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Histological Study of the Chicken Retina and Associated Ocular Structures.

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ABSTRACT

Comparative ocular and histological studies is the high degree of development of the anatomical elements and superior visual acuity make the avian eye (particularly, the domestic hens) an ideal model to study. This study aimed to study the retina and other ocular structures of the domestic chicken (Gallus gallus domesticus) in histological point of view. Twenty samples of the eyeballs were taken out of healthy hens of approximately 40 days old and the weight range was 1.5-2.0 kg. The samples were processed like any other normal biological material, whereby they were placed in 10 percent neutral buffered formalin, dehydrated, and embedded like any other normal histological material. The overall architecture of the tissues in the sections was observed regarding hematoxylin and eosin (H&E) stain.

At low and high magnification microscopic examination revealed the typical multilayered structure of the avian retina. Besides the five layers of cornea, other eye body tissues were discovered and documented including sclera (dense collagen fiber), scleral supportive cartilage, skeletal muscle bundles within sclera and the adjacent vascularized pigmented choroid to the retina. This research increases our understanding of the anatomy of chicken eye tissues and provides a foundation with which comparative and veterinary research on chicken eyes can build on.

Introduction

The special features of the bird eyes enable them to feel color, recognize movement and observe finer detail. Chickens (Gallus gallus domesticus) possess superior visual ability due to the elaborate make-up of the retinas (1). Chickens like all birds possess numerous layers in the retinas where they process the visual information. Six major layers are considered characteristic of a normal retina and these include the inner limiting membrane, the photoreceptor complex, the nerve fiber layer, the inner and outer plexiform layers, the inner and outer nuclear layers and the photoreceptor complex (2). The chicken retina has cone cells and rod cells that coordinate two processes, color discrimination and light intensity measurement. Chickens have the tetrachromatic system of vision that enables them to see many parts of the spectrum, including UV light (3). The wavelength detection is enhanced by dispersing oil droplets in cone cells and this serves to improve color discrimination (4). The adaptive capabilities have assisted the chickens a lot on their social and foraging life. Retinal pigmented epithelium (RPE) attributes to the development of vision pigments, absorption of excess light and metabolic sustenance of photoreceptors, which are invaluable to the functions of the photoreceptors (5). The lower supply of blood to your choroid, which is under the retina as accorded by the recent medical studies, makes the transport of oxygen and nutrients to the retinal tissues difficult. Its melanin pigment increases the clearness of pictures and decreases the dispersion of images (6). The most significant refracting surface in the eye is the cornea; it allows light into the eye and is a major element of focusing light rays on the retina. The anatomy of the eye has the following layers: epithelium, stromal, Descemet, Bowman and endothelium (7). The cartilaginous and bony structures in the sclera of birds keep its eyes stable and supported (8). In order to research the development of vertebrate vision systems, one will have to histologically analyze chicken retinas. The two fields that depend on such investigations include ophthalmology and vision science especially (9). It is possible to examine the very structure of this retina with the help of staining it with hematoxylin and eosin (H&E) and studying its structural components and the location of the connective tissues and cells of the retina in different parts of the eye (10). Primate species as well as other diurnal vertebrates have similar cones that are concentrated in their retinas relative to the bird species (11) compared with the nocturnal species whose retinas have more rods compared with the cones. The retina development depends on retinal glial cells, M, to be precise, as they regulate ions and provide metabolic supplies (12). Chicken retinal tissue is commonly used in human retinal disease model (such as retinitis pigmentosa and macular degeneration). Human foveas can be studied well using chicken fovea since it, too, is highly similar to the human foveas (13). One area of histological research of interests in the study is the development of new vision restoration processes, which include stem cell therapy, and retinal prosthesis (14). A careful histological analysis of the chicken eye and associated retina can reveal essential data not only in areas of avian vision, but also evolutionary architecture, and potential medical opportunities (15). The main idea of the given research is to discover more about the structure and functioning of the system and receive the in-depth histological examination of chicken retina and other ocular structures. This study aims to enhance the understanding of the visual system of birds through histological examination of the chicken eye. This can contribute to comparative anatomical research and vision science. In addition, given the economic value of chickens as the main source of poultry meat and eggs, and therefore, the results may have practical implications for improving health and production in poultry.

Materials and Methods

Biological Sample

This study included 20 eyeball samples collected from local domestic chickens (Gallus gallus domesticus). The birds were approximately 40 days of age and weighed between 1.5 and 2 kilograms. Only healthy chickens with no visible ocular abnormalities were included. The collected samples were used for routine histological evaluation of the eye, with particular attention to the retinal layers and other ocular structures.

Chemicals and Reagents

Fixative for 10% Neutral Buffered Formalin (NBF) for tissue preservation. Dehydration Agents for Ethanol series (70%, 80%, 90%, 95%, and 100%) for tissue dehydration. Tissues require this agent to remove alcohol content (Xylene) during the de-alcoholification process before embedding. The laboratory provides an Embedding Medium for Paraffin wax, which is used for sectioning procedures.

Staining Reagents

Hematoxylin and Eosin (H&E) stain for general histological description. Hematoxylin and Eosin (H&E) staining protocol is a common histological staining method employed in examination of paraffin-embedded sections so as to observe the tissue structure and cell morphology. It starts with the cutting of sections of a thickness of 4-6 m by a rotary microtome, floating them in a warm water bath (40-45 C), flattening them, mounting them on clean glass slides and drying them either overnight at 37-40 C or under 60 C within 30-60 minutes. Deparaffinization initiates the process of staining by placing slides in two or three changes of xylene 3-5 minutes each to dissolve paraffin wax, and the process continues by re hydrating the slides using descent alcohol (100 percent, 95 percent and 70 percent) after which the slides are rinsed in distilled water. To stain nuclei nuclei, the slides are immersed in hematoxylin solutions-Harris, Mayer s, or Gill s, depending on which formulation is used, and 5-8 minutes later rinsed under running tap water 3-5 mins. Differentiating is done by momentary dipping the sections (35-5 seconds) in 1 percent acid alcohol to remove superfluous nuclear stain and washing in running tap water 1-2 minutes. Bluing Slides are blued by placing stain-free slides in a 0.2 percent ammonia water or Scott tap water substitute between 30 seconds to 1 minute and rinse again in neutral stainless water 2 to 3 minutes to fix the nuclear stain. Sections are then stained in and placed in 0.51 percent eosin Y solution (aqueous or alcoholic with acetic acid added) in approximately 30 seconds up to 2 minutes in order to provide the cytoplasm and extracellular elements with pink to red color. Sections are dehydrated using successive mixtures of ethanol (95 and 100 percent) and then cleared with two to three changes of xylene 2-3 min each, before mounting in a synthetic resin (DPX) and covering with a glass coverslip. Nuclei observed under the microscope should be sharp blue to purple, cytoplasm pink to red, collagen fibers pink to orange and red blood cells bright red or orange. Proper timing, correct handling of reagents, and removal of all excess wax is critical to ensuring even staining, and the amount of staining or differentiation time would be adjusted to correct light nuclei, excessive background staining, or light cytoplasm staining.

Sample Collection and Fixation

A total of 20 eyeball samples were collected from local domestic chickens (Gallus gallus domesticus). The birds were obtained from licensed poultry slaughterhouses located in Salah Al-Din Governorate, Iraq, where the heads were collected immediately after commercial slaughter, with no storage involved before eye extraction. The chickens used in this study were approximately 40 days in age, with body weights ranging between 1.5 and 2.0 kilograms. The sex of the birds was not considered, as it was not relevant to the objectives of the study. The collected heads were transported directly to the Animal House Facility at the University of Tikrit, where the whole eyeballs were carefully enucleated. Immediately after removal, the eyes were immersed in 10% neutral buffered formalin for fixation. The fixed samples were then transferred to the Histology Laboratory for routine tissue processing and microscopic examination

Tissue Processing

The fixed tissue specimens went through a stepwise pattern of dehydration with changing concentrations of ethanol from 70% to 100%. Xylene solution served as the clearing agent because it eliminated remaining ethanol from the samples. A station for embedding paraffin wax allowed scientists to place the tissues in molten paraffin wax. The paraffin material solidified at room temperature before preparing sections. A rotary microtome produced sections 6 µm thick from trimmed paraffin blocks for microtomy. A warm water bath with a temperature of 40°C enabled the tissue sections to become flat enough before they were placed onto glass slides.

The method involved Hematoxylin & Eosin Staining as the staining technique.

The tissue sections went through xylene deparaffinization, followed by ethanol rehydration as part of the process. Research tissue sections remained inside hematoxylin stain solutions 5 minutes to achieve optimal nucleus visibility. The technique consists of differentiation with acid alcohol (1% HCl in ethanol), followed by tap water washing. The eosin staining lasted for a time period of 3 minutes to achieve sufficient cytoplasmic component coloration. The sample was dehydrated through successive ethanol solutions before being cleared with xylene and received final consolidation with DPX mounting medium.

Microscopic Examination

Microscopic analysis occurred under X10 and X40 magnification to examine both retinal layers as well as choroid and sclera, and corneal ocular structures through stained slides. Photos of the

examination were taken as part of the documentation and analysis.

Results and discussion

The chicken retina presented a properly structured, multiple-layered anatomical arrangement. The retina showed its layers starting from the inner limiting membrane through nerve fiber layer, then ganglionic cell layer, followed by inner plexiform layer and inner nuclear layer until outer plexiform layer before reaching the outer nuclear layer and outer limiting membrane, while ending with photoreceptor rods and cones as the final layer. The last layer in the retinal stack was located at the retinal pigmented epithelium (RPE) (Fig. 1).

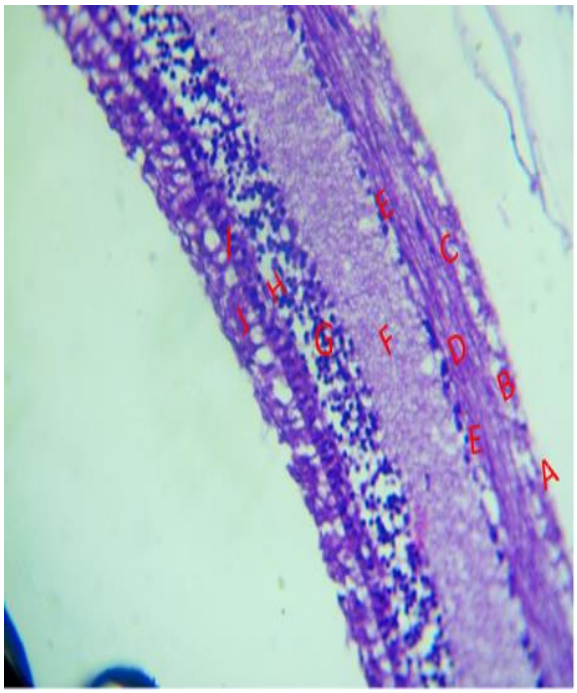
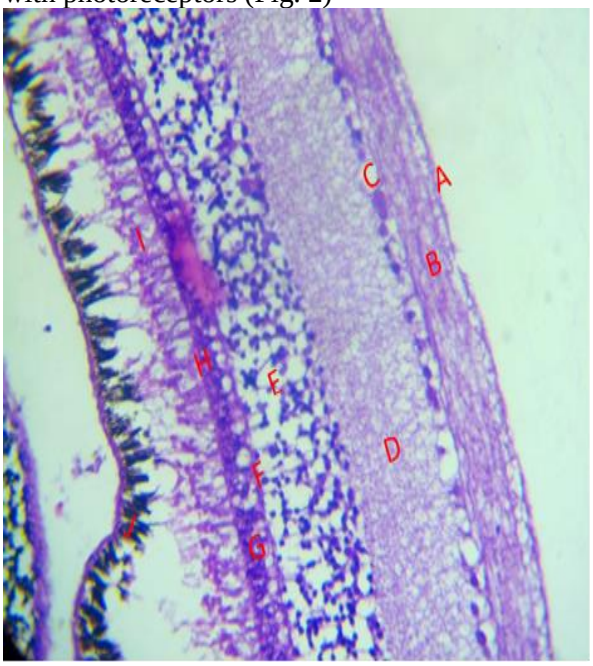


Figure (1): Retina of chicken, inner limiting membrane (A) nerve fiber layer (B) ganglionic cell layer (C) inner plexiform layer (D) inner nuclear layer (E) outer plexiform layer (F) outer nuclear layer (G) outer limiting layer (H) photoreceptors (I) retinal pigmented epithelium (J) H&E (X10).

The chicken retina consists of layers that correspond to standard vertebrate retinal arrangements that link neurons between the inner nuclear layer and outer nuclear layer, and the ganglionic layer properly. The organization serves an essential role in proper signal processing, together with visual perception (16). The numerous cones within the photoreceptor layer enable chickens to detect an extensive spectrum of colors, which extends to ultraviolet wavelengths. A significant trait in chicken vision is tetrachromatic sight, which serves multiple roles in both food search activities and social relationships (17). The retinal pigmented epithelium (RPE) maintains photoreceptors through its dark color and serves to protect the receptors by absorbing light and recycling pigments while providing metabolic support for photoreceptors (18).

The whole rows of retinal tissues were well demonstrated which are the inner limiting membrane, bundle of nerve fiber, ganglionic cell layer which had prominent nuclei, extensive zone of inner plexiform layer which synapse with the nerve bundle of the outer plexiform layer via inner nuclear layer, The outer limiting membrane was appeared as a line nearby the nuclei of photoreceptors of the rods and cons in turn coated by retinal pigmented epithelial cell appeared as dark pigmented cells with processes associated with photoreceptors (Fig. 2)



(Fig 2): Retina of chicken, inner limiting membrane(A) nerve fiber layer(B) ganglionic cell layer (C) inner plexiform layer (D) inner nuclear layer (E) outer plexiform layer (F)outer nuclear layer (G) outer limiting layer (H) photoreceptors (I) retinal pigmented epithelium(J) H&E (X10).

The structure of the chicken retina adopts a standard vertebrate organization, which includes ordered neural pathways linking the inner nuclear layer to the outer nuclear layer as well as the ganglionic layer. The proper functioning of signal processing alongside visual perception depends on this organizational structure, which can be found in the photoreceptor layer (19). The high cone concentration in this layer allows chickens to view an extensive spectrum that includes ultraviolet light. The animal benefits from four-color vision because it improves its ability both to locate food and to connect with other individuals (20). The

retinal pigmented epithelium (RPE) functions as a critical element of photoreceptor support through its dark coloring to absorb extra light and execute photoreceptor recycling and metabolic functions (21). The choroid layer of the eyeball was formed by pigmented epithelium, which was darkly pigmented, enriched with melanin stain, the other region of the choroid was loose C.T, enriched with venous plexus and other blood capillaries with multiple dots of melanin (Fig. 3).

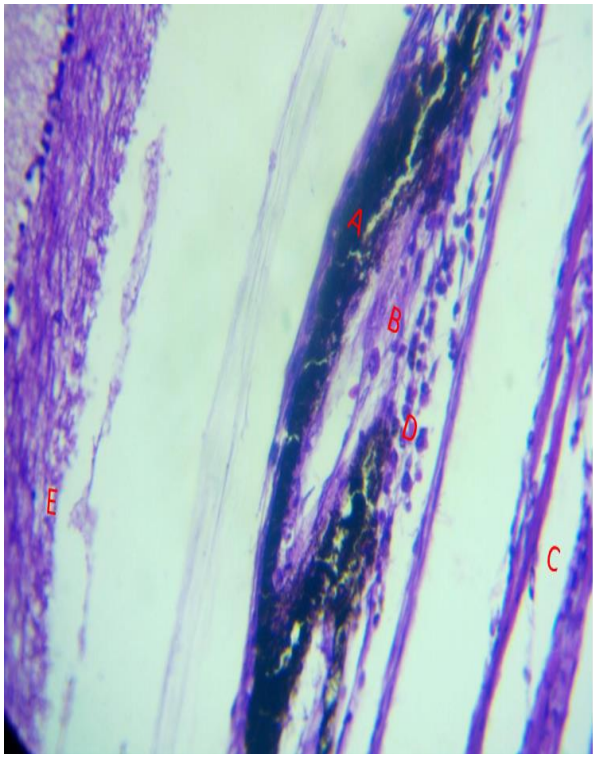


Fig (3): choroid with pigmented melanin (A) venous plexus (B) loos C.T of choroid (C) multiple melanin dote (D) retinal epithelium (E) H&E (X10)

The choroid contains multiple vessels that supply the retinal layers with nutrients and oxygen due to their metabolically active characteristics. The findings from other birds validate that choroidal venous plexus, along with melanin granules, support thermoregulation and light absorption functions in this tissue (22). The choroid improves visual clarity through its melanin-rich epithelial cells, which minimize light scatter. Birds heavily depend on visual abilities for their navigation and predator detection, and food search activities, so this adaptation plays a crucial role for them (23).

The avian eye uses a particular adaptation through cartilage in its sclera that exists only in birds, along with certain reptiles. The adaptation serves two functions: maintaining eye shape and supporting quick vision movements (Fig. 4).

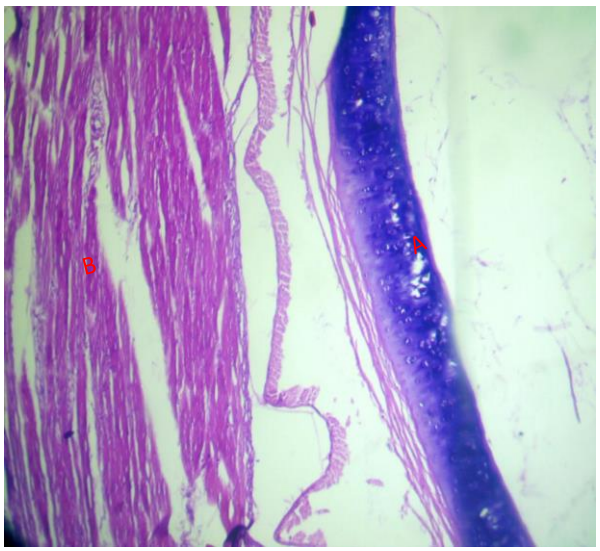


Fig (4): corneal Epithelium (A) Bowman's membrane layer (B) dissociated stroma (C) Descemet's membrane (D) Endothelium (E) (H&E X10)

Transparency together with refractive properties derives from the multiple layers, including epithelium and Bowman's membrane and stroma, Descemet's membrane, and endothelium (24). The corneal strength, along with optical clarity, depends on the highly organized bundles of collagen found within the stroma. The optics of vision become compromised when abnormal patterns appear within corneal fibers (25).

The sclera contained the hyaline cartilage, which was formed by matrix and lacuna with chondrocytes, and this bar of cartilage was invested by perichondrium, collagen bundles were adhered to the cartilage with its fibroblasts (Fig. 5, 6).

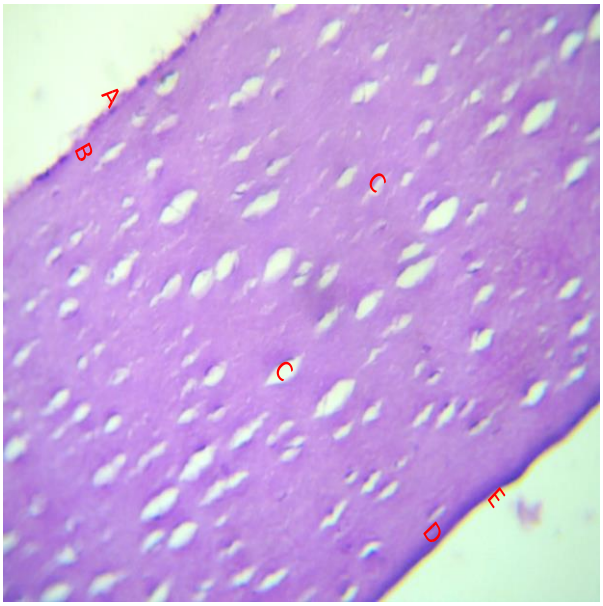


Fig (5): scleral bar of hyaline cartilage (A). Collagen bundles (B) (H&E X10)

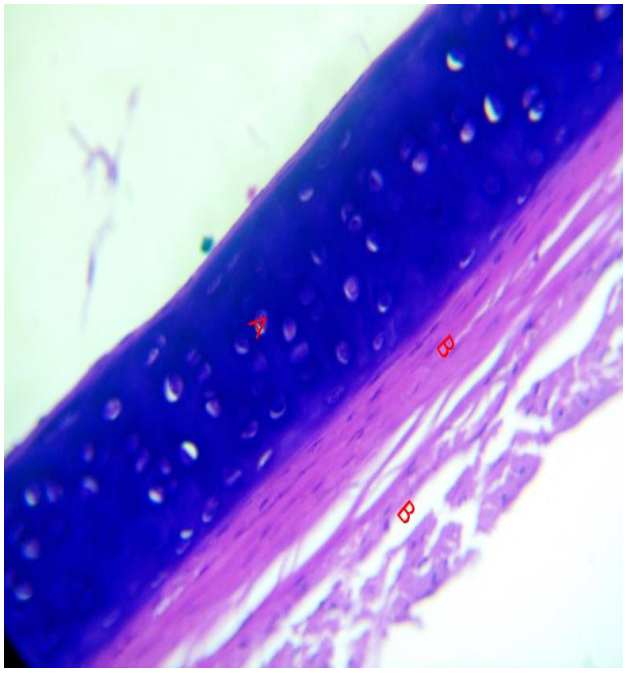


Fig (6): sclera Bar of hyaline cartilage (A), collagen bundles (B) (H&E X40)

The cartilaginous tissue that makes up the sclera serves as a distinctive feature of birds because it supports the eye structure while managing eyeball shape during different internal pressure levels (26). Rapid eye movement and stabilization adaptations are possible due to the skeletal muscle fibers along with collagen bundles present in the outer sclera tissue, which support the dynamic vision needed by birds (27). The stroma of the sclera was formed by multiple bundles of collagen fibers, and the loose C.T in between those bundles is the blood capillaries with few W.B.Cs (fig 7).

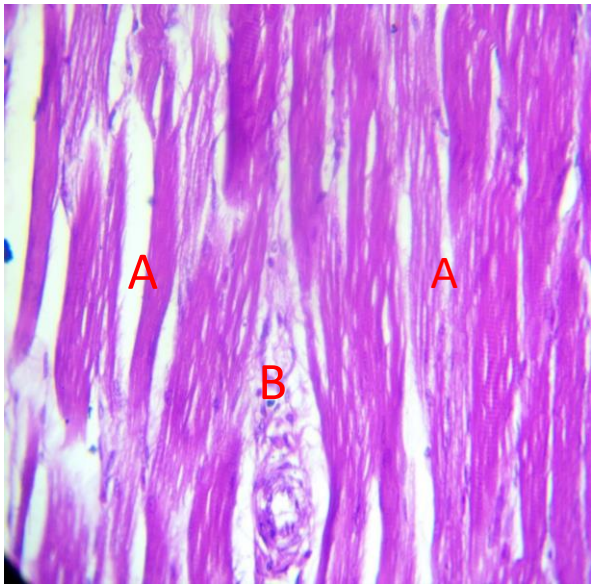


Fig (7): sclera collagen bundles of sclera (A), loose C.T. with blood capillaries with few WBCs (B) (H&E X40)

The sclera contained scattered skeletal muscle fibers at the periphery of the sclera, nerve fibers were demonstrated in between the skeletal muscle fibers, with the presence few blood capillaries (Fig. 8).

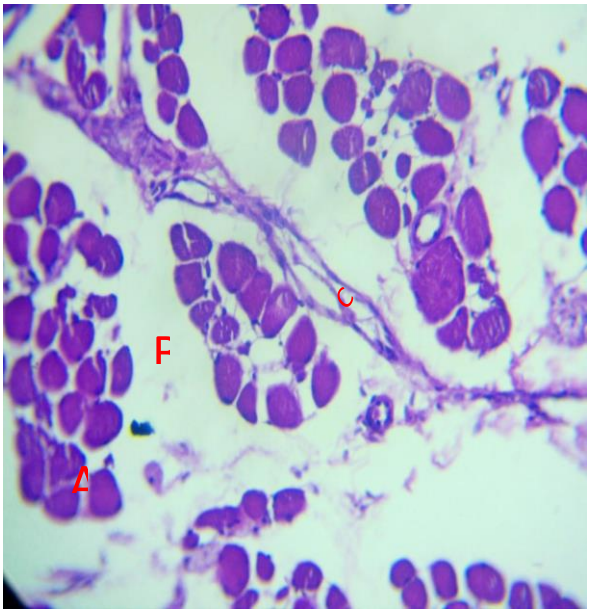


Fig (8): Periphery of sclera with scattered skeletal muscle fibers (A) Loos C.T (B) nerve fibers (C) (H&E X10).

The skeletal muscle fibers in the peripheral sclera help birds achieve better mobility and stability in their vision, which is vital for their fast and coordinated eye movements (28). Further evidence supporting this idea comes from the observed nerve fibers alongside skeletal muscles, showing neural control similarities seen across visually dependent species. The higher density of cone cells in the chicken retina surpasses that of mammalian retinas, enabling improved color perception (29). Although the retinas of vertebrates active at night consist of many rod photoreceptors, studies show that most of the retina of the chicken is composed of cones as opposed to rods to enable day time visibility (30). Another peculiarity of the adaption of birds and reptiles is the appearance of cartilaginous sclera; this characteristic is absent in mammals (31).

Conclusion

Histological study on the retinal structure and eye anatomy of chicken has led to discovery of adaptations to enhance their visual performance, fast movement of the eyeballs and ability to see color. Studies show that chicken retina has high concentration of cone photo receptors implying that these birds are the best in viewing through natural light. Retinal pigmented epithelium (RPE) is fundamental in maintaining photoreceptors and

soaking of light hence ensuring no impairment in the visual operations. The choroid layer contains abundant vascular networks and melanin, providing essential support for retina metabolism while improving light refraction. The collagen arrangement in the cornea creates a clear and efficient light path, improving image quality. Birds have developed the sclera with hyaline cartilage and collagen bundles to ensure eyeball stability and provide mechanical support. Skeletal muscle fibers, along with nerve fibers located in the peripheral sclera, enable rapid eye movements that are vital for navigation, foraging, and threat detection. These findings offer valuable baseline histological data for the avian eye and may serve as a reference for future anatomical, comparative, and pathological studies in poultry and other bird species.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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دراسة نسيجية لشبكية العين وهياكل العين المرتبطة بها

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فرع التشريح والأنسجة ، كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

الملخص

الدراسات العينية والنسيجية المقارنة هي الدرجة العالية من تطور العناصر التشريحية. وحدة البصر الفائقة تجعل عين الطيور (وخاصة الدجاج المنزلي) نموذجا مثاليا للدراسة. تهدف هذه الدراسة الى دراسة شبكية العين وهياكل العين الاخرى للدجاج المنزلي من وجهة النظر النسيجية. تم اخذ عشرون عينة من مفل العيون من الدجاج السليم والتي يبلغ عمرها حوالي اربعين يوما وكانت الاوزان تتراوح من واحد ونصف الى اثنين كغم. تمت معالجة العينات مثل اي مادة نسيجية طبيعة اخرى حيث تم وضعها في محلول الفورمالين المخفف بنسبة عشرة بالمئة ومن ثم التجفيف والتضمين مثل اي مادة نسيجية عادية اخرى. لوحظ الترتيب الهندسي للأنسجة في المقاطع النسيجية فيما يتعلق بصيغة الهيماتوكسيلين ايوسين. في الفحص المجهري التكبيري المنخفض والعالي لوحظ او تم الكشف عن الهيكل النموذجي متعدد الطبقات لشبكية عيون الطيور. الى جانب الطبقات الخمس من القرنية تم اكتشاف وتوثيق انسجة العين الاخرى بما في ذلك الصلبة (الياف الكولاجين الكثيفة) والغضروف الداعم للصلبة والمشيمية المجاورة لشبكية العين. يزيد هذا البحث من فهمنا لتشريح عين الدجاج ويوفر اساسا يمكن ان تبنى عليه الابحاث المقارنة والبيطرية حول عيون الدجاج.

الكلمات المفتاحية: شبكية العين، المستقبلات الضوئية ،علم الأنسجة،المشيمية،القرنية ،الصلبة ،رؤية الطيور.