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Electron microscopic studies on the nervous layer of the eye in donkeys

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ABSTRACT

The microanatomy of the donkey eye is important to understand because pathological disorders affecting them are relatively common. The current study aimed to document the cellular components of donkey's retinae using light and electron microscopic studies. Ten donkey retinae were dissected and processed for semi-thin sections and electron microscopic studies. The photoreceptor layer was made up of the outer and inner segments of rods and cones. The outer segments were filled with invaginations of cell membranes that form stacks of membranous disks. Shed discs of photoreceptor outer segments could be seen in the photoreceptor layer as well as near the Müller cells. The inner segments of cones were conical in shape, while those of rods were slim rod-shaped. Both were filled with long thin mitochondria and free ribosomes. Three rows of photoreceptor cell nuclei made up the outer nuclear layer. The rod nuclei had more electron-dense chromatin than those of the cones. There were two rows of cell nuclei in the inner nuclear layer that represent the following four cell classes: horizontal cells, bipolar cells, amacrine cells, and Müller cells. Bipolar cells constitute the bulk of the inner nuclear layer. They were elongated in shape and had thick branched dendrites. Amacrine cells were in the inner face of the INL. It could be observed within IPL and known as displaced amacrine cells. Muller glial cells were irregular elongated in shape with many cytoplasmic processes. They were distributed in the INL among the bipolar cells. Müller cells were observed in the inner plexiform layer, the ganglion cell layer, and the nerve fiber layer. In conclusion, this study characterized the detailed cytological organization and ultrastructure of the healthy donkey retina. which maintains the fundamental laminar architecture characteristic of other mammalian retinas, and consists of 10 distinguishable layers. When compared to previously described retinal morphologies in domestic species, some distinctive characters were observed in donkey retinal cells.

Introduction

The retina is composed of a network of glial and neuron cells and constitutes the innermost layer of the eyeball (Fine and Yanoff, 1979). This is a highly specialized sensory organ that is able to convert light into electrical signals, which are then sent to the brain's visual centers via the optic nerve. After being absorbed by the visual pigment in the photoreceptors, photons are converted into a biochemical message and then into an electrical signal that can excite all of the retina's subsequent neurons. The highly ordered structure of the retinas of all vertebrates is made up of two layers of synapses and three layers of cells (Crispin *et al*., 1990). Cone and rod photoreceptor cell bodies make up the outer nuclear layer; radial Mueller glial cells and neurons (horizontal, bipolar, and amacrine cells) make up the inner nuclear layer; and ganglion cells make up the ganglion cell layer (Marc, 1999). Together, these cells have significant functions in the processing of visual stimuli, including color, motion, intensity, and directionality (Masland, 2001). The nuclear layers are separated by the outer and inner plexiform synaptic layers. The convergence of the ganglion cell axons at the exit of the optic nerve forms the nerve fiber layer (Glasgow and Foos, 1993). All vertebrates share a general similarity in retinal microanatomy. However, owing to the various species' various environmental circumstances, there are species-specific variances (Crispin *et al*., 1990).

It became clear during the research of retinal pathology in ocular illnesses in donkeys that there isn't a thorough description of the morphological structure of this area of the eye in the literature. Even though the general structure of the individual layer sequence is almost the same in all vertebrates, a number of significant morphologic differences were found when comparing the retinal structure of other domestic animals, which prompted a more thorough morphologic analysis using light and electron microscopic studies.

Materials and methods

Animals and histological sample preparation

Ten mature donkey eyes were obtained from Donkeys that were euthanized Faculty of Veterinary Medicine at Assiut University, Egypt. The donkeys' eyes were placed in 10% paraformaldehyde (PFA). The retina was dissected and prepared for routine histological procedures according to previous studies (Attaai *et al*., 2022; Hussein *et al*., 2022; Semiekaa *et al*., 2023).

Ethical approval

The Ethical Committee in the Faculty of Veterinary Medicine at Assiut University, Egypt has approved this study (06/2023/0082).

Scanning electron microscopy

Small retinal specimens were fixed in mixture of paraformaldehyde solution (2.5%) and glutaraldehyde solution (2.5%) in phosphate buffer (pH 7.3) for 24 hours. The samples were washed in 0.1M phosphate buffer, dehydrated in ascending grades of ethanol, critical point-dried in liquid carbon dioxide, and then coated with gold palladium in sputtering device. The samples were then examined and photographed using JSM-5400LV Scanning electron microscope operated at 20 KV in the EM center

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of Assiut University, Egypt.

Semithin sectioning and transmission electron microscopy

Small retinal specimens were kept at 4°C for 48 h by immersing them in a solution of 3% paraformaldehyde–glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 (Morris, 1965). The samples were rinsed in the same buffer before being postfixed for 2 h at room temperature in 1% osmic acid in 0.1 M sodium cacodylate buffer. The specimens were dehydrated in an ethanol gradient, followed by propylene oxide, before being embedded in an Araldite–Epon mixture. Toluidine was used to stain 1 µm-thick semithin sections. A Leitz Dialux 20 microscope was used to view stained sections, and images were taken with a Canon digital camera (Canon Power shot A 95). At Assiut University's Electron Microscopy Unit, ultrathin sections (70 nm) were stained with lead citrate and uranyl acetate and photographed using a JEOL 100 II transmission electron microscope (JEOL, Tokyo, Japan).

Digital colorization of TEM images

To increase the visual contrast between several structures on the same electron micrograph, we digitally colored specific elements (e.g., bipolar cells, amacrine cells, horizontal cells, Müller cells, and ganglion cells) to make them more visible to the readers. All elements were meticulously hand-colored in Adobe Photoshop software version 6 (Abdel-Maksoud *et al*., 2019; Mokhtar *et al*., 2019; Hussein and Abdel-Maksoud, 2020; Hussein *et al*., 2020; Mokhtar *et al*., 2022; Abdel-Maksoud *et al*., 2023a; Abdel-Maksoud *et al*., 2023b; Fadl *et al*., 2023).

Results

The retina of donkeys was composed of ten layers: (1) retinal pigment epithelium, (2) photoreceptor layer, (3) outer limiting membrane, (4) outer nuclear layer, (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglion cell layer, (9) nerve fiber layer, and (10) inner limiting membrane (Fig. 1 A, B and Fig. 2). The retinal pigmented epithelium (RPE) was made up of a single layer of cuboidal polygonal cells that contained numerous pigmented melanin granules (Fig. 1B).

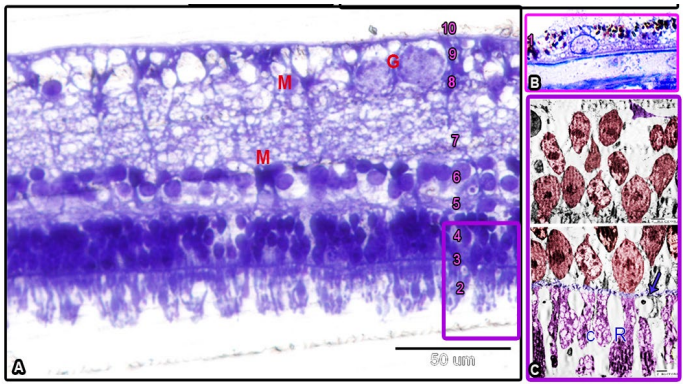

Fig. 1. General view of the donkey retina. A, B: Semi-thin sections stained with toluidine blue Showing the different layers of donkey retina: 1: PRE, 2: Photoreceptor layer, 3: Outer limiting membrane, 4: Outer nuclear layer, 5: Outer plexiform layer, 6: Inner nuclear layer, 7: Inner plexiform layer, 8: Ganglion cell layer, 9: Nerve fiber layer (NFL), 10: Inner limiting membrane. Notice: Ganglion cell (G); Muller cells (M). C: Digitally colored image of TEM showing the outer nuclear layer (brown), the outer and inner segments of rods (R) and cones (C). Notice; outer limiting membrane (arrow).

The photoreceptor layer was made up of the outer and inner segments of rods and cones. The outer segment regions of both rods and cones were filled with invaginations of cell membranes that form stacks of membranous disks where photopigments exist. Shed discs of photoreceptor outer segments could be seen in the photoreceptor layer. They resemble whorls of membranes. The inner segments of cones were conical in shape, while those of rods were slim rod-shaped. Both were filled

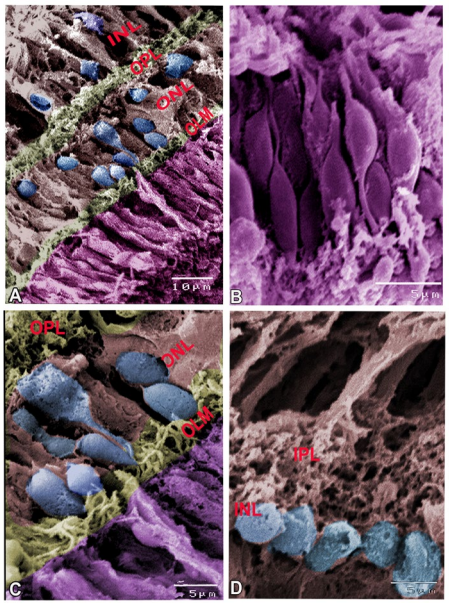

Fig. 2. General view of the donkey retina. A–D: Digitally colored SEM images showing the various layers of the donkey retina: photoreceptor layer (violet), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL).

with long thin mitochondria and free ribosomes. The inner segments lied close to the outer limiting membrane which separates the photoreceptors from their nuclei in the outer nuclear layer. The cell bodies of the two types form the outer nuclear layer (ONL), which is made up of approximately three rows of cell nuclei. The rod nuclei had more electron-dense chromatin than the cone nuclei. Cone nuclei were found near the outer limiting membrane, whereas rod nuclei were found at all levels of the ONL (Figs. 1C and Fig. 3).

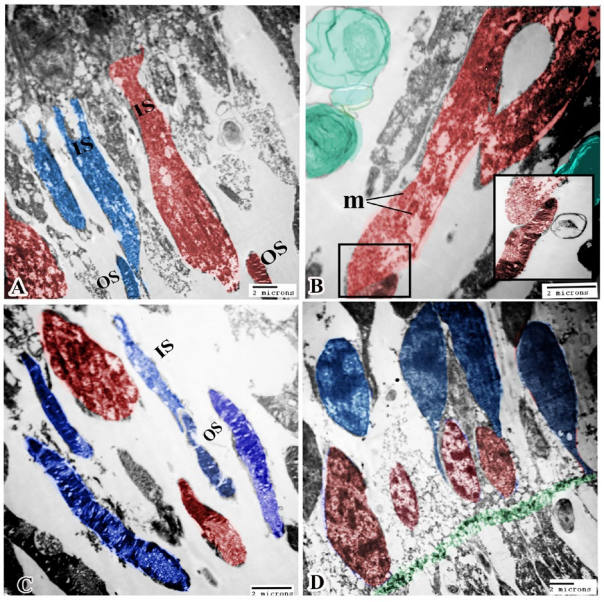

Fig. 3. Digitally colored TEM images. A–C: The photoreceptor layer is made up of the outer (OS) and inner (IS) segments of rods (blue) and cones (brown). The inner segments are filled with long thin mitochondria (m). The black square showing the connection between the outer and inner segments. Take note of the shed discs of photoreceptor outer segments (green). D: the outer nuclear layer is made up of the cell bodies of rods and cones. The rod nuclei (blue) have more electron-dense chromatin than the cone nuclei (brown) which are found near the outer limiting membrane (green).

The outer and inner nuclear layers were separated by the outer plexiform layer (OPL). The inner nuclear layer (INL) consisted of horizontal cells, bipolar cells, amacrine cells, and Müller cells (Figs. 4 and 6).

Horizontal cells: They were found on the INL's outer margin. They had large vesicular nuclei with regularly distributed loose chromatin. There are numerous vacuoles in the cytoplasm and extend two large cytoplasmic processes (Fig. 4 A, B).

Bipolar cells: They constituted form the bulk of the inner nuclear layer. They were elongated in shape and had thick branched dendrites that projected toward the OPL and thin axons that projected into the IPL (Fig. 4 A-C).

Amacrine cells: They were in the inner face of the INL. It could be observed within IPL and known as displaced amacrine cells (Fig. 4 B-D).

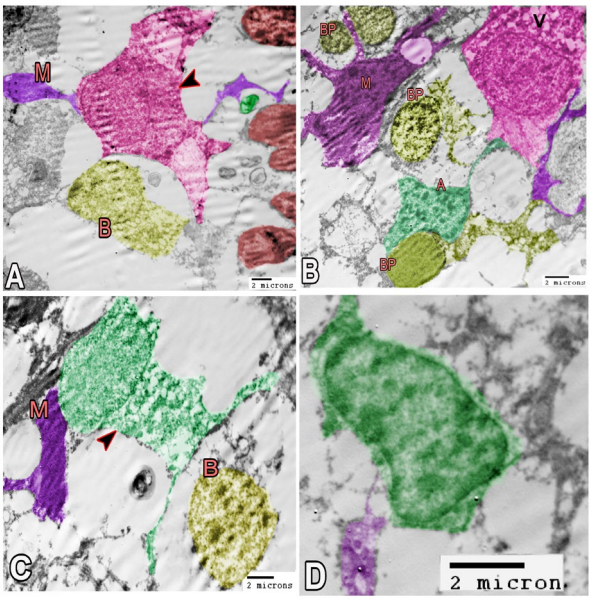

Fig. 4. Horizontal, bipolar cells and amacrine cells in the retina of a donkey. A-D: Digitally colored TEM images showing the inner nuclear layer is made up of horizontal cells (pink), bipolar cells (BP, yellow), amacrine cells (A, green), and Müller cells (M, violet). Horizontal cells show large vesicular nuclei and small vacuoles (V) in their cytoplasm.

Muller and ganglion cells: Muller glial cells were irregular elongated in shape with many cytoplasmic processes. They were distributed in the INL among the bipolar cells. Müller cells were observed in the inner plexiform layer, the ganglion cell layer, and the nerve fiber layer (Fig. 5). By TEM Muller cells were darkly stained with long processes and vacuoles. Their processes were in close contact with Rods and cones (Fig. 6A), as well as rod and cone bipolar cells (Fig. 6 B-D). Shed discs of photoreceptor outer segments could be seen near the Müller cells (Fig. 6 B). The ganglion cells synapsed with Müller cells (Fig. 6E). Large ganglion cells were rounded in shape. They had round pale nucleus. The cytoplasm contained numerous Nissl's granules (Figs. 1 A& 5).

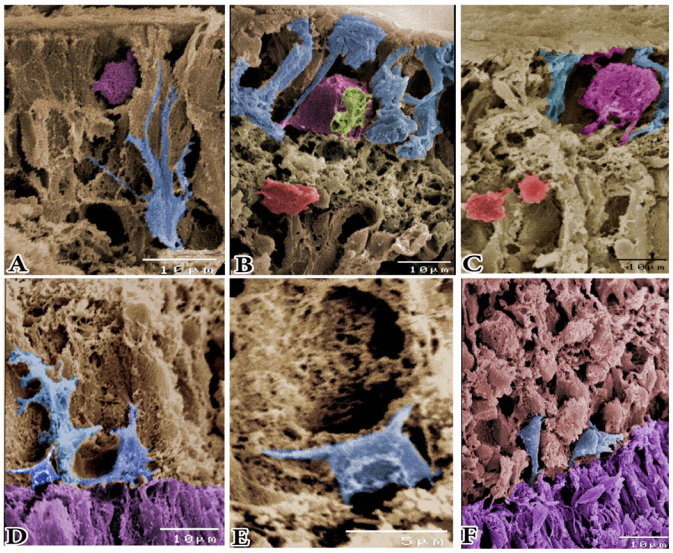

Fig. 5. Digitally colored SEM images showing the distribution of Müller cells (blue) in various retinal areas: A: in the inner plexiform layer, B, C: in the ganglion cell layer and nerve fiber layer. Notice, Ganglion cells (violet), glial cells (green) partially cover the ganglion cells displaced amacrine cells (red) in the inner plexiform layer. D–F: in the outer nuclear layer. Notice, the photoreceptor layer (violet).

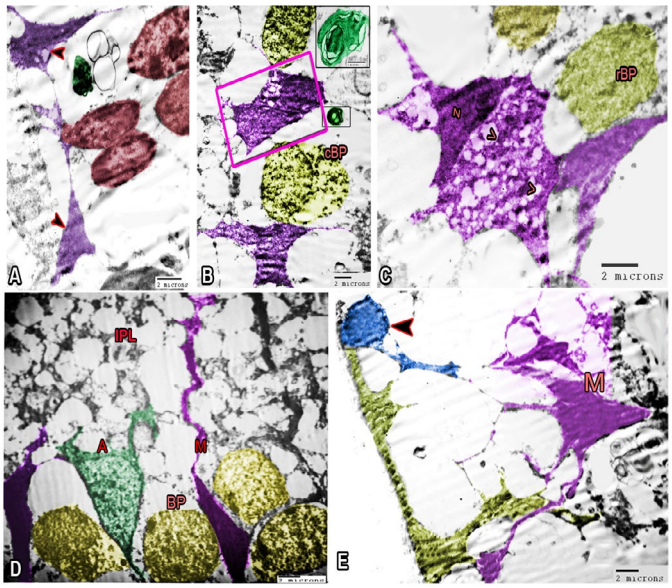

Fig. 6. Digitally colored TEM images of Müller and ganglion cells in the retina of a donkey. A-D: Müller cells (violet) are darkly stained with long processes and vacuoles (V). Their processes are in close contact with Rods and cones (brown), as well as rod and cone bipolar cells (yellow). Notice: Shedded discs of photoreceptor outer segments (green colored) in form of whorls of membranes (black squares). Displaced amacrine (A, green). E: The ganglion cells (blue) synapse with Müller cells (M).

Discussion

A thorough understanding of retinal cytoarchitecture serves as an essential foundation for identifying and interpreting retinal abnormalities. However, descriptions of donkey retinal morphology remain limited, precluding in-depth investigation and histopathological analysis of retinal diseases in this species. Additionally, inter-species variations in retinal structure necessitate species-specific characterization. In agreement with the previous studies in different species (Dowling and Boycott, 1966; Dowling, 1970; Hoon *et al*., 2014), the current study showed the retina histologically consists of ten layers. The inner nuclear layer houses the bipolar, horizontal and amacrine cells. The ganglion cell layer contains the soma of retinal ganglion neurons. Interposed between the nuclear layers are the outer and inner plexiform layers, which contain synapses between the different retinal neuron types. In current study the photoreceptors appeared by TEM consists of inner and outer segments. The detection of light begins at the photoreceptors, which are the specialized neuronal cells located in the outermost nuclear layer of the retina - the outer nuclear layer. Photoreceptors initiate visual phototransduction by converting light signals into electrical signals that are transmitted to inner retinal neurons. There are two types of photoreceptors in the retina: rods and cones. Rods are highly light-sensitive photoreceptor cells optimized for dim-light, scotopic vision. They contain the photopigment rhodopsin, which allows rods to function under low-illumination conditions like at night. Cones, on the other hand, have lower light sensitivity than rods but are able to distinguish color as they contain photopigments (such as S-cone opsin, M-cone opsin, L-cone opsin) that are specific for different wavelengths of light in the visible spectrum. As such, cones are responsible for high acuity, photopic color vision during daylight conditions (Hussey *et al*., 2022). Photoreceptors release the neurotransmitter glutamate at synaptic contacts with bipolar cells located within the outer plexiform layer. Bipolar cell bodies reside in the inner nuclear layer as recorded in the current study, just deep to this synaptic layer. Bipolar cells thus act as relay interneurons, transmitting the photoreceptor signal to the inner retina while providing a means to segregate the ON and OFF pathways involved in light/dark detection. Their synaptic connections within the inner plexiform layer are vital for signal processing and the generation of the neural impulse that is conveyed to the brain (Ichinose and Habib, 2022). In the current study, amacrine cells were also observed using transmission electron microscopy (TEM). Amacrine cells reside in the inner nuclear layer and have processes that arborize extensively within the inner plexiform layer. They modulate the excitation of retinal ganglion cells through either direct contact with ganglion cell dendrites or indirect contact via bipolar cell axon terminal bulbs (Bloomfield and Xin, 2000). Horizontal cells were also observed in the present study. These cells have long slender dendrites that arborize mainly in the outer plexiform layer, where they form synaptic contacts with photoreceptor terminals. Horizontal cells function to modulate the communication between photoreceptors and bipolar cells through a negative feedback mechanism called lateral

inhibition (Poché and Reese, 2009). Finally, the current work was reported the presence of the Müller cells. These are the principal glial cells of the retina and play an integral role in retinal function and homeostasis. Müller cells span the entire width of the retina from the inner limiting membrane to the outer limiting membrane. In doing so, their processes cover and make contact with nearly all retinal neurons, including photoreceptors, bipolar cells, amacrine cells and ganglion cells. Müller cells perform vital supporting functions such as recycling neurotransmitters from the synaptic clefts, preventing glutamate neurotoxicity, supplying nutrients to neurons, and maintaining the blood-retinal barrier. They are also involved in forming the outer limiting membrane (Bringmann *et al*., 2013). Surprisingly, shed discs of photoreceptor outer segments were observed around the Müller cells in our current study. This backs up previous findings that Müller cells phagocytize outer segment discs shed from cones (Long *et al*., 1986; Wang and Kefalov, 2011).

Conclusion

This study characterized the detailed cytological organization and ultrastructure of the healthy donkey retina. The results demonstrate that the donkey retina maintains the fundamental laminar architecture characteristic of other mammalian retinas, consisting of 10 distinguishable layers. When compared to previously described retinal morphologies in domestic species, some distinctive characters were observed in donkey retinal cells.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdel-Maksoud, F.M., Fadl, S., Abou-Elmagd, A., Saleh, A.M.M., 2023a. Post-hatching developmental changes in the adrenal gland of the Japanese quail (*Coturnix coturnix japonica*): Histological, immunohistochemical, and electron microscopic studies. Microsc. Res. Tech. 86, 1461-1474.

Abdel-Maksoud, F.M., Hussein, M.T., Attaai, A., 2019. Seasonal Variation of the Intraepithelial Gland in Camel Epididymis with Special Reference to Autophagosome. Microsc Microanal 25, 1052-1060.

Abdel-Maksoud, F.M., Zayed, A.E., Abdelhafez, E.A., Hussein, M.T., 2023b. Seasonal variations of the epididymis in donkeys (*Equus asinus*) with special reference to blood epididymal barrier. Microsc Res Tech. 87, 326-338.

Attaai, A.H., Hussein, M.T., Aly, K.H., Abdel-Maksoud, F.M., 2022. Morphological, immunohistochemical, and ultrastructural studies of the Donkey's eye with special reference to the AFGF and ACE expression. Microscopy and Microanalysis 28, 1780-1793.

Bloomfield, S. A., Xin, D., 2000. Surround inhibition of mammalian AII amacrine cells is generated in the proximal retina. J. Physiol. 523 Pt 3, 771-783.

Bringmann, A., Grosche, A., Pannicke, T., Reichenbach, A., 2013. GABA and Glutamate Uptake and Metabolism in Retinal Glial (Müller) Cells. Front Endocrinol (Lausanne) 4, 48.

Crispin, S.M., Matthews, A.G., Parker, J., 1990. The equine fundus I: examination, embryology, structure and function. Equine Veterinary Journal 22, 42-49.

Dowling, J.E., 1970. Organization of vertebrate retinas. Invest. Ophthalmol. 9, 655-80.

Dowling, J.E., Boycott, B.B., 1966. Organization of the primate retina: electron microscopy. Proc. R Soc. Lond B Biol. Sci. 166, 80-111.

Fadl, S., Saleh, A.M M., Abou-Elmagd, A., Abdel-Maksoud, F.M., 2023. Prehatching development of the adrenal gland in Japanese quail (*Coturnix japonica*): Histological, immunohistochemical, and electron microscopic studies. Microsc. Res. Tech. 87, 727-739

Fine, B.S., Yanoff, M., 1979. Ocular histology: a text and atlas. Publisher. Medical Dept., Harper & Row.

Glasgow, B.J., Foos, R.Y., 1993. Ocular cytopathology. Butterworth-Heinemannn Boston.

Hoon, M., Okawa, H., Della Santina, L., Wong, R.O., 2014. Functional architecture of the retina: development and disease. Prog. Retin. Eye Res. 42, 44-84.

Hussein, K., Hussein, M.T., Attaai, A., Ragab, L., Semieka, M., 2022. Effect of Nictitans gland and third eyelid excisions on ocular surface integrity, pH, and tear production in dogs. Journal of Advanced Veterinary Research 12, 90-98.

Hussein, M.T., Abdel-Maksoud, F.M., 2020. Structural Investigation of Epididymal Microvasculature and Its Relation to Telocytes and Immune Cells in Camel. Microscopy and Microanalysis 26, 1024-1034.

Hussein, M.T., Mokhtar, D.M., Hassan, A.S.J.P., 2020. Melatonin activates the vascular elements, telocytes, and neuroimmune communication in the adrenal gland of Soay rams during the non-breeding season. Protoplasma 257, 353-369.

Hussey, K.A., Hadyniak, S.E., Johnston, R.J., 2022. Patterning and Development of Photoreceptors in the Human Retina. Front. Cell Dev. Biol. 10, 878350.

Ichinose, T., Habib, S., 2022. On and off signaling pathways in the retina and the visual system. 2.

Long, K.O., Fisher, S.K., Fariss, R.N., Anderson, D.H., 1986. Disc shedding and autophagy in the cone-dominant ground squirrel retina. Exp. Eye Res. 43, 193-205.

Marc, R.E., 1999. Kainate activation of horizontal, bipolar, amacrine, and ganglion cells in the rabbit retina. Journal of Comparative Neurology 407, 65-76.

Masland, R.H., 2001. The fundamental plan of the retina. Nature neuroscience 4, 877-886.

Mokhtar, D.M., Hussein, M.T., Hussein, M.M., Abd-Elhafez, E.A., Kamel, G., 2019. New Insight into the Development of the Respiratory Acini in Rabbits: Morphological, Electron Microscopic Studies, and TUNEL Assay. Microsc. Microanal. 25, 769-785.

Mokhtar, D.M., Sayed, R.K.A., Zaccone, G., Albano, M., Hussein, M.T., 2022. Ependymal and Neural Stem Cells of Adult Molly Fish (Poecilia sphenops, Valenciennes, 1846) Brain: Histomorphometry, Immunohistochemical, and Ultrastructural Studies. Cells 11.

Morris, J.K., 1965. A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy. J. cell Biol. 27, 1A-149A.

Poché, R.A., Reese, B.E., 2009. Retinal horizontal cells: challenging paradigms of neural development and cancer biology. Development 136, 2141-51.

Semiekaa, M., Abdelbaset, A., Hussein, M.T., Hussein, M.K., Attaai, A.H., Hamed, M.A., 2023. The Comparative Effect of Total Versus Partial Surgical Excision of Nictating Membrane on The Aqueous Tear Production and Ocular Surface Health in Donkeys (*Equus asinus*). Journal of Advanced Veterinary Research 13, 166-173.

Wang, J.S., Kefalov, V.J., 2011. The cone-specific visual cycle. Prog. Retin. Eye Res. 30, 115-28.