

ORIGINAL ARTICLE

Gross, histochemical and electron microscopical characterization of the Pecten oculi of Baladi ducks (*Anas boschas domesticus*)

Attia A. A. Moselhy, Enas El-Hady

Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Egypt

ABSTRACT

Objective: As pecten oculi had great functional significances for ornithology, pecten oculi of Baladi duck was well-deserving of intensive morphological study. So, the aim of this study was to throw light on some anatomical and histological formation of the pecten oculi of Baladi ducks as well as use of scanning electron microscopy.

Materials and Methods: Twenty eyeballs of 10 adult Baladi ducks were used to fulfill this work. Ten eyes were used to study the gross anatomy of pecten oculi, including the location, shape, and numbers of pleats. Five samples were embedded at 10% neutral buffered formalin. The specimens were examined by regular histological procedures. The latter five samples were applied for electron microscopy.

Results: Grossly, the pecten oculi is formed of three portions: the base, emerged from the optic disk; the pleats, sorted in fan shape; and the bridge. The essential histological ingredients of Baladi ducks' pecten oculi are the blood vessels, lymph vessels, pigment cells, and hyalocytes.

Conclusion: The current work explains the primary macro- and micro-morphological features of pecten oculi in Baladi duck and collates these features to those formerly explained in other birds. Generally, pecten oculi of Baladi duck was analogous to that of the diurnal birds.

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Introduction

The duck is generally a diurnal prey bird that exhibits a global distribution. Duck belongs to the family *Anatidae*, genus *Anas*. The diversities of duck (*Anas boschas domesticus*) come down from the mallard (*Anas platyrhynchos*) [1]. The pecten oculi is a thin, extremely vascular and pigmented organ specific to the eye of birds [2]. It is located above the optic disk and it is protruded from the retina to vitreous body [3]. The anatomical forms of pecten oculi are classified as three items pleated, vaned, and conical [4]. The conical kind is present in the brown kiwi; the vaned kind is in ostriches, and rheas and pleated type is located in most avian species such as quail [3], mallard [5], pigeon [6], and jungle crow [7]. The histological structure of the avian pecten is essentially formed of great numbers of capillaries, melanocytes, and connective tissue [8]. The avian retina is thicker in comparison to animals without retinal blood vessels [9]. The function of the

pecten appears to provide nutrition by diffusion through the vitreous body to avascular retina [2], and formation of a blood-retinal barrier [9]. The difference in numbers and size of folds in pecten oculi are noticed in nocturnal and diurnal birds [4]. As pecten oculi had a great functional significances for ornithology, pecten oculi of Baladi duck was well-deserving of intensive morphological study using stereo, light, and scanning electron microscopy. The purpose of the current work was to carry out anatomical, histochemical, and electron microscopical investigation of pecten oculi structure of the Baladi duck and to expose the variations.

Materials and Methods

Ethical approval

The handling of birds in this work followed the guidelines of the Institutional Animal Care and the Research

Correspondence Attia A. A. Moselhy ✉ attiaanatomy@gmail.com 📧 Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Egypt.

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Ethics Committee of the Zagazig University, with an ethical approval number of ZU-IACUC/2/F/161/2019.

Birds used

This work was performed on 20 eyeballs of 10 adult ducks (*Anas domesticus*) of both sexes and their age ranged from 6 to 10 months, which were brought from Zagazig villages. The ducks were slaughtered in the abattoir, the eyeballs were sliced with each other with optic nerve away from orbit, and cleaned.

Macroscopic examinations

Ten eyeballs were cut from the equatorial region. The caudal part of the eye containing the pecten oculi was examined as fresh cadavers. The position, shape, and numbers of pleat of pecten oculi were exposed; apical and basal lengths as well as height were gained. The forced light stereomicroscope (Odrrect, Toko, Seiwa Optical No.6075000) by the objective lens X5 was used.

Light microscopic examinations

The posterior regions of five eyeballs including the pecten oculi were processed for paraffin embedding technique and tissue segments paraffin blocks were sectioned in 5-micron thickness and stained with Hematoxylin and Eosin stain, PAS and Masson's trichrome [10]. The stained sections were examined under the light microscope and photographed by a digital Dsc-W 130 camera joined Olympus BX 21 light microscope in the Histology and Cytology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Scanning electron microscopy

The pecten oculi structure of five eyeballs was fixed immediately after slaughter in 2.5% of gluteraldehyde solution for 48 h. After that washed by 0.1 M phosphate buffer solution twice. The tissues were kept in a 1% of osmium tetroxide solution for 1 h, then handled by acetone sequences and dehydrated by CPD. The specimens were plated by Gold-Palladium and studied by a scanning electron microscope at various magnifications, gauges linked to pecten oculi formation and melanin pigment. The specimens were examined with JSM-6510LV scanning electron microscope (JEOL CO, USA) at an accelerating voltage of 20, 30 kV. This section was performed at Faculty of Agriculture, Mansoura University.

Results

The gross anatomical features

Grossly, the pecten oculi of Baladi duck was intraocular, thin, fine, and comb like with bruch borders. The base of the pecten oculi attached to the linear optic disk and the free border (apex) protruded freely to the gel-like vitreous

body (Fig. 1A and B). It had black color due to intensive melanin pigment especially at the apex. It was located at the posteroventral aspect of the eye, very delicate, and velvety in consistency (Fig. 1C). The anatomical type of the pecten oculi of Baladi duck was pleated and it was shown as a folded organ consisted of 14–15 slender pleats which confluent together at the apex in the form of an accordion pattern (Fig. 1A–D). These folds connected apically to form a bridge (Fig. 1A and C).

There are no gender variation. The mean length of the pecten at the base was 5.4 ± 0.11 mm while at the bridge measured 3.6 ± 0.13 mm. The mean height from the first to the last pleat was 1.3 ± 0.11 to 2.6 ± 0.12 mm, respectively. The mean distance between two pleats ranged from 48 ± 1.15 μ m to 320 ± 11.5 μ m. The measurements of pecten oculi are achieved by electron microscope as seen in Table 1.

Histochemical structures

Microscopically, the pecten oculi of duck emerged from the core of optic nerve and got progression as a garland of blood capillaries as accordion folds at the posterior side upto half way inside of the cup of the eyeball. The base of pecten oculi was secured alternately ventrally to the linear optic disk while its apex was positioned liberally into the vitreous humor. At the apex of the pecten, the pleats were fixed jointly by a darkly pigmented "bridge". Accordion folds began its course from the base and fold-away to attach the apex in snaky fashion. At the anterior end of the summit of pecten and optic nerve, the pleural numbers of prominent blood capillaries were also present with either side retinal layers, combine layers of choroid and sclera (Fig. 1D, 2A–C).

In serial transverse sections, the pecten partitioned into three equal parts; bridge (apex), base, and middle part. The pectineal folds consisted of many capillaries usually arranged in two layers around the larger vessels (arterioles and venules). The distance between two pectineal folds and the dimension of capillaries were larger at the middle part of pecten (Fig. 2D and E, Table 1).

The apex of pecten oculi had shorter and narrower folds, ended as thickened triangular-shaped structure which known as the bridge. In vertical sections of the bridge surface had tight depressions, sometimes profound and coiled, which gave connection for the fibers of vitreous body. It comprised of a network of anastomosing cords of polymorphic melanocytes and few capillaries with less dimension, lined by a thick or thin endothelial cells (Figs. 2D, F, G, 3A, B).

The polymorphic melanocytes were the second prominent cell in the pecten and it was responsible for its dark black coloration. It either presents independently or in clusters in between different blood vessels. Melanin dissemination was the highest at the apex and even in other parts of the pecten (Figs. 2E, F, G, 3A, B, C, F, G).

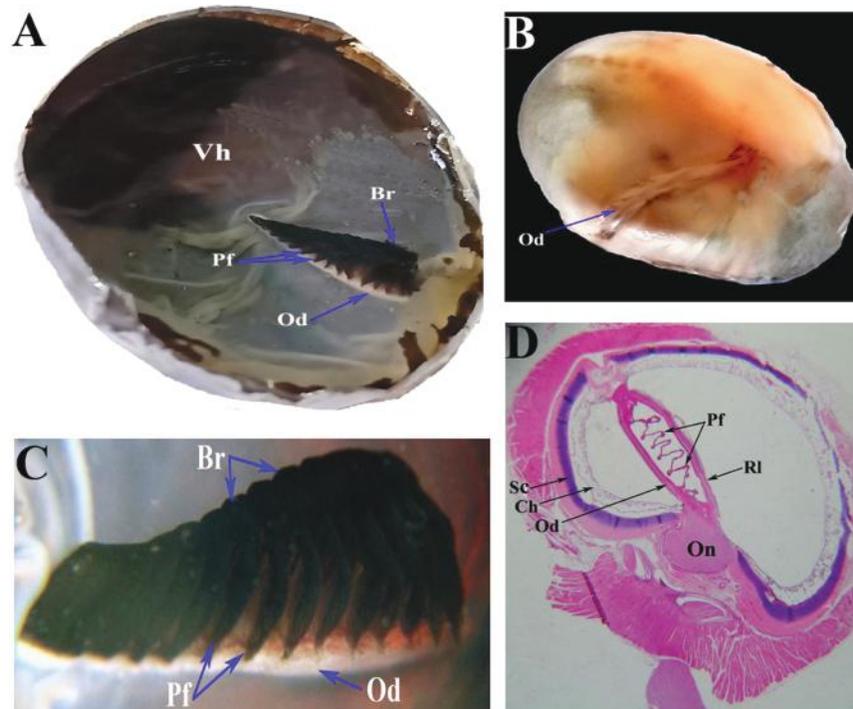


Figure 1. (A) A photograph equatorial section of the eye of Baladi duck showing the pecten parts. (B) A photograph equatorial section of the eye of Baladi duck after removal of eye contents. (C) A photograph of Baladi duck pecten oculi. A photograph equatorial section of the eye of Baladi duck after removal of eye contents. (D) Stereomicroscopic image of pecten oculi 5X. Vh = Vitreous humor, Pf = Pecten fold, Od = Optic disk, Br = Bridge, Sc = Scleral cartilage, Ch = Choroid, On = Optic nerve, Rl = Retinal layer

Table 1. The measurements of the pecten oculi of Baladi duck.

The mean length at base	5.4 ± 0.11 mm
The mean length at bridge	3.6 ± 0.13 mm
The mean height of first pleat	1.3 ± 0.11 mm
The mean height of last pleat	2.6 ± 0.12 mm
The small distance between 2 pleats	48 ± 1.15 μm
The large distance between 2 pleats	320 ± 11.5 μm

Both blood vessels and capillaries had thick basement membrane that give a positive reaction to PAS and trichrome stain. The blood capillaries were lined by endothelial cells (simple squamous epithelium with rounded or flat nucleus). The lumen of blood capillaries appeared either contained nucleated erythrocytes or empty (Fig. 3D–G).

Interestingly, the presence of lymphatic capillaries in accompany with the blood capillaries. These lymphatic capillaries linked the blood capillaries of the pleats in the vicinity of the vitreous membrane (Fig. 3C).

Other cells existed in pecten oculi such as hyalocytes which was appeared as a subtype of blood-borne

macrophages. Also, some lymphocytes were present in a few numbers (Fig. 3C).

Scanning electron microscopy

A scanning electron examination at low magnification indicated that the pecten oculi formed from 14 to 15 pleats. The pectineal pleats were connected together apically by a bridge and distally attached to a linear optic disk (Fig. 4A and B). The outer surface of the pectineal pleats appeared as cord-like structures and contained a dense vascular connection (Fig. 4C). This network had spheroidal bodies which recognized as pigmented cells (melanocytes) (Fig. 4D). The cross section of the pecten oculi showed numerous blood vessels with different diameters (Fig. 4E and F). There are no gender variation in Baladi duck concerning macro and micromorphology.

Discussion

The gross anatomical features

The morphometrical measurements of pecten oculi are in the same range with that of Orhan et al. [3]. The pecten oculi

is involved with nutrition of avascular retina of birds [11]. The pecten oculi consists of three portions; the base, the folds, and bridge [12,13]. The site of the pecten oculi in duck conformed that recorded in other avian species. The pecten is a fanlike shape in accordance with Pourlis [11] in Quail.

The numbers of pleats differs among the birds which have a pleated type pecten. Usually, diurnal birds had larger and more pleated pectens compared to nocturnal birds [9]. By comparing Baladi duck to other diurnal bird, it had 14–15 pleates. However, mallard had 12–14 [5], American duck had 13–14 [4], 10–12 in budgerigar [12], and 11–12 in sparrow hawk [14]. The pigeon had 12, turkey had 21–22, starling had 17 [4], domestic fowl had 16–18 [9], Guinea fowl had 13–17 [8], black kite had 12–13 [2,9], seagulls had 18–21 [15], quail contains 19 [3], common buzzard contains 17–18 [16], 20–25 in Australian galah [17], and jungle crow has 24–25 [7], pleats in pecten. Although nocturnal birds have small pectens as barred owl which contains 8–10 [18] and spotted eagle owl that has 5–6 [9], folds in pecten. On the basis of physics, a black body absorbs the heat of those radiations of all wavelength

which fall on it. Melanosomes, which was providing the black color to the pecten oculi through its different parts, helps in absorbing this heat [8].

Except the pecten of kiwis, the pectens of all birds are folded or pleated. The intention of this folding is clearly to increase the surface area of the pecten in addition to produce strength as suggested by Tucker [19]. As the pecten is very delicate, if it was straight or linear, it might be torn when the bird exposed to any concussion [19].

Histochemical structures

The histological structure of the pecten of duck was curiously analogous to that of other birds. It consisted of a slender, folded plexus of slightly big capillaries grasped by a template of pigmented cells [3,20,21]. The extraordinary vascular structure and specific capillary morphology are essential characteristic to be a trophic organ the retina substituting the absence of central retinal artery.

We noticed capillaries and blood vessels of different sizes, the capillaries were more dominant in pecten oculi

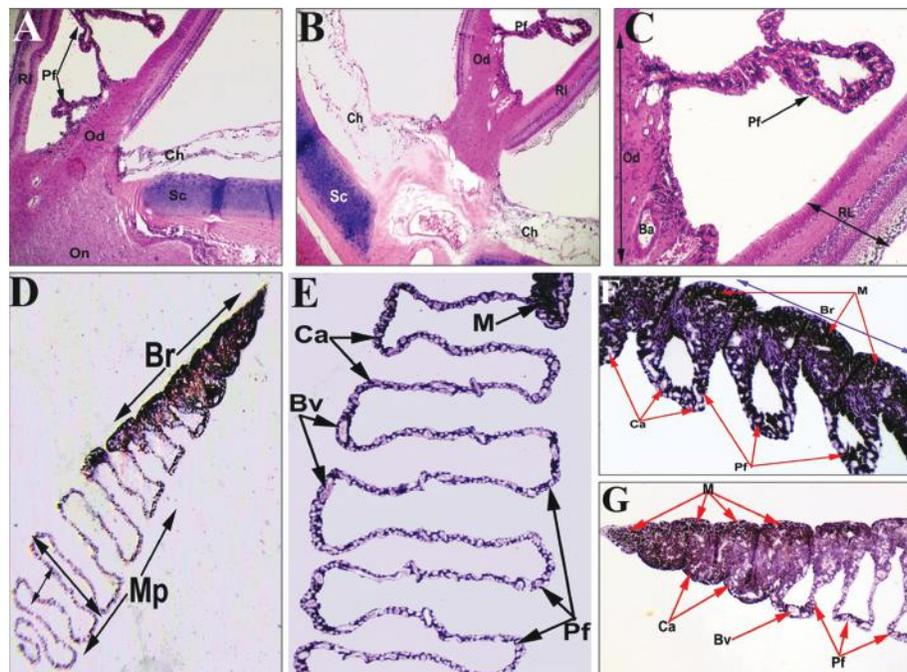


Figure 2. Light micrograph of pecten oculi in Baladi duck with Hematoxylin and Eosin staining technique (A, B, E, G X 40), (C, F X100). (D X 5) Stereo-microscopic image of pecten oculi 5X. (A, B, C) showing the location of the pecten on the ventral part of the optic disc with its surrounding structures. D–G Transverse section showing the different parts of the pecten appearing as a thin, folded lamina in the form of accordion pattern. Its middle part has a maximum length of folds and maximum distance between two pectineal folds (D, E). The apex the pecten oculi (bridge) thickened and highly pigmented mass located along the free edge of the pecten (F, G). Pf = Pecten fold, M = Melanocyte, Ca = Capillary, Bv = Blood vessel, Od = Optic disk, Ch = Choroid, Sc = Scleral cartilage, On = Optic nerve, Rl = Retinal layer, Br = Bridge, Ba = Basal artery, Mp = Middle piece of pecten

of Baladi duck, similar findings were observed by Yilmaz et al. [22] in common barn owl. The capillaries were ringed by a dense basal membrane in agreeing with that described in previous studies [5,6,17,21]. It is proposed of dense basal laminae that may assist the delicate endothelial cells [6,17].

In accordance to Yilmaz et al. [22] in domestic fowl confirmed that PAS positive reaction in was greater or lesser extent in the entire pecten. The collagen fibers of the blood capillaries, their basal lamina, and even the adjacent connective tissue septa of the bridge were showing positive activity with trichrome stain in agreement with Mishra and Meshram [8] in guinea fowl.

The melanocytes were the second prominent cell type of the detected in between the capillaries pecten oculi of Baladi duck. The accumulated melanocytes especially on the bridge and the pecten surface were responsible for its dark black coloration and somewhat the velvety texture in agreement with Mishra and Meshram [8]. This shows evidence for light absorbption by the pecten and protection of the eye from harmful effects of ultraviolet light [2,17,21].

That directed to propose that pecten's function equivalent to the light baffles in the camera [23,24].

The distribution of melanocytes and capillaries also described in the black kite [2], quail [3], ostrich [20], and jungle crow [7]. In this study, the melanin concentration was the highest at the apex and even in other parts of the pecten, as reported by others [13,25]. The melanocytes are proposed to provide a structural support to the pecten for holding it tightly straight in vitreous humor [17,26]. In accordance with Scala et al. [27] and Corona et al. [28] in duck, small lymphatic capillaries accompanied the blood capillaries. Conversely, these lymphatic capillaries were not evident in other species [12,13,22]. Interestingly, the existence of lymphatic vessels in the choroidal coat to drain intraocular fluids from the caudal part of the orb may elucidate that the pecten oculi performs an eminent part in the product of these fluids [29].

The hyalocytes have been observed by light microscopy in the connective tissue between the blood capillaries, similar findings were reported in some species such

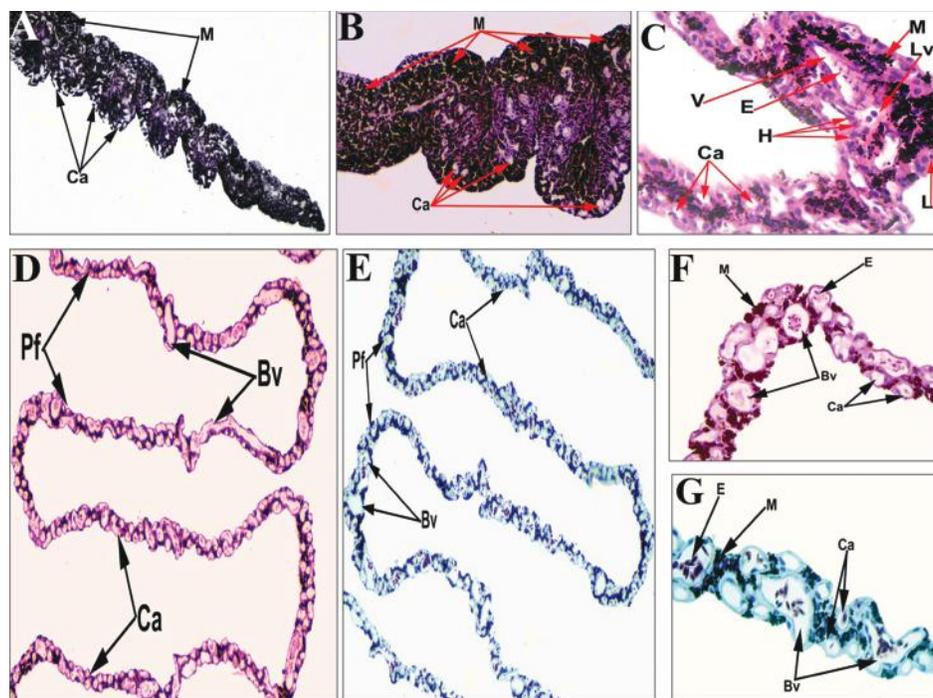


Figure 3. Light micrograph of pecten oculi in Baladi duck with Hematoxylin and Eosin staining technique (A, B, C), PAS (D, F), and trichrome stain (E, G): (A X40), (B, D, E X100), (C, F, G X 400). (A, B) The black coloration of the bridge due to high concentration of melanocytes. (C) High-power photomicrograph of the pecten oculi fold showing numerous and polymorphic melanocytes either individually or in clusters in between the blood capillaries, the latter accompanied by lymph vessels with few numbers of lymphocytes and hyalocytes. (D–G) The blood capillaries had thick basement membrane, lined by endothelium and their lumen appeared contained nucleated erythrocytes. V = Venule, E = Erythrocyte, M = Melanocyte, Lv = Lymph vessel, H = Hyalocyte, L = Lymphocyte, Ca = Capillary, Bv = Blood vessel, Pf = Pecten fold

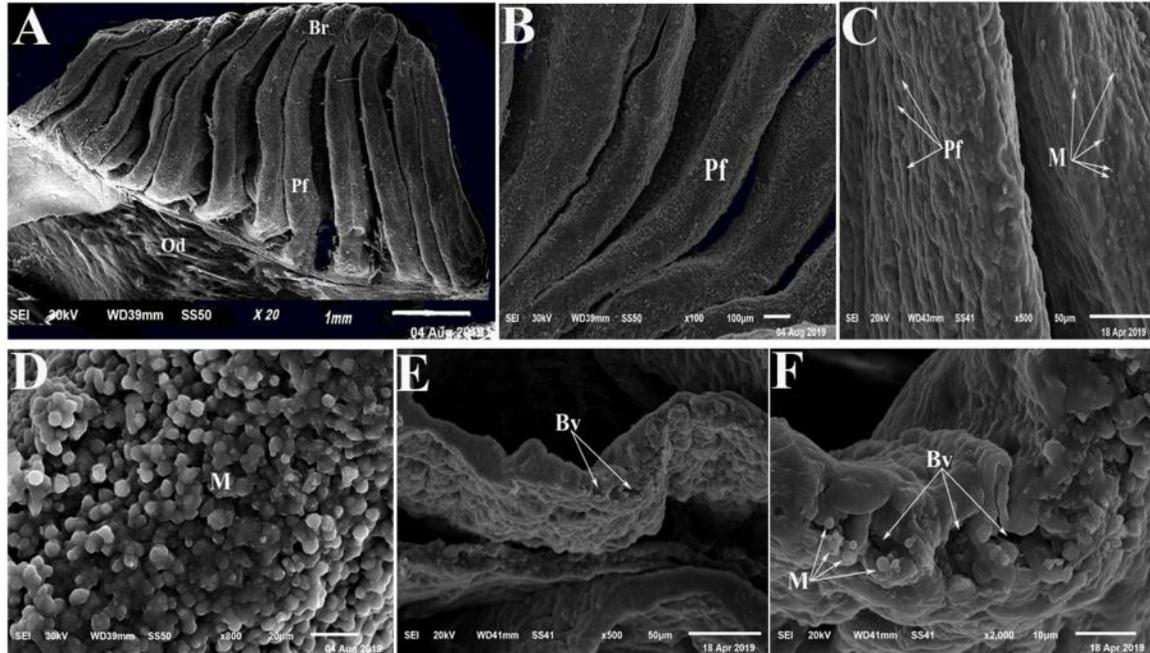


Figure 4. (A, B, C, and D) Scanning electron micrograph of the pecten oculi at different magnifications. E and F: Scanning electron micrograph of the cross cut surface of the pecten oculi. Od = Optic disk, Pf = Pecten fold, Br = Bridge, Bv = Blood vessel, M = Melanocyte

as chicken [30,31], budgerigar [12], quail [3,11], mallard [5], and emu [21]. On the contrary, hyalocytes were absent in other species such as the pigeon [6], red tailed hawk [26], or nighthawk [25]. In contrary to Yilmaz et al. [22] in common, barn owl hyalocytes have been seen on the surface of the pleats only via scanning electron microscope and not detected by light microscopy. Also, the presence of fewer numbers of lymphocyte, together with the macrophage-like hyalocytes fend off diseases and infection, this indicate that the pecten plays a role in immunity [8].

Scanning electron microscopy

Pectineal pleats display the various densities and shape of blood capillaries and the nucleated erythrocytes were clarified [13]. In contrast with Poulis [11] in Quail, demonstrated, the limiting membrane which covers the pecten by scanning electron microscopy. The pecten oculi of duck had a similar structure to that of other avian with some substantial discrepancies; such as number of the folds, volume, frame, and density of the capillary basal lamina [17]. These variations rely on the avian attitude in relation to their general action and the optical activity of the species.

Beside, a nutritive role of retina by blood capillaries of the pecten, it also provides structural support and protection of ocular component, from ultraviolet light and oxygen radicals might be possible by the presence of melanin [8,12].

Conclusion

The current work explains the primary macro- and micro-morphological features of pecten oculi in the Baladi duck and collates these features to those formerly explained in other birds. Generally, pecten oculi of the Baladi duck was more analogous to that of the diurnal birds. This study is the first trial to study pecten oculi in Baladi duck (sudani) in Egypt through gross, histochemical, and electron microscopical investigation and collates these morphological features to those explained previously.

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Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Both authors designed plan of work, collected samples, carried out research, wrote, and revised manuscript.

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