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The Histological Structure of the Pecten Oculi in the Ostrich (*Struthio camelus australis*)

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SUMMARY

The present study was aimed at investigating the pecten oculi of the ostrich by light and electron microscopy. The pecten oculi of 5 healthy adult ostriches, obtained from a slaughterhouse, constituted the material of the study. When examined macroscopically, the pecten oculi of the ostrich was observed to be a highly pigmented and relatively large fan-like shaped (vaned) structure consisting of approximately 20-24 folds. It was situated on the ventral surface of the eye and extended into the vitreous body. Light microscopic investigation revealed two or more blood vessels and numerous capillaries in each pecteneal fold. The capillaries had a thick basal lamina and numerous melanocytes and the pecten was surrounded by the vitreo-pecteneal limiting membrane. Hyalocytes were not observed outside of the vitreo-pecteneal membrane. At transmission electron microscopic (TEM) examination, endothelial cells were observed to possess a thin cytoplasm and the organelles were localized to the center of the cells. The capillary endothelium exhibited a large number of luminal and abluminal folds. Pericytes were seen lying beneath the basal lamina. In the present study, ostrich pecten oculi was examined by light and electron microscopic and compared with other poultry species and their differences were revealed.

Key Words: *Ostrich, Electron microscopy, Light microscopy, Pecten oculi*

ÖZET

Devekuşunda (*Struthio camelus australis*) Pekten Okulinin Histolojik Yapısı

Yapılan bu araştırmada devekuşu pekten okulisinin ışık ve elektron mikroskobik olarak incelenmesi amaçlandı. Çalışmada et tüketimine sunulan ve mezbahanede kesilen 5 adet erişkin ve sağlıklı devekuşundan alınan pekten okuli örnekleri kullanıldı. Makroskobik incelemeler sonucu, devekuşunda pekten okulinin gözün ventral yüzeyinden vitreusa doğru uzanan, koyu pigmentli, kendi üzerinde yaklaşık 20-24 adet kıvrım yapan, oldukça büyük bir yapı olduğu görüldü. Işık mikroskobik incelemelerde pekten okulinin her bir kıvrımında çok sayıda kapillar ve melanosit yanında az sayıda arter ve ven de bulunmaktaydı. Melanositlerin pekten kıvrımlarının perifer kısmında, damarların etrafında yoğunlaştığı görüldü. Yapının etrafını pekteno-sınırlayıcı membran çevrelemekteydi. Devekuşu pekten okulisinde hyalositlere rastlanmadı. TEM'de (Transmission Electron Microscope) endotel hücrelerinin ince stoplazmaya sahip olduğu ve organellerin hücrenin merkezinde, çekirdek etrafında yer aldığı gözlendi. Kapillar endotelinin çok sayıda luminal ve abluminal kıvrımı bulunmaktaydı ve kalın bir bazal laminaya sahipti. Bu laminanın altında ise perisiler gözlendi. Sunulan çalışmada devekuşu pekten okulisi ışık ve elektron mikroskobik olarak incelendi ve diğer kanatlı türleriyle karşılaştırılarak farklılıkları ortaya kondu.

Anahtar Kelimeler: *Devekuşu, Elektron mikroskop, Işık mikroskop, Pekten okuli*

INTRODUCTION

Avians have a well-developed visual capability owing to their eyes, which are rather large in proportion to the size of their head (Nickel et al. 1977; Smith et al. 1996). It is known that vision begins in the retina. Different from the others, the avian retina is completely avascular (De Schaepdrijver 1989).

Avascular retina is supply of the pecten oculi, which is a structure special to the avian eye (Braekevelt 1994; Kiama et al. 1997; Kiama et al. 1998). Pecten oculi is associated with nutrition or retinal circulation (Braekevelt 1988; Braekevelt 1991a), form the blood-retinal barrier (Wolburg et al. 1999) and contribute to the regulation of the intraocular pH (Eichhorn and Flugel 1988).

The pecten oculi extends from the retinal entrance site of the optic nerve to the vitreous body. This structure has been classified into three types on the basis of its shape. The conical type has been recognized in the kiwi, the vaned type in the ostrich, and the pleated type in other avian species (Braekevelt 1993; Kiama et al. 1997).

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Besides its shape, the pecten oculi also varies with respect to its size and number of folds.

Although its shape varies among bird species, the basic structure of the avian pecten oculi is the same. It has been reported that numerous capillaries and melanocytes exist within each fold of the pecten oculi (Tucker 1975; Braekevelt 1984; Braekevelt 1991; Dayan and Ozaydın 2013).

The pecten oculi is surrounded by a membrane referred to as the vitreo-pecteneal membrane. There are hyalocytes on the vitreo-pecteneal membrane of some species (Uhera et al. 1996; Orhan et al. 2011). These cells are reported subtype of macrophage (Korkmaz and Kum 2016)

The present study was aimed at investigating the pecten oculi of the ostrich by light and electron microscopy.

MATERIALS and METHODS

In the present study, we used specimens collected from the pecten oculi of the eyes of 5 healthy adult ostriches slaughtered at the Sincan slaughterhouse in Ankara-Turkey. After the animals were slaughtered, their eyes were enucleated and slit open at the equator. The posterior half of the eye then was removed and the pecten and its underlying retinal tissue were dissected carefully. Tissue samples were fixed in 10% neutral formalin for 24 h. After fixation, samples were dehydrated through a series of graded alcohols, cleared in xylene and embedded in paraffin. 6 µm thick paraffin serial sections are taken from tissue samples for light microscopic examination. After deparaffinization and rehydration, 6 µm paraffin sections were stained with Masson's trichrome stain (Denk et al. 1989).

Electron microscopic tissue samples were first prefixed in glutaraldehyde-paraformaldehyde (pH 7.4), as described by Karnovsky (1965), and subsequently were fixed for a second time in 1% osmic acid solution for 2 h. Following the second fixation, tissue samples were maintained in 1% uranyl acetate for 2 h, dehydrated through an ascending series of graded alcohols and propylene oxide, and embedded in Araldite M. The semi-thin 1-µm-thick sections cut from these blocks were stained with toluidin blue, and following the marking of the targeted area, thin sections of 30-40 nm thickness were cut. These sections which were taken on grids stained with uranyl acetate and lead citrate as described by Veneable and Coggeshall (1965), and were examined with the transmission electron microscope.

RESULTS

The pecten oculi of the ostrich was observed to extend from the retinal entrance site of the optic nerve to the vitreous body. In the ostrich, the pecten oculi is settled on a wide base. The retina decreases through the optic nerve, continues on the pecten oculi a little further and then disappears. Macroscopic examination revealed this structure to be black or brown in color. While the connective tissue, connective tissue fibrils and cells are highly dense in the sections near the choroidea they decrease throughout the vitreus. Only the ostrich has a vaned pecten oculi in terms of morphological classification. The pecten oculi of the ostrich consists of 20-24 folds that converge at the apex (Figure 1a). These apically extending folds were determined to display a empty cone-like form as they converged by a connective tissue bridge (Figure 1b).

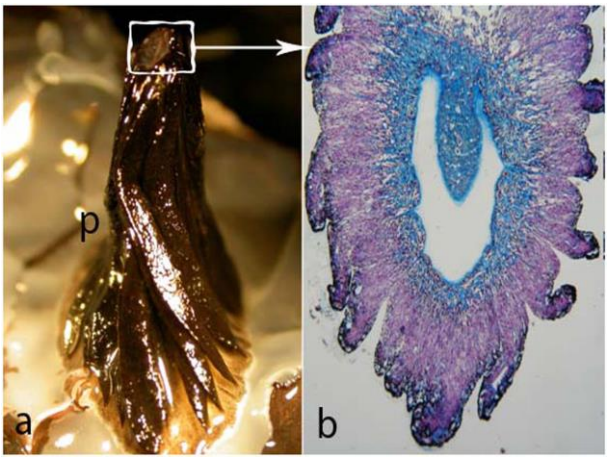

Figure 1. a) Macroscopic image of pecten oculi. square: overhead of pecten, p: pecten oculi; b) Overhead of pecten oculi. Crossman's modified triple staining (X20)

Light microscopic examination revealed the presence of numerous capillaries and two or more larger blood vessels within each pectineal fold of the ostrich. The capillaries within the pectineal folds were particularly dense in the peripheral zone. In ostriches, which have a large pecten oculi in proportion to the size of their eyes, the basal lamina is quite thick. In the present study, it was observed that the thick basal lamina, which surrounded the capillaries, was itself surrounded by many melanocytes. Only the larger blood vessels were observed in the middle of the folds. Melanocytes were determined to detach the capillaries by surrounding them. The pecten oculi was surrounded by an external membrane, which was the continue of the internal limiting membrane of the retina (Figure 2).

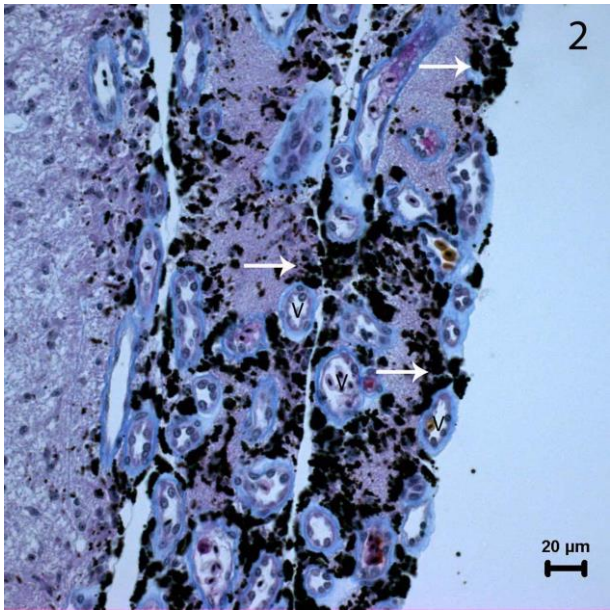

Figure 2. Pecteneal fold. arrow: melanocyte, V: vessel, Crossman's modified triple staining (X40)

Electron microscopic examination demonstrated the presence of long cytoplasmatic folds on both the luminal and abluminal surfaces of the capillaries. These folds were numeous in the peripheral zone of the endothelial cells, but were scarce in the wide central zone, where the nucleus was located. The cell organelles were generally localized around the nucleus. In the ostrich, the luminal folds were determined to be longer and greater in number, in comparison to the abluminal folds. The nucleus was

observed to be large and heterochromatic. Two or more endothelial cells were observed around the capillaries. These cells were determined to be attached to each other with a zonula occludens-type strong cellular junction. The thick basal lamina surrounding the capillaries was observed to detach the capillaries not only from each other but also from the connective tissue surrounding them (Figure 3).

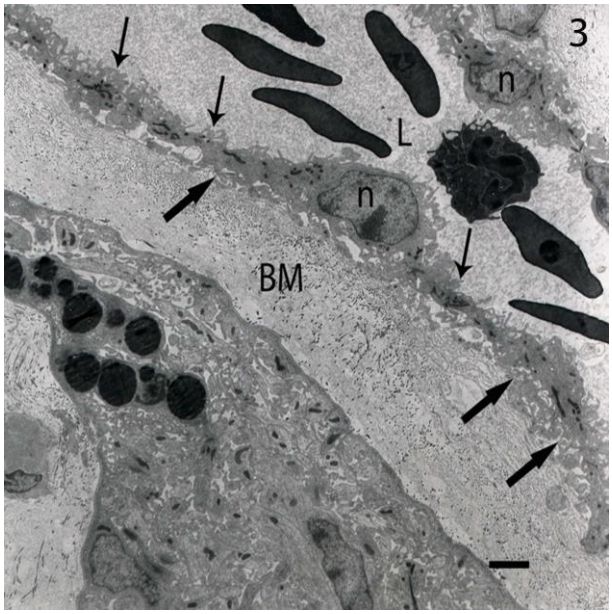

Figure 3. Electron microscopy of pecten oculi. thick arrow: abluminal folds, thin arrow: luminal folds, n: nucleus, L: lumen, BM: basal membrane Bar: 0.5 µm

Another cell type surrounding the capillaries of the pecten oculi was the pericytes. The pericytes were observed to be located in-between the capillary endothelial cells and the basal membrane. There were one or two pericytes around a capillary. The pericytes differed from the endothelial cells in that they were not microfolds and contained less organelles (Figure 4).

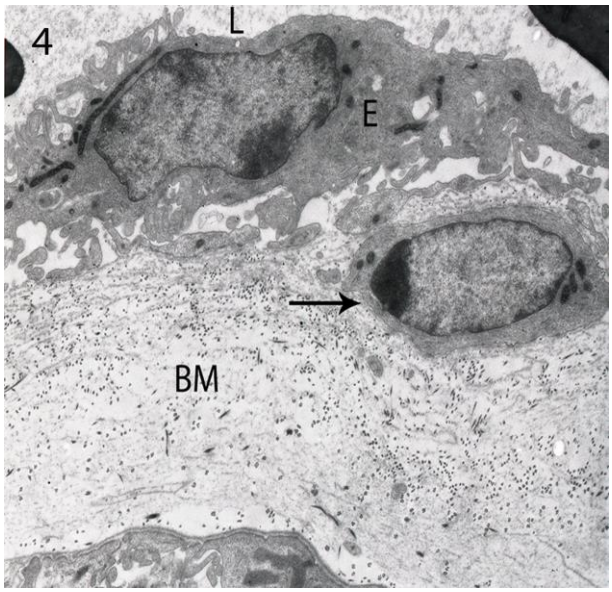

Figure 4. Electron microscopy of pecten oculi. Arrow: pericytes, L: lumen, E: endothelial cell, BM: basal membrane Bar: 0,5 µm

Melanocytes were also detected within the pecten oculi of the ostrich. A large and euchromatic nucleus was observed in these cells. Abundant melanosomes were seen in the perinuclear zone of the cells. Only large melanomas were established inside the cells. Similar to the endothelial cells, cytoplasmic folds were also observed in the melanocytes (Figure 5).

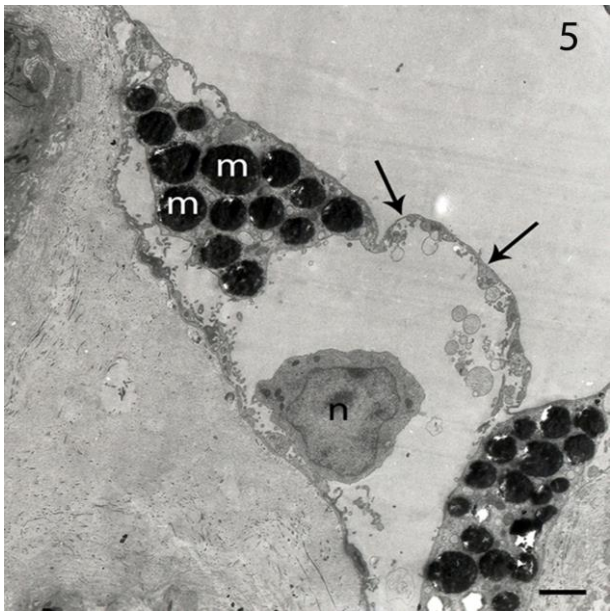

Figure 5. Electron microscopy of pecten oculi. arrow: vitreo-pecteneal limiting membrane; n: melanocyte nucleous; m: melanosomes; Bar: 0.6 µm

The external surface of the pecten oculi of the ostrich was surrounded by a vitreo-pecteneal limiting membrane (Figure 5). The microscopic examination of the serial sections not observed of hyalocytes in the vitreo-pecteneal limiting membrane of the ostrich.

Different from other species, in the ostrich, the pecten oculi folds converge at the apex by connective tissue, and thus resemble a empty cone in form.

DISCUSSION

The pecten oculi of the avian eye has been classified into three morphological types; conical (kiwi), vaned (ostrich) or pleated (other birds) (Braekevelt 1993; Kiama et al. 2009; Dayan and Ozaydin 2013). The results of the present study demonstrated that the pecten oculi of the ostrich is of the fan-like (vaned) type.

Research has shown that the pecten oculi differs among species for its size, number of folds and thickness of the basal lamina surrounding the pectineal capillaries (Braekevelt 1984; Braekevelt 1988; Braekevelt 1994). These differences are believed to be directly related to the visual requirements or diurnal activity of different species. The size of the pecten oculi is relatively larger in avian species with a well-developed visual activity; whereas, it is smaller in birds that are less dependent on visual ability. The nighthawk has the smallest pecten with the fewest number of detected folds (4-5 folds) (Braekevelt 1984). The pecten of the pigeon is medium-sized and consists of 17 folds (Braekevelt 1988). The largest pecten, consisting of 22-25 folds, has been detected in the American crow (*Corvus brachyrhynchos*) (Braekevelt 1994) and jungle crow (*Corvus macrorhynchas*) (Rahman et al. 2010). In the present study, we detected 20-24 large folds in the pecten oculi of the ostrich, which suggests that the ostrich has a highly developed visual ability.

Liebner et al. (1997) observed very strong cell junctions between the endothelial capillary cells in a study they

performed on the pecten oculi capillaries of the chicken. In the present study, a zonula occludens-type of junction was detected between the capillary endothelial cells. In previous studies performed in chickens, Gerhardt et al. (1996) observed strong interactions between the endothelial cells and specific barrier proteins in the cell cytoplasm, and thus, suggested that endothelial cells join the blood-retinal barrier. Braekevelt (1998) determined that the capillary endothelium of the pecten oculi of the emu was surrounded by microfolds and that the cell organelles were generally located in the perinuclear zone. Kiama (2009) reported that the microfolds in the endothelial cells of the ostrich were short and few in number. In our study, we observed many long microfolds in the luminal zone, and short and scarce microfolds in the abluminal zone of the capillary endothelial cells of the ostrich. Fewer microfolds were observed in parts of the plasma membrane, which were in close association with the cell nucleus.

A thick basal lamina has been reported to surround the capillaries of the pecten oculi in the chicken. The thick basal lamina is believed to contribute to the transport of material (Amemiya 1985). In previous research performed in various avian species, a thick basal lamina has been reported in birds with a large pecten, such as the rooster (Amemiya 1985), blue heron (Braekevelt 1991) and red-tailed hawk (Braekevelt 1991a), whereas a thin basal lamina has been reported in birds with a small pecten such as the nighthawk. Furthermore, a moderately thick basal lamina has been reported in birds with a medium-sized pecten such as the pigeon. We observed a thick basal lamina and a large pecten oculi in the ostrich.

Pericytes have been reported to be found in-between the capillary endothelial cells and basal lamina and to surround the capillaries in the nighthawk (Braekevelt 1984). Furthermore, research in pigeons has shown that pericytes regulate the blood flow rate with the help of the contraction of the endothelial cells (Braekevelt 1988). In the present study, we detected one or two pericytes just below the capillary endothelium.

In the pecten oculi of the emu (Braekevelt 1998) and the black kite (Kiama et al. 1994), the melanocytes surrounding the capillaries were reported to increase in number at the bridge or towards the apex of the pecten. Melanocytes were suggested to regulate the heat of the pecten oculi by absorbing light with the aid of the pigments they contain and thus, to serve a metabolic function. The increase observed in the number of melanocytes at the bridge or towards the apex of the pecten oculi was suggested to be an indication of this function (Kiama et al. 1994; Braekevelt 1998). In the present study, melanocytes were also observed to increase at the apex of the pectineal folds in the ostrich.

Research has shown that, in the chicken, the vitreo-pecteneal limiting membrane, which externally surrounds the pecten oculi, is the continue of the internal limiting membrane of the retina (Braekevelt 1984; Uhera et al. 1996; Llombart et al. 2009). Hyalocytes, which are located on the outer surface of the membrane and exhibit amoeboid features, have been observed to perform phagocytosis in the chicken (Uhera et al. 1996) and quail (Llombart et al. 2009). In the ostrich, hyalocytes were not observed on the vitreo-pecteneal limiting membrane.

The ostrich has large eyes in proportion to its head. The presence of a large pecten oculi is considered as an indicator of a highly developed visual ability in this species. While the presence of numerous microfolds on the luminal surface of endothelial cells is considered to be associated with intensive material exchange, the thickness of the basal lamina is related to selectivity for materials. In view of the pecten oculi features detected in the ostrich in the present study and in other bird species in previous research, it is suggested that this structure has an impact on visual function, whether directly or indirectly.

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