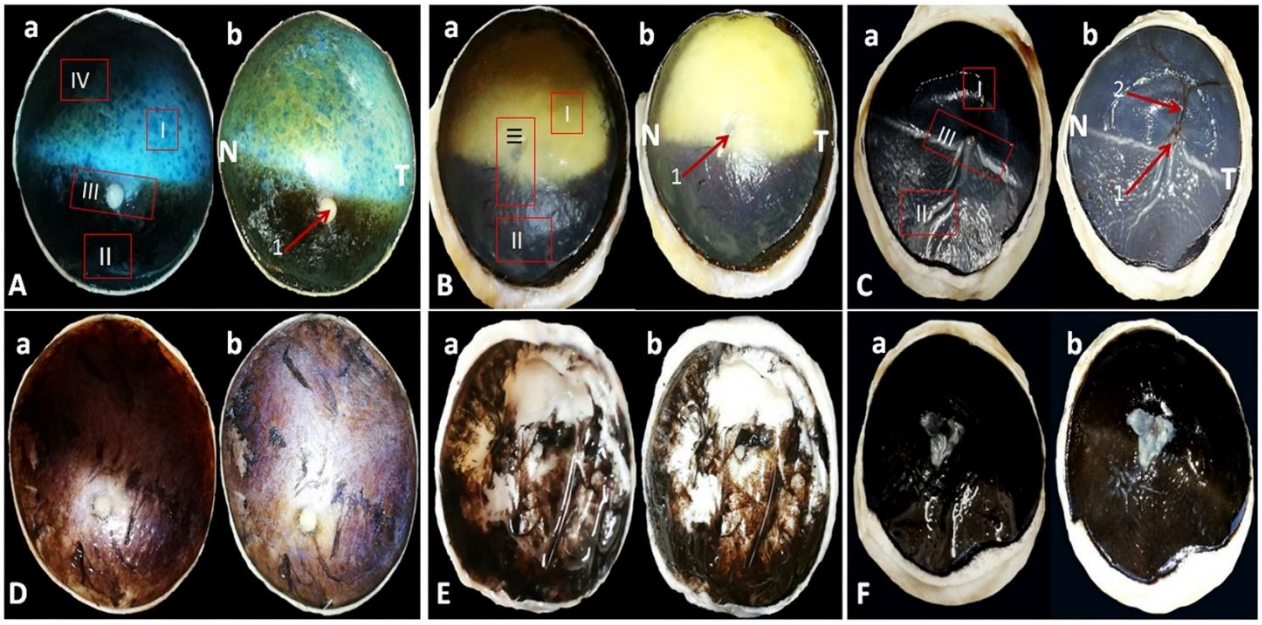

Figure 1: Photomacrographs of equatorial sections of ocular fundus with nasal (N) and temporal (T) ends of donkey without flash (Aa) and with flash (Ab), cat without flash (Ba) and with flash (Bb) camel without flash (Ca) and with flash (Cb) showing the different sites of cutting section for histological examination, colour and shape of Tapetum Lucidum, equatorial sections of ocular fundus after removal of retina of donkey without flash (Da) and with flash (Db), cat without flash (Ea) and with flash (Eb) camel without flash (Fa) and with flash (Fb) showing the differences in colour of the fundus with changes in the degree of illumination

I. Above the optic disc, the indigo colored area with dark blue spots, the yellow area and the dark area in donkey, cat and camel respectively.

II. Under the optic disc, the black area in donkey and cat and the light area in camel.

III. The junction between I and II contained the optic disc.

IV. Above the optic disc, the black area in donkey (changed into green with flash).

The specimens were dehydrated in ascending grades of alcohols, cleared in three changes of Xylol and embedded in paraffin wax for obtaining sections of five microns thickness. The sections were mounted and stained with the following stains: Hematoxylin and Eosin (H&E) stain for general histological demonstration, Crossman's trichrome stain for collagen and muscle fibers and Orcein stain for elastic fibers (15).

Results

Anatomical findings

In daylight, the donkey's pupils appeared black, centrally situated and horizontal in the direction in the brown iris (Figure 2A). Under flash, the light condensed centrally and the color changed into green or blue according to the direction of the light (Figure 2B). Moreover, the cat's pupils were oval vertically slit-like (Figure 2C) and colored yellow to red orange under flash (Figure 2D). The camel's pupils were similar to that of the donkey and the iris was darker in color in daylight (Figure 2E) while under flash there was a small spot of light centrally (Figure 2F).

Figure 2: Photomacrographs of eyes of donkey under day light (A) and with flash (B), eyes of cat under day light (C) and with flash (D) and eye of camel under day light (E) and with flash (F) showing the eye pupil was horizontal in donkey and camel while vertical in cat. The variation of the responsibility to the strong light in the examined species exhibited as green or blue colour in donkey, yellow or orange red in cat and a small light spot in camel

In donkey, *Tapetum* under the weak light was horizontal triangular in shape and was located dorsal to the optic disc with the nasally situated apex (Figure 1A). In the cat, *Tapetum* appeared semicircular in outline (Figure 1B). It was occupied the dorsal half of the fundus and it contained the optic disc in its ventral part (Figure 1B). In camel, the fundus appeared divided into two halves by a line passing below the optic disc (Figure 1C). *Tapetum* was indigo with dark blue spots and yellow in colour in donkey and cat respectively (Figure 1A and B). In donkey, the ventral edge didn't affect by the degree of light, while the dorsal one was indistinct under weak light and was rounded to give *Tapetum* its semicircular appearance under strong light (flash) (Figure 1A). With increasing the illumination degree, the green color appeared in the two third of *Tapetum* toward the nasal end (Figure 1A). In the cat, the ventral edge was horizontal, extended below the optic disc and more curved (bulged) distally at the temporal end (Figure 1B). In camel, under the weak light, the proximal half of the fundus appeared dark and the distal one was lighter. Under flash, the two halves appeared transparent white with large blood vessels extended dorsally and ventrally from the optic disc (Figure 1C). In donkey, the retina was closely attached to the underlying layers. After removing of the retina, the fundus of the eye appeared brown in colour with a rounded white ring around the optic disc by weak light (Figure 1D). While under the strong light it appeared violet and the light concentrated above the optic disc (Figure 1D). While in cat, the retina was easily detached from the underlying layer and the fundus of the eye was dark brown in color (Figure 1E). Also, in camel the retina was easily

detached and had several corrugations (Figure 1C), after the removal of the retina, the underlying layer appeared dark brown in color with a semicircular appearance at the distal half. The dorsal edge of it was horizontal just below the optic disc (Figure 1F).

Histological findings

Tapetum lucidum was located between the choroid layer (Choroidea) externally and retinal epithelium internally in donkey and cat instead, there was a brush's membrane in the same location in camel (Figure 3A, B and C). There was a single layer of large blood vessels between *Tapetum* and the choroid. These vessels send small branches which penetrated *Tapetum* and formed a single layer of capillary network termed the choriocapillaries layer separating *Tapetum* from the retinal epithelium (Figure 3D). In donkey, the tapetal tissue was fibrous in its texture, consisted of collagen fibers with fibroblast cells (Figure 3E and F). The collagen fibers were in the form of parallel bundles (Figure 3F) crossed by the blood vessels to form choriocapillaries layer above its surface (Figure 3D). In the cat, *Tapetum* appeared as brick-like rows of rectangular cells (Figure 3G). The tapetal cells had homogenous cytoplasm and the nucleus was large, oval, well stained centrally located. Two to four nucleoli were seen inside each nucleus. The latter was lightly stained with small dark stain areas scattered throughout the nucleus and inside the nuclear envelope (Figure 3G and H). While in camel, the brush's membrane was a single layer of elastic collagen fibers (Figure 4A). In donkey, the thickness of the tapetal tissue differed according to the region of *Tapetum*. Above the optic disc according to the macroscopic anatomy, *Tapetum* was thick in the region I (Figure 1A) and no pigmentation in the retinal epithelium (Figure 4B). The tapetal thickness decreased in the region (IV) (Figure 1A) and the pigmentation of the retinal epithelium began to appear (Figure 4C). When the tapetal layer abruptly decreased in its thickness in the region II (Figure 1A), the retinal epithelium was highly pigmented (Figure 4D). The nuclear layer of the retina was extended above the optic disc in the area of the junction (region III) (Figures 1A and 4E). In the cat, the tapetal layer appeared thinner at the periphery and above the optic disc (Figure 4F and G), then increased gradually in thickness at the center. *Tapetum* was 10-14 layers of cells in the thickest part. The most external layer of *Tapetum* formed of many cells separated from each other leaving large spaces with few numbers of scattered nuclei (Figures 3G and 4H). The outer dorsal layer (choriocapillary) had flattened nucleus and some cells with an oval nucleus (Figure 5A). The retinal epithelium was unpigmented allover the tapetal layer except for a little extent before the disappearance of the tapetal layer (Figures 4G, H and 5B). The latter was expanded on the optic disc (Figure 4G). In camel, the brush's membrane characterized by rounded dark (heterochromatin) nucleus with one pale nucleolus centrally located (Figures 4A and 5C). In the dark region (I) (Figure 1C) of the fundus, the retinal epithelium was heavily pigmented with few, spaced out and darkly stained melanocytes in the choroid layer (Figure 5D and E). While the light part (region II) (Figure 1C) characterized by few pigmentations in the retinal epithelium with a lot of regular (evenly) distributed melanocytes in the choroid (Figure 5F and G). At the area of the junction and above the optic nerve (*N. opticus*), the nuclear layer of the retina which was present in the donkey was represented by retinal ganglionic layer (Figure 5H).

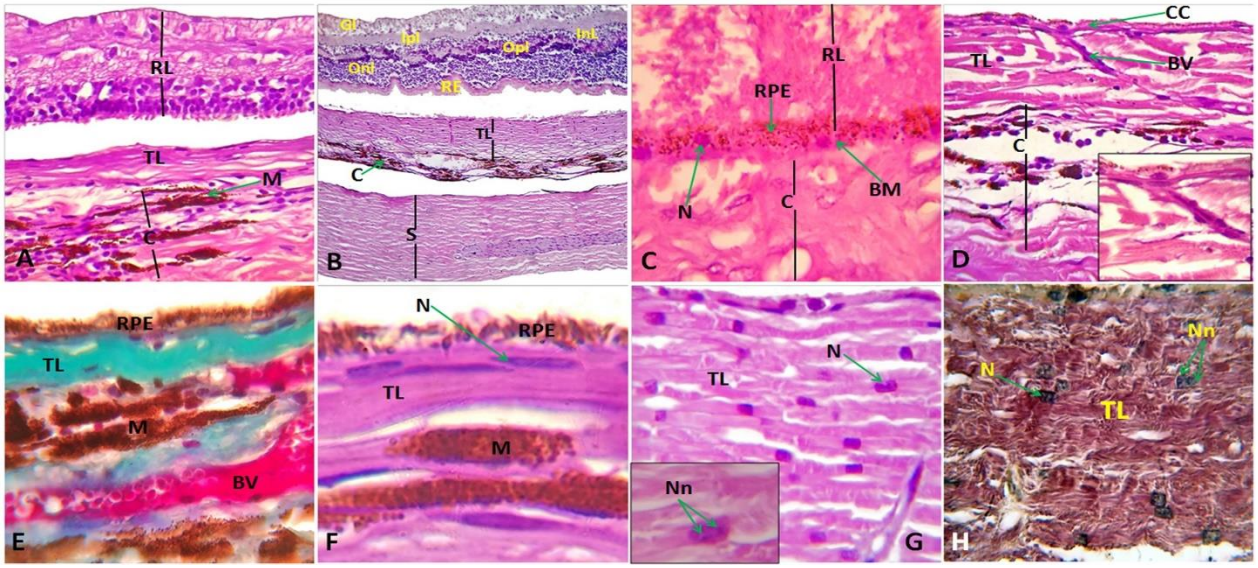

Figure 3: A photomicrograph showing: Retinal Layers (RL), Tapetum Lucidum (TL), Choroid (C), Sclera (S), Retinal Epithelium (RE), Retinal Pigmented Epithelium (RPE), Outer nuclear layer (Onl), Outer plexiform layer (Opl), Inner nuclear layer (Inl), Inner plexiform layer (Ipl), Ganglionic layer (Gl), Brush's Membrane (BM), Melanin granules (M), Blood vessels (Bv), Choriocapillary (CC), Nucleus (N) and Nucleolus (Nn). (A) Donkey and cat (B) Stain: H&E. Obj.x 10: Oc.x 10. (C) Camel Stain: H&E. Obj.x 100: Oc.x 10. (D) Donkey Stain: H&E. Obj.x 40: Oc.x 10 with insert showing higher magnification. (E) Donkey Stain: Crossmon's trichrome Obj.x 100: Oc.x 10. (F) Donkey Stain: H&E. Obj.x 100: Oc.x 10. (G) Cat Stain: H&E. Obj.x 40: Oc.x 10 with insert showing higher magnification of the nucleus. (H) Cat Stain: Crossmon's trichrome Obj.x 100: Oc.x 10

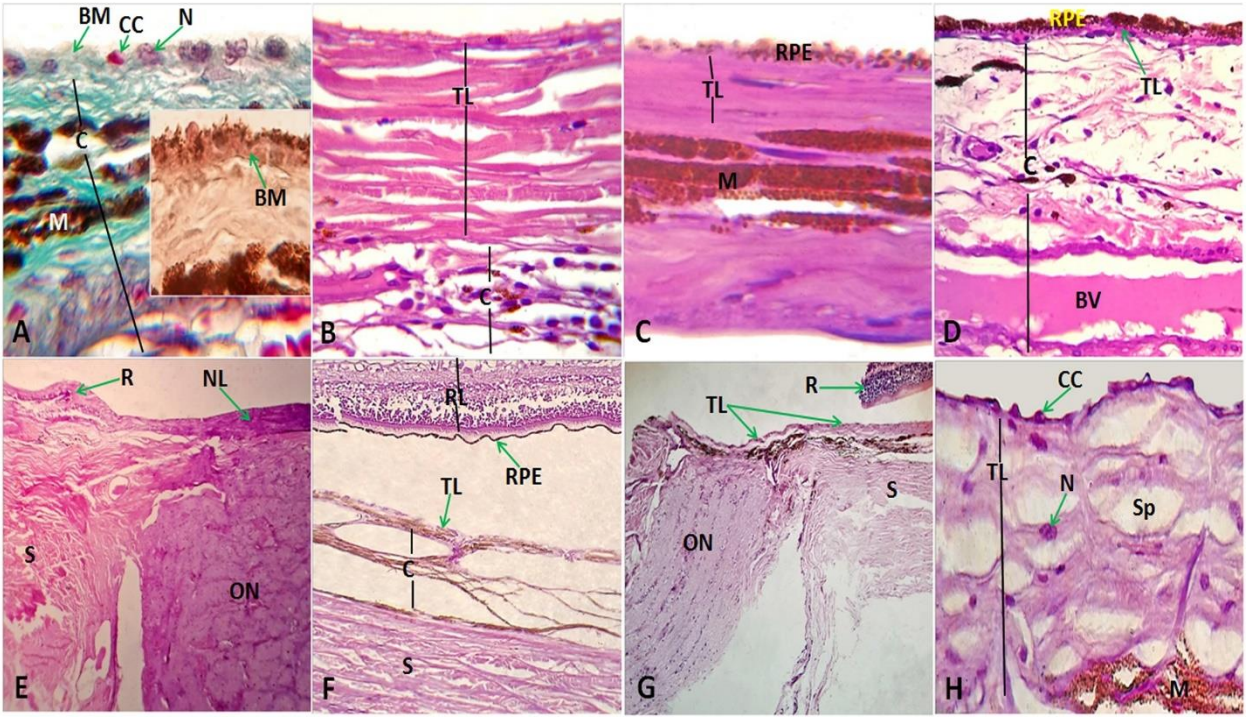

Figure 4: A photomicrograph showing: Retina (R), Tapetum Lucidum (TL), Choroid (C) and Sclera (S) Retinal Layers (RL), Retinal Pigmented Epithelium (RPE), Nuclear Layer (NL), Brush's Membrane (BM), Melanin granules (M), Blood vessels (Bv), Choriocapillary (CC), Nucleus (N), Optic Nerve (ON) and Spaces (Sp). (A) Camel Stain: Crossmon's trichrome with insert showing brush's membrane contained elastic fibers Orcein stain Obj.x 100: Oc.x 10. (B) Donkey Stain: H&E. Obj.x 40: Oc.x 10, (C) Obj.x 100: Oc.x 10, (D) Obj.x 40: Oc.x 10 and (E) Obj.x 4: Oc.x 10. (F) Cat Stain: H&E. Obj.x 10: Oc.x 10, (G) Obj.x 4: Oc.x 10 and (H) Obj.x 100: Oc.x 10

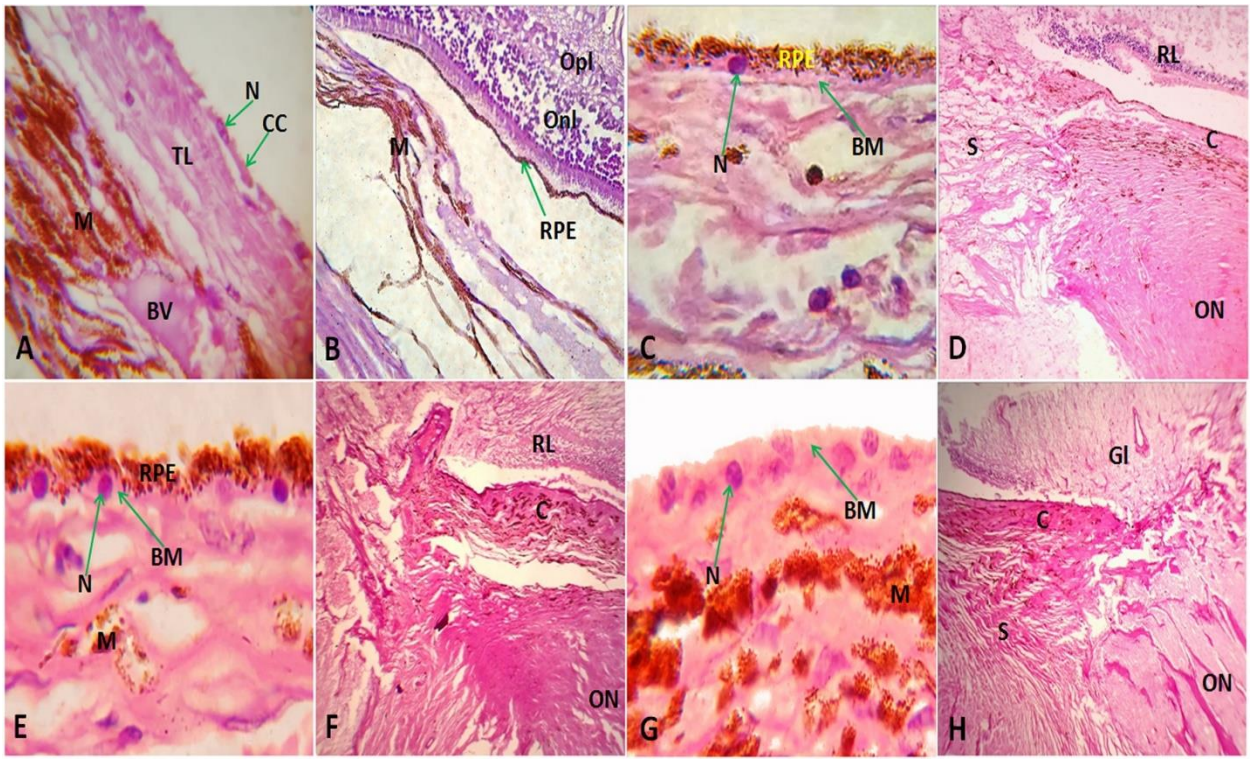

Figure 5: A photomicrograph showing Retinal Layers (RL), Tapetum Lucidum (TL), Choroid (C), Sclera (S), Retinal Pigmented Epithelium (RPE), Outer nuclear layer (Onl), Outer plexiform layer (Opl), Ganglionic layer (Gl), Brush's Membrane (BM), Melanin granules (M), Blood vessels (Bv), Choriocapillary (CC), Nucleus (N) and Optic Nerve (ON). (A) and (B) Cat Stain: H&E. Obj.x 40: Oc.x 10. (C) Camel Stain: H&E. Obj.x 100: Oc.x 10, (D) Obj.x 4: Oc.x 10, (E) Obj.x 100: Oc.x 10, (F) Obj.x 4: Oc.x 10, (G) Obj.x 100: Oc.x 10 and (H) Obj.x 4: Oc.x 10

Discussion

The shape and direction of the eye pupils were differed according to animal species as in donkey and camel, it was elongated and horizontal but in cat, it appeared oval with a vertical direction *Tapetum lucidum* which came in the same line of (16) in the cat.

The colored reflection of the light as green or blue in donkey, yellow to red orange in cat and a small spot of light in camel under strong light were in accordance with the macroscopic findings of the posterior fundus in the present work. Thus, confirmed the presence of *Tapetum lucidum* in donkey and cat and its absence in camel. *Tapetum lucidum* improved the night vision. Humans had poor vision in the night with the comparison to many animals, in part because humans lacked *Tapetum lucidum* (17). The color of *Tapetum lucidum* had been distinguishing in various animal species; yellow in cat, yellow green or green blue in the adult dog, blue green in bovine, sheep and horse (16,18-22).

Regarding the shape and location of *Tapetum lucidum*, in sheep, it appeared as a horizontal strip with lower edge passed through the optic disc while in dog and cat, it was a rounded equilateral triangle with upward apex (18). In our result, *Tapetum* was a horizontal triangular with nasally situated apex by weak light changed into semicircular by flash in the donkey. So, the degree of light affected on the tapetal shape as observed by Shinozaki et al. (20) in sheep and in the horse (21). The tapetal layer in the cat was semicircular and the optic disc was present in its ventral part which disagreed with our result in donkey and Alina et al. (18) who reported that the optic disc of dog was outside the tapetal outlines. On the contrary in the dog (23) clarified that the base of triangular *Tapetum* was in contact with the optic disc.

On the contrary, with our findings in donkey and cat, the posterior fundus of the camel in our

result appeared equally divided with the same color and two different degrees of brightness above and below the optic disc. The clear blood vessels were observed in the fundus of camel more than donkey and cat. In the Sulawesi bear cuscus, which lacked *Tapetum lucidum*, the posterior fundus was dark in color after removal of the retina (24) as our investigations in camel. In our work, the removal of the retina was very easy in cat and camel than in donkey. The dark color of the camel's fundus after retinal removal and the disparity in the degree of coloration after exposure to weak or strong light could be explained as due to the different amounts of melanin granules in the choroid.

The location of *Tapetum lucidum* next to the choroid and separated from the retinal epithelium by choriocapillary layer in donkey and cat in the present work was similar to (3) in cow, sheep, goat and horse (18) in bovine, sheep, dog and cat (22) in Barbary sheep and (25) in sheep and goat.

In the present work, donkey had *Tapetum fibrosum* while in cat, it was *cellulosum* one that came in the same classification manner of (4). While in camel, there was no *Tapetum* on the contrary of the brush's membrane which was well marked the same as Young et al. (26) in the red kangaroo. The presence of the brush's membrane was independent to the presence of *Tapetum*. The choroid consisted of five layers, Suprachoroid layer, Vessel layer, Tapetal layer, Choriocapillary layer and Basal complex or Bruch's membrane (27) in domestic animals, (28) in buffalo, (29) in bovine, (30) in domestic animals and (31) in adult Marwari goat. The histological structure of donkey's *Tapetum* in our study was similar to the general architecture of the fibrous type in bovine and sheep (18), in sheep (20), in horse (21) and in adult Marwari goat (31). In the present work, the cat's *Tapetum* consisted of rectangular cells arranged in layers that were reported to be symmetrical to the microscopic examination of *Tapetum cellulosum* of carnivores (18) and in dogs (19, 23). The brush's membrane of camel had the basic histological structure of this membrane that was reported also by Ramkrishna et al. and Banks (28,32) in some mammals.

Concerning the layers of *Tapetum cellulosum* (18) reported that the tapetal cell layers in the center were 18-20 in dog and 15-20 in cat. These layers also counted by Yamaue et al. (23) in dog and ranged in-between 9 to 12 layers. While in the present study, the layers of cat's *Tapetum* were 10-14 layers of cells in the thickest part. In some areas of *Tapetum* other than the center characterized by an increased thickness that might be due to the spaces between its layers.

The macroscopic appearance of *Tapetum* expressed its functional state and affected by the degree of illumination, pigmentation of the retinal epithelium and the thickness of *Tapetum.* The functional *Tapetum* was noticed macroscopically by weak light. This functional area was microscopically characterized by unpigmented retinal epithelium. Lacking pigmentation in retinal epithelium let light to cross through it then reflected by existed *Tapetum* that was agreed with Previous findings (20) in sheep, (21) in horse and (33) in bovine. Increasing the tapetal thickness was leading to more reflection of light so the horizontal triangle in the posterior fundus of the donkey was the functional part of *Tapetum* which characterized by unpigmented retinal epithelium and the highest thickness. While in cat it was represented by the whole yellow semicircular area of the fundus.

Activity style and the daytime when the animal was conscious and dynamic that decided the quantity of light accessible for vision, considered as a key selective control on the evolution of the vertebrate visual system. Earlier research explained that the functional requirement for vision under photopic and scotopic status selected for divergent eye morphologies in nocturnal, cathemeral and diurnal species because the vertebrate eye under the latter conditions couldn't be optimized for high-quality vision (34, 35). When we analyzed the lifestyle of examined animals with the presence or absence of *Tapetum* and its functional state, we observed that in case of camels as diurnal animals and their eyes were adaptive for improved visual acuity (36), *Tapetum lucidum* was absent. In donkeys that were diurnal animals but active also in other

day times as evening; adapted with human needs; they used the triangular area of *Tapetum* in normal light. Then if the light changed to be strong, *Tapetum* increased in its area and appeared semicircular that agreed with Dwyer (37). He reported that sheep were mainly diurnal but showed some activity as of dawn to evening. According to the shape of its functional tapetal area, sheep got the best mesopic vision in horizontal and antero-inferior visual fields. While the cats usually expressed crepuscular behavior, bordering nocturnal, active at dusk and dawn (38) and their eyes were adaptive for enhanced visual sensitivity (36). They used the whole area of *Tapetum*, which was histologically adapted with their activity pattern and their lifestyle.

Conflict of interest

The authors declare that they have no conflict of interest.

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