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The Light and Scanning Electron Microscopic Structure of Pecten oculi in the Goose (Anser anser)

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Abstract

This study aims to establish the light and electron microscopic structure of the pecten oculi in the goose (*Anser anser*). For this purpose, 12 samples of pecten oculi extracted from 6 goose eyes were used. In the study, it was found that the goose pecten consists of 13-14 pleats. The maximum transversal length of the eye was approximately 10 mm, the corneal diameter was 5 mm, the basal length of the pecten was 7 mm, the apical length was 1.5 mm, and the height of the pecten was 5.55 mm (n=6). In pecten pleats, the mean diameters of two separate vessels, primary and secondary, were 48.94 and 23.36 µm respectively. The primary vessels located at the centre of the pecten pleats were surrounded by the secondary vessels. It was observed that the melanocytes in pleats gradually intensified from basal to apical regions. Pecten covered to the vitreo-pecteneal limiting membrane and, hyalocytes were found on this part. This study revealed that the goose pecten has a structure similar to the avian species in the waterfowl family.

Keywords: Goose, light microscope, Pecten oculi, scanning electron microscope, structure.

INTRODUCTION

Birds have rather large eyes compared to their heads (Baumel et al., 1993). This reveals the importance of vision for birds to perform vital activities (Burton, 2008).

The eye is not just the organ of vision. It also plays a key role in balancing the inner eye pressure by retinal and choroid circulation (Yu et al., 2014). In all vertebrates, the choroid is the primary vascular source of the outer retina (Nickla and Wallman, 2010). In numerous vertebrates, a second vascular system supplies the internal retina, a supplementary blood flow or retinal circulation flow and has several forms. One of them is the pecten oculi, which is seen in the avian species (Mcmillan and Harris, 2018).

Pecten oculi is a highly vascular and pigmented organ (Braekevelt, 1991; Yilmaz et al., 2017; Cevik Demirkan et al., 2018). In the embryonic period, it extends from the choroid (optic) fissure to the retina and from there to the vitreous body in the part where the optic nerve reaches the retina (Korkmaz and Kum, 2016; Elghoul et al., 2022). It is hypothesized that pecten oculi has many functions such as providing oxygen to the retina, regulating of intraocular pH (Mishra and Meshram, 2019), adjusting the intraocular temperature (Murphy and Dubielzig, 1993), acting as the bird's magnetic field sensor region (Southern et al., 1982), protecting the retina from UV rays and oxygen radicals (Bennett and Cuthill, 1994).

Hyalocytes or peripectinate cells are placed on vitreopecteneal membrane. It is believed that they are a subtype of macrophages (Korkmaz and Kum, 2016). It is considered that pecten melanocytes play roles in the absorption of sunlight and regulation of the temperature in the eye (Bennett and Cuthill, 1994). Geese are an avian species that belongs to the Anseriformes family. Although there are many different goose species, Anser anser (*Eastern Greylag Goose*) is the most common species in Turkey. Goose farming is performed near the city of Kars in Turkey and despite being largely herbivores, these animals can feed on small insects and algae These diurnal animals hunt during the day and rest during the night (Buckland and Guy, 2002; Carboneras and Kirwan, 2020).

This study aims to establish the macroanatomical and histological features of the pecten oculi in goose (Anser anser) using light and scanning electron microscopy (SEM).

MATERIALS AND METHODS

A total of 12 eye samples of six geese were used in this study. While 6 of these eyes were used for light microscopic examinations, three eyes were used for stereomicroscopic examinations and 3 eyes were used for SEM examinations.

The ethical approval (Date: 02/02/2018 and Number: 51025321-050.05.04) was obtained from the Harran University Animal Experiments Local Ethics Committee.

Morphometric analysis

The three eyeballs were cut equatorial and revealed the pecten in the eye. Stereomicroscope (Olympus-SZX7, Olympus Optical, Japan) and camera attachment (Olympus Cam-SC50) connected to it were used for morphometric measurements in the pecten. Nomina Anatomica Avium (Baumel et al., 1993) was used for terminology. Morphometrically, the maximum transverse length of the eye, maximum corneal length, pecten length and width were measured.

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Histologic analysis

For the histological examinations, 12 eye samples of 6 geese were used. The pecten oculi samples removed from the equatorially transected eyes were fixated in 10% formalin solution for 24 hours and then washed for 24 hours. After washing, the tissues were treated with xylol series after gradually increasing degrees of alcohol series (50%, 70%, 80%, 96% and 100%) for dehydration. Afterwards, they were embedded in paraffin blocks. Five serial sections of 5 µm thickness were taken from the blocked tissues. These sections were stained with Crossmon's modification of Mallory's Trichrome method (Crossmon, 1937). Serial sections were obtained and examined by Olympus BX53 research microscope, and photographs were taken. Besides, six regions were randomly selected from each section and the primary and secondary vessel diameters in 30 different for each animal (a total 180 measurements in all animals) were measured using the cellSens image software analysis program (Olympus).

Scanning electron microscope (SEM) analysis

For the SEM examinations, pecten oculi removed from 3 eyes of goose were used. After removing the eyeball, the pecten oculi samples were washed twice in phosphate-buffer solution (0.1 M, pH: 7.4) and fixated for 48 hours in 2.5% glutaraldehyde solution. The samples were then treated with 1% osmium tetroxide for one hour. To dehydrate the tissues, samples were subjected to gradually increasing concentrations of acetone series (25%, 50%, 75%, 100%, washed 3 times each). The samples were then dried to the critical point for drying (EMS 850) (Dunlap and Adaskaveg, 1997). The dried samples were covered with old-palladium (EMS 550) and examined at different magnifications with SEM (Zeiss, Evo 50). Investigations were made in Harran University Central Laboratory (HUPTAM).

RESULTS

The stereo microscopic examinations revealed that the goose had brown pecten oculi consisting of 13-14 pleats (Figure 1-A). When the goose eyes were examined, it was found that the maximum transversal length of the eye was approximately 10 mm, the corneal diameter was 5 mm, the basal length of the pecten was 7 mm, the apical length was 1.5 mm, and the height of the pecten (between the base and the apical's highest point) was measured at 5.55 mm (n=6) (Figure 1-B). On the other hand, it was observed that the width of the basal part and apical part of the pecten was about 7.09 mm and 1.32 mm, respectively (Figure 1-C). The height of the pecten oculi.

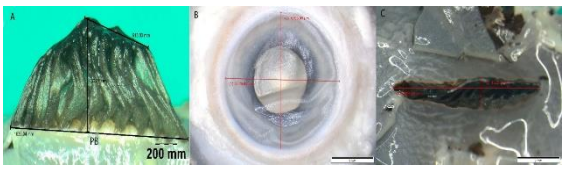

Figure 1. A: Image in the pecten oculi **PB**: Pecten base, **B:** Corneal diameter, **C:** Pecten length.

In histological examinations, as in all avian animals, it was seen that the pecten oculi in the goose extends towards the vitreous body at the entrance of the nervus opticus to the retina. In this part, it was found that the retina was interrupted, and in this region the basal portion of the pecten occurred. Pecten consisted of three parts: the base (or basal), bridge (or apical), and pleats. The vessels and melanocytes of the pecten oculi, sitting on a wide base, arose from the base of the pecten (Figure 2). It was seen that the pecten pleats stretched upwards and joined to form a bridge at the top (Figure 3 A-B).

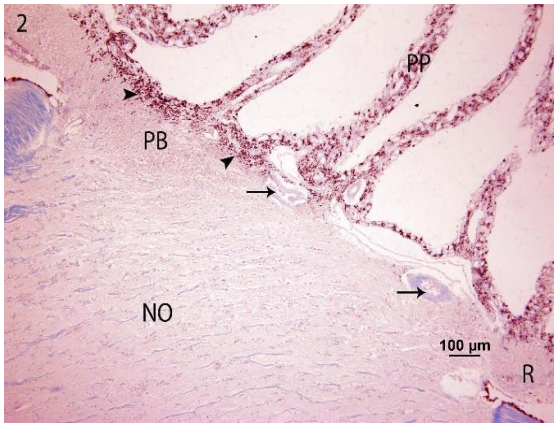

Figure 2. Histological view of pecten base **NO:** Nervus Optikus, **PB:** Pecten Base, **R:** Retina, **PP:** Pecten Pleate, **arrows:** vessels, **arrowheads:** Melanocytes. Crossmon Trichrome stain. Bar: 100µm

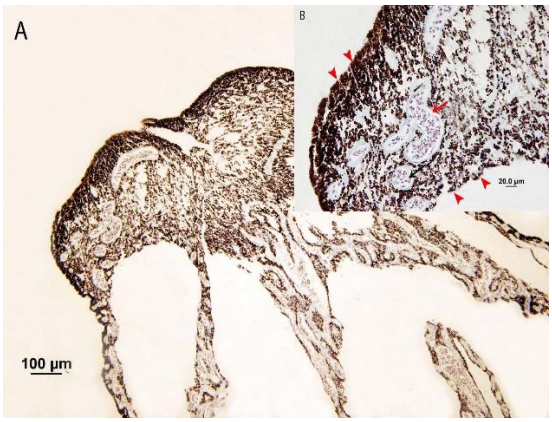

Figure 3. Histological view of pecten **A:** Pecten bridge Bar: 100µm Trichrome stain, **B:** Pecten bridge **arrow:** vessel, **arrowheads:** Melanocytes. Crossmon Trichrome stain. Bar: 20 µm

When the pecten pleats were examined, it was seen that there were two capillary vessels of different diameters in each pleat. The larger vessels are called primary capillary vessel whereas the smaller vessels are known as secondary capillary vessels. In the middle of each pleat, it was found that the primary capillary vessels and the secondary capillary vessels were in the periphery of the pleat and around the primary capillary vessels (Figure 4). Hyalocytes were located on the pecten pleats (Figure 5). The mean diameters of two separate capillary vessels, primary and secondary, in pecten pleats were average 48.94±10,83 and 23.36±5,77 µm respectively (Table 1) (Figure 6) (n=6). This vascular glomerulus was observed to extend into the vitreous body, leaving the density to melanocytes in the bridge area where the pleats join. Also,

the melanocytes contained in the pleats surrounded both the primary and secondary blood vessels. In the apical portion of the pecten, the primary capillary vessels were replaced by the secondary capillary vessels and melanocytes, which concentrated in this part (Figure 3 A-B). The vitreo-pecteneal limiting membrane surrounded the pecten oculi. Outside this membrane, there were macrophage-like cells called hyalocytes (Figure 5).

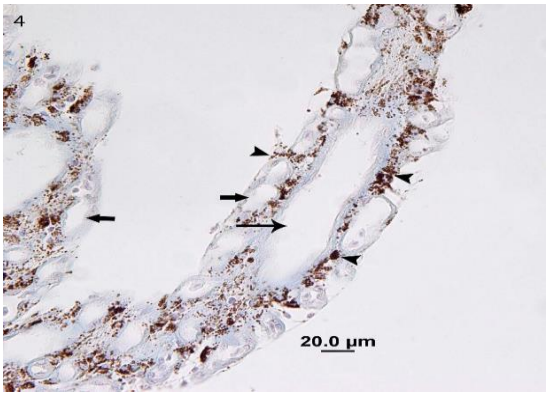

Figure 4. Histological view of pecten pleate **thin arrow**: Primary vessel, **thick arrows**: Secondary vessels, **arrowheads**: Melanocyte. Crossmon Trichrome stain. Bar: 20 µm

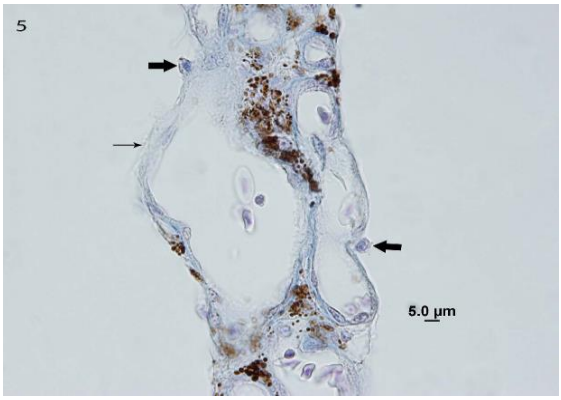

Figure 5. Histological view of pecten pleate **thick arrow:** Hyalocytes **thin arrow**: Pecteneal limiting membrane. Crossmon Trichrome stain. Bar: 5 µm

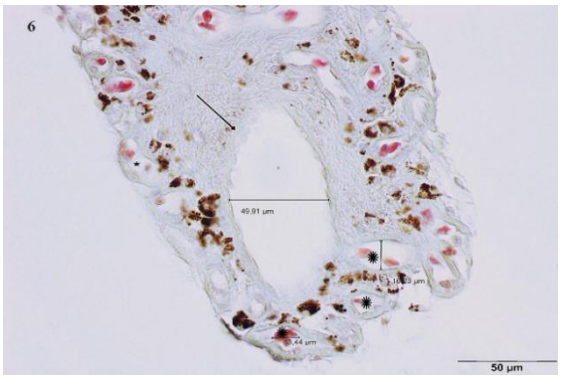

Figure 6. Histological view of pecten pleate. Vessels diamater. **arrow:** Primary vessel **star**: Secondary vessel. Crossmon Trichrome stain. Bar: 50 µm

Table 1. Primary and secondary vessels diamater in the Goose pecten oculi

	Goose	
	PR (average)	**SC (average)**
Goose 1	47,83	22,20
Goose 2	48,84	22,47
Goose 3	48,66	23,48
Goose 4	49,76	25,04
Goose 5	50,72	24,78
Goose 6	47,83	22,20
	Average	
	48,94±10,83	23,36±5,77

PR: primary vessel, **SC:** secondary vessel

In the SEM examinations, it was found that the pecten rested on a wide base and extended towards the vitreous body. Pecten pleats merged at the top to form a bridge (Figure 7). When the pecten pleats were examined, it was seen that the pleats consisted of veins of various sizes. The melanocytes filled the space among these vessels (Figure 8 A). In the SEM examinations, it was observed that hyalocytes were located on the pecteneal limiting membrane (Figure 8 B).

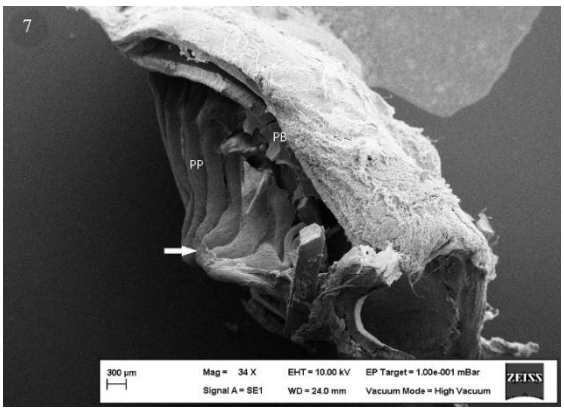

Figure 7. SEM view of pecten oculi. **PB:** Pecten Base, **PP:** Pecten pleate, **arrow:** Pecten bridge.

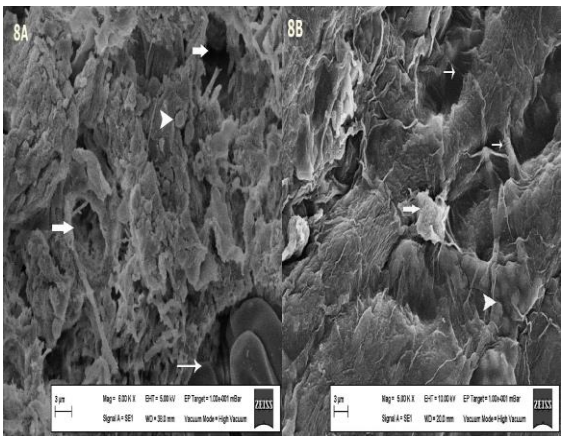

Figure 8. A: SEM view of pecten pleate **thin arrow:** Primary vessel, **thick arrows:** Secondary vessels **arrowhead**: Melanocyte. **B:** SEM view of pecten pleate **thick arrow**: Hyalocyte, **thin arrow:** Vitreo-pekteneal limiting membrane, **arrowhead**: Melanocyte.

DISCUSSION AND CONCLUSION

Pecten oculi is an organ first defined by Perrault in 1676, playing a role in nourishing the avascular retina and presenting only in the eyes of avian animals. Many studies on different avian species have attempted to reveal the structure of the pecten oculi (Yilmaz et al., 2017; Korkmaz and Harem, 2021). This study with goose (Anser anser) grown in Turkey aimed to establish the morphological, morphometric, and histological structure of the pecten oculi with light and electron microscopic findings and determine the similarities and differences to other avian species.

In the pecten oculi studies conducted to date, it has been found that each avian species has a different size of pecten. The table below shows the pecten oculi measurements taken from different animal species and the number of pecten pleats. Merlin (Cevik Demirkan et al., 2018), barn owl, a member of the carnivorous bird family (Yilmaz et al., 2017), a seagull (Gezer Ince et al., 2017), pigeon (Orhan et al., 2011), great blue heron, a large wading bird in the heron family Ardeidae (Braekevelt, 1991) and the duck, a member of waterfowl family (Moselhy and El-Hady, 2019) were compared in this table. The number of pecten pleats was 13-14, pecten length was approximately 7 mm, the width was about 1.5 mm, and the height were about 5 mm in the goose. Compared with other avian animals, although these data of the geese are similar to waterfowls in terms of the number of pleats in the pecten, there are small differences in the pecten measurements (Table 2).

Table 2: In the table, the pecten oculi measurements taken from different animal species and the number of pecten pleats are given**.**

Avian Name	Pecten pleate	Pecten height	Pecten length
Merlin	17-18	5.83 ± 0,12 mm	7.97 ± 0,08 mm (Cevik Demirkan et al., 2018)
Common Barn Owl	8	2.741 ± 0.008 mm	1.447±0.06 mm (apex) 4.431±0.009 mm (base) (Yilmaz et al., 2017)
Pigeon	19	2.14 mm	3.36 mm (apex) 4.95 mm (base) (Korkmaz, 2017)
Seagull	18-21	6.4±0.62 mm	5.77±0.56 mm (apex) 9.01±1.35 mm (base) (Gezer Ince et al., 2017)
Great blue heron	14-15	4-5 mm	8-10 mm (base) (Braekevelt, 1991)
Duck	14-15	1.3±0.11mm (first pli) 2.6±0.12mm (last pili)	3.6±0.13 mm (apex) 5.4±0.11 mm (base) (Moselhy and El-Hady, 2019)
Goose	**13-14**	**5.55 mm**	**1.32 mm (apex)** **7.09 mm (base)**

This information shows that there are large differences in the size of the pecten in birds with different habitats; in similar families (such as goose and duck) the pecten is larger in size and the number of pleats in the pecten.

The ocular bulbus diameter is 22.07±1.18 mm in the seagull (Gezer Ince et al., 2017). Corneal diameter in the owl is 11.95±0.17 mm (Yilmaz et al. 2017). Eyeball diameter is 8-9 mm in the budgerigar (Micali et al., 2012). In the vulture, the corneal diameter has been reported as 9.02 mm and the eye diameter as 22.4 mm (Lisney et al., 2013). In this study, it was found that the transversal length of the eye was 10 mm, and the corneal diameter was 5 mm in the goose (Table 2).

In histological examinations, as in all avian animals (Braekevelt and Richardson, 1996; Gezer Ince et al., 2017; Moselhy and El-Hady, 2019), it was seen that the pecten oculi in the goose extend from the entrance area of the nervus opticus to the vitreous body. In this part, it was found that the retina was disappeared and the basal portion of the pecten formed the region.

Vessels are reported to be the most common structures seen in the pleats of the pecten. It was reported that in the Baladi duck, the large vessels in the centre of the pecten pleats are surrounded by capillaries (Moselhy and El-Hady, 2019). Korkmaz, (2017) detected three types of vessels with different diameters in the pigeon, and the diameter of the vessels is gradually reduced from basal to apical regions. Similarly, Onuk et al., (2013) reported that afferent and efferent, large, and small vessels were found on storks. In the present study, it was found that there are vessels with two different diameters in each pleat and the secondary vessel is located around the primary vessel.

In a comparative study (Dayan and Ozaydin, 2013), the diameter of the vessels in the pecten was compared between different animal species, and it was reported that the mean vessel diameters in ostrich, duck, pigeon, turkey and starling were 20.23, 14.34, 11.78, 12.58 and 12.78 μm, respectively. In pigeons, however, primary vessel diameters were found to be 110.91±24.88 μm on average, secondary vessels diameters 55.03 ± 12.51 μm on average and tertiary vessel diameters 17.62 ± 3.48 μm on average (Korkmaz, 2017). In the study conducted in geese, it was found that there are primary and secondary vessels, and the average diameters of these vessels were 49.17±10.83 and 23.77±5.77 μm, respectively.

Melanocytes are the most common cell type in the pecten oculi of all avian animals, and these cells are generally concentrated in the apex or bridge region of the avian species. (Moselhy and El-Hady, 2019). It is reported that although their intensity varies, melanocytes, are rare in the pigeon (Korkmaz, 2017) and dense in the loon (Braekevelt, 1986). In this study, although melanocytes were detected intensely across the pecten, it was mostly seen in the apex of the pecten.

In all avian animals, pecten is surrounded by a connective tissue membrane. (Braekevelt, 1993; 1994). This membrane, called the vitreo-pecteneal limiting membrane, was also found in the goose pecten. In some avian species, budgerigar (Micali et al., 2012), Baladi ducks (Moselhy and El-Hady, 2019) and chicken (Uhera

et al., 1996) macrophage-like structures were defined on this membrane (Henis et al., 2015; Korkmaz and Kum, 2016), and these cells were called pectineal hyalocytes. However, in some avian species, American Crow (Braekevelt, 1994), Australian Galah (Braekevelt and Richardson, 1996) and Ostrich (Elghoul et al., 2022), the presence of the pectineal hyalocytes was not mentioned. In this study, pectineal hyalocytes were found in both light and electron microscopic examinations.

The geese, a member of the waterfowl family, are diurnal and herbivorous animal. Compared to the pecten oculi of the birds such as duck (Mallard duck, Baladi duck), heron, loon, which are close relatives of goose pecten, all have been found to bear 13-15 pleats. However, some differences were identified between the lengths and widths of the pecten. A literature review revealed that the pecten measurements were performed using different methods. While some authors measured the length of the pecten separately as basal and apex (Orhan et al., 2011), some researchers only performed basal (Braekevelt, 1991) or apex measurements (Cevik Demirkan et al., 2018). Some researchers calculated the height of the pecten by measuring the first and last pleat separately (Moselhy and El-Hady, 2019), while others measured the middle part (Yilmaz et al., 2017). Although the researchers performed the measurements macroscopically, they made measurements using different methods (Yilmaz et al., 2017; Cevik Demirkan et al., 2018). For all these reasons, the similarity or differences among the avian animals cannot be fully established. However, in the literature searches, it was seen that most of the researchers compared the avian species they studied with other avian species according to their diurnal and nocturnal nature.

By considering the findings obtained from this study, it has been found that the goose pecten is similar to the other avian species that belong to the waterfowl family. These results indicate that pecten functional morphology correlates with the lifestyle and functional need of bird and supports the pecten's nutritional role. However, further comparative studies are needed to reach a more accurate conclusion.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: D.K., Design: D.K., Data Collection or Processing: D.K., I.S.H., Analysis or Interpretation: D.K., I.S.H., Literature Search: D.K., Writing: D.K.

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