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Light and Electron Microscopical Studies on the Hyalocytes of Turkey *(Meleagris Gallopavo)*

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ABSTRACT

The present study aimed to investigate the light and electron microscopical structure of the hyalocytes in turkey. This study was applied on a total number of 15 (10 males and 5 females) clinically healthy turkeys of Bronze black species, collected from a local farm in Assiut Governorate, Egypt. For sampling and fixation, 30 turkey's eyeballs were enucleated and subjected to study. The hyalocytes appeared as large cell with different shapes (rounded, oval or elliptical). They located within ambushes found along the outer surface of the retino-pecteneal membrane. In these cells, present numerous cytoplasmic vacuoles and large oval nucleus located near the internal part of the cell. There were many cytoplasmic processes that joined each other as a fine meshwork enclosing several vesicles or parts of foreign materials along the external portion of the cell. On the internal or deep surface of the cell present numerous filopodia, which extended to occupy the depressions found on the outer surface of the retino-pecteneal membrane. The presence of ingested foreign materials and the appearance of filopodia in a moving condition along the internal surface of the cell insure that hyalocytes are considered highly active phagocytic cells.

Introduction

The pecten oculi mainly consists of three cell types: endothelial cells, pigmented glial cells and hyalocytes, which are situated on the inner limiting membrane in close relationship with blood vessels (Hamburg, 1959; Uehara *et al*., 1999). Despite the existence of many publications devoted to the avian pecten, little information can be found concerning the origin, morphology and function of pecteneal hyalocytes as reported by Uehara *et al*. (1990, 1996); Navascués *et al*. (1995).

According to Uehara *et al*. (1990) the localization and distribution of hyalocytes on the inner limiting membrane and between the pleats was similar in quail to those in chicken. Uehara *et al*. (1996) stated that hyalocytes in normal chickens were mainly found on the pleats of the pecten oculi and on the ciliary body, while they were absent on the retina. Thus a close relationship exists between the vasculature in the tissues surrounding the vitreous chamber and the distribution of hyalocytes.

These hyalocytes which were predominantly spindle shaped or oval in contour, displayed a ruffled surface with occasional blebs, filopodia and lamellipodia. Flattened hyalocytes with relatively few and short pseudopodia were frequently observed, especially on the ciliary body as recorded by Uehara *et al*. (1996).

According to Liombart *et al*. (2009) the hyalocytes are considered as a subtype of blood-borne macrophages. In most species including the great blue heron, however these hyalocytes have not been reported (Braekevelt, 1984, 1986, 1988). Hyalocytes are occasionally noted adherent to the outer surface of the limiting membrane of the pecten of the Australian Galah (*Eolophus roseicapillus*) (Braekevelt and Richardson, 1996). The present study aimed to study the light and electron microscopical structure of the hyalocytes in turkey.

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Materials and methods

This study was applied on a total number of 15 (10 males and 5 females) clinically healthy turkeys of Bronze black species, collected from a local farm in Assiut Governorate, Egypt.

For sampling and fixation, 30 turkey's eyeballs were carefully enucleated, washed in normal saline several times and 20 of them were immersed into 4% glutaraldehyde solution and the others were immersed in 10% formalin. Each eyeball was pierced behind the corneoscleral junction before immersion to allow the fixative penetrating the vitreous chamber. After 12 hours in case of gluteraldehyde fixation and 24 hours in case of 10% formalin fixation, the posterior half of the eyeball was dissected and the vitreous body was removed carefully. Each pecten was carefully handled and subjected to the routine protocol of histological and scanning eletron microscopical examination.

For the scanning electron microscopical examination, the posterior half of the eyeball was sectioned and the vitreous body carefully removed, then each pecten was carefully dissected out and washed several times in normal saline and acetic acid 2% then fixed in the same fixative for 24 hours, after that pecten was postfixed in 2% buffered osmium tetraoxide. The fixed samples were washed in 0.1 M cacodylate buffer at pH 7.3 and then dehydrated in ascending grades of ethanol, critical point dried in liquid carbon dioxide, and mounted on metal stubs then coated with gold palladium in sputtering device. Specimens were examined and photographed by using JSM_4500 LV scanning electron microscope (SEM) operated at 20 KV.

For histological investigation; after proper fixation specimens were washed for 24 hours under running tape water, then dehydrated in ascending graded concentrations of ethanol. The samples were cleared in methyl benzoate and embedded in paraffin wax, 3 um thick sections were cut, mounted on glass slides, and stained with haematoxylin and eosin (H&E) stain for general histological examination (Harris, 1900) and crossmon trichrome stain for differentiation of connective tissue and muscle fibers (Crossmon, 1937). Morphometrical studies were applied on stained histological sections and semithin sections using Leica Q 500 MC image analyzer.

For electron microscopical examination, small pieces of tissues 2 mm^2 of specimens were postfixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer and contrasted in 0.5% uranyl acetate in 0.05 M maleate buffer. Dehydration was done as for paraffin embedding and the ethanol gradually replaced with propylene oxide and finally infiltrated and embedded in epoxy resin. Semithin and ultrathin sections were cut using an ultramicrotome. The semithin sections were collected on glass slides, stained with 0.5% toluidine blue, and viewed under a light microscope. The ultrathin sections were picked on 200- mesh carbon-coated copper grids, stained with lead citrate, and observed with a JEOL JEM-100 CX II electron microscope 300 transmission electron microscope under an accelerating voltage of 60 KV.

Results

Observations of SEM in the present work (Fig. 1) revealed that some oval to rounded hyalocytes

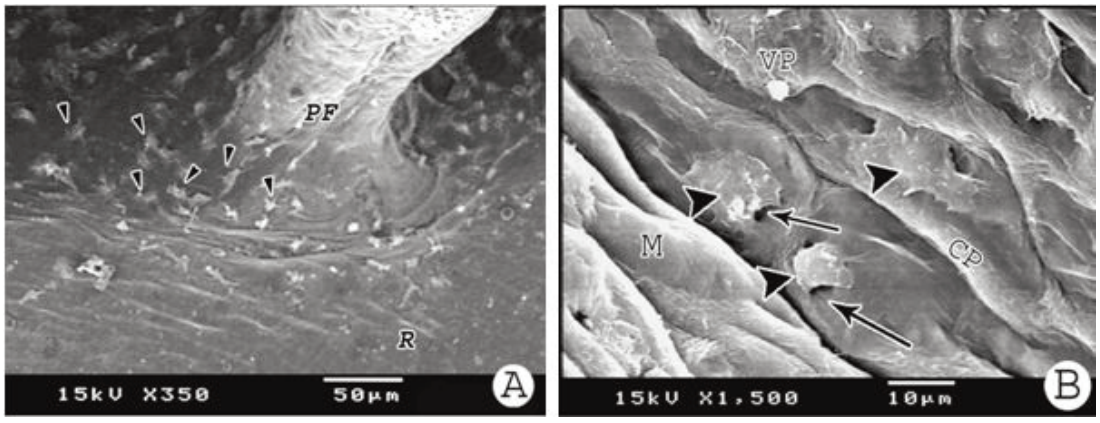

Fig.1. (A) SEM at low magnification shows the distribution of hyalocytes (arrow head) on the retino-pecteneal membrane. The retina (R) and the beginning of the pecteneal fold (PF) are indicated. (B) SEM at the lateral aspect of the pecteneal plate showing hyalocytes (arrowhead) are covering pores (arrow). The pecteneal capillaries (CP), melanocytes (M) traces of vitreous body (VB) are illustrated.

with numerous cell processes were noticed along the surface of the pecten, some of them were lodged with some traces of the vitreous on the surface of the pores and covered it by their processes.

Hyalocytes were noticed attached to the outer surface of the pecten, which were few in number (Fig. 2). But in one of the examined samples, which were apparently healthy, it was noticed that there were numerous hyalocytes along the outer surface of the pecten forming a continuous sheath adherent to the outer pecteneal surface with high condensation of pigment cells along the pecteneal fold (Fig. 3). This finding supports the fact that hyalocytes may have a phagocytic function and are involved in the defense mechanism.

Hyalocyte appeared as large cell with different shapes (rounded, oval to elliptical) located within ambush along the outer surface of the retino-pecteneal membrane with numerous cytoplasmic vacuoles and large oval nucleus located near the internal part of the cell (Fig. 4, 5).

There were many cytoplasmic processes, which

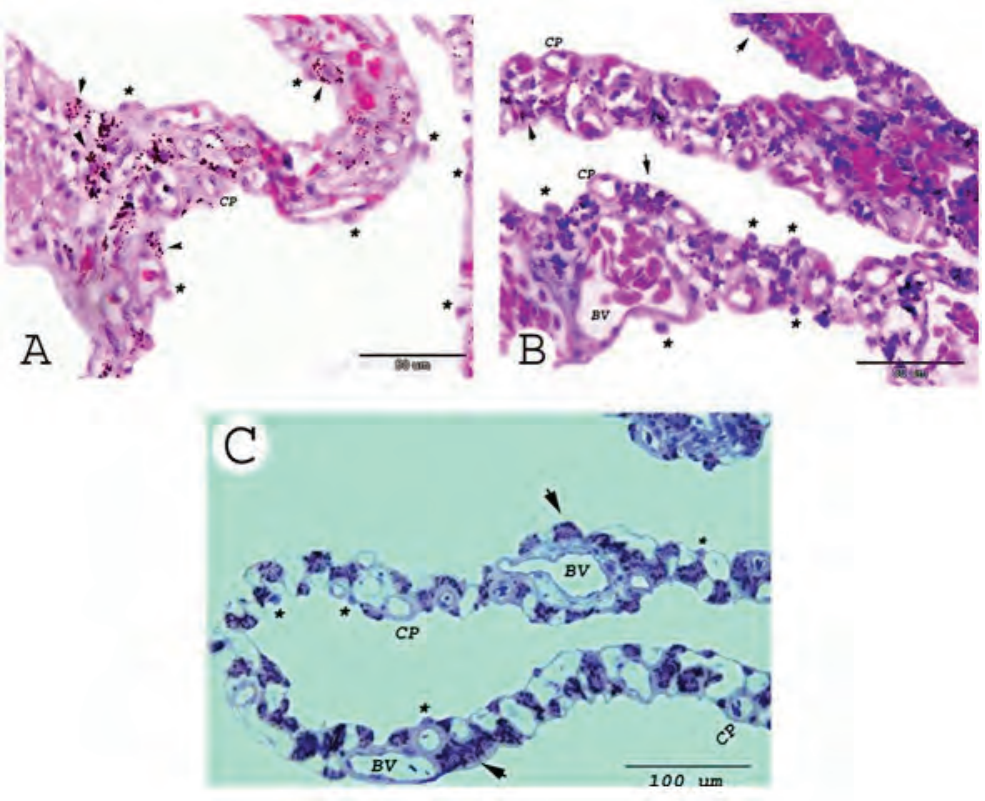

Fig. 2. (A) and (B) Transverse histological sections of the pecteneal fold stained by H&E and (C) semithin section by toluidine blue showing hyalocytes (*) on the outer surface of the pecteneal membrane., The blood vessels (BV), pecteneal capillaries (CP) and melanocytes (arrowhead) are illustrated.

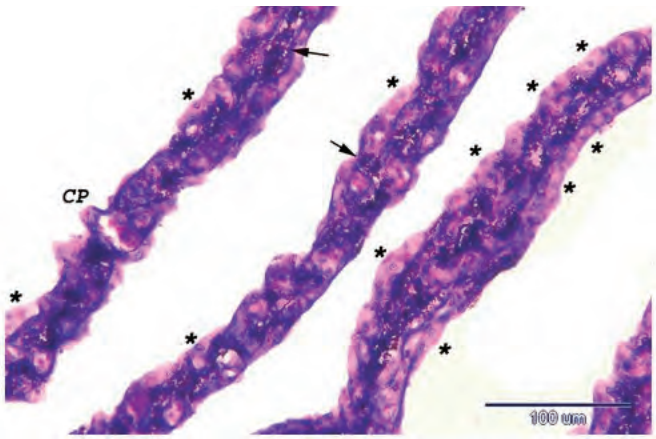

Fig. 3. Photomicrograph showing large number of hyalocytes (*) along the outer surface of the pecteneal plates which may indicate an inflammatory case. The pigment cells (arrow) with large number of pigment granules filling almost the middle of the plate. Pecteneal capillary (CP) H&E.

joined each other as a network or a rete enclosing several vesicles or parts of foreign materials along the external surface of the cell (Fig. 4, 6). Along the internal surface of some cells there were numerous filopodia, which extend and occupy the depressions on the outer surface of the retino-pecteneal membrane (Fig. 5). The presence of the cytoplasmic processes along the external surface of the cell; numerous cytoplasmic vesicles or ingested foreign materials, localization of the nucleus toward the internal part of the cell and the appearance of filopodia in a moving condition along the internal surface of the cell insure that hyalocytes are considered highly active phagocytic cells.

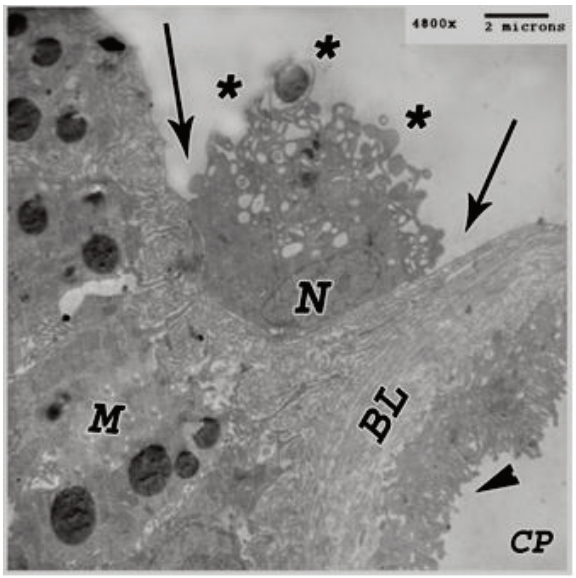

Fig. 4. Transmission electron micrograph shows rounded to oval hyalocyte located within ambush or depression along the outer surface of the retino-pecteneal membrane (arrow) with numerous cytoplasmic processes which join each other as a network or a rete enclosing several vesicles or parts of foreign materials along the external surface of the cell (*) . The large oval nucleus (N) located near the internal part of the cell. The basal lamina (BL) and luminal microvilli of the pecteneal capillary (CP) and melanocytes (M) are illustrated.

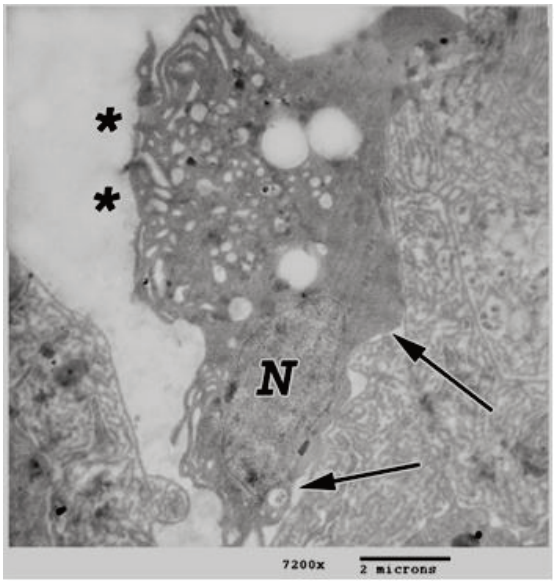

Fig. 5. Transmission electron micrograph shows oval to elliptical hyalocyte located within depression along the outer surface of retino-pecteneal membrane with numerous cytoplasmic vacuoles and processes which join each other as a network or a rete enclosing several vesicles or parts of foreign materials along the external surface of the cell (*). The large oval nucleus (N) located near the internal part of the cell. A long the internal surface of the cell there are numerous filopodia extend and occupy the depressions on the outer surface of the retino-pecteneal membrane (arrow) and the whole cell appears in a moving condition.

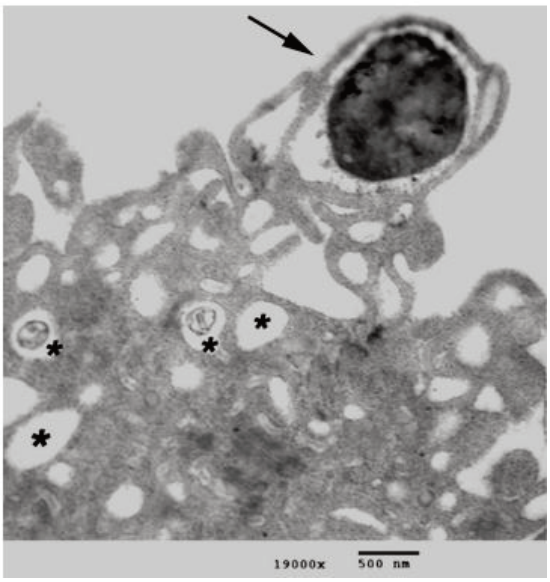

Fig. 6. Transmission electron micrograph at higher magnification for hyalocyte shows numerous cytoplasmic vacuoles containing ingested foreign materials (*) and numerous cytoplasmic processes which join each other as a network or a rete enclosing several vesicles or parts of foreign materials along the external surface of the cell (arrow).

Discussion

The pecten oculi in chicken consists mainly of three cell types: endothelial cells, pigmented glial cells and hyalocytes, which are situated on the inner limiting membrane in close relationship with blood vessels (Hamburg, 1959; Uehara *et al*., 1996 and Gerhardt *et al*. 1999). Despite the existence of many publications devoted to the avian pecten, little information can be found concerning the origin, morphology and function of pecteneal hyalocytes (Uehara *et al*. 1990; Navascués *et al*., 1995 and Uehara *et al*., 1996).

By using SEM, Uehara *et al*. (1996) showed a characterized distribution of the hyalocytes in chicken. They were plentiful on the pecten oculi (in the depressions of the pleats, occurring singly or in clusters and on the elongated optic nerve head which corresponds to the base of the pecten), sparse on the ciliary body and infrequent on the retina. This finding differs from that of Hamburg (1959), who reported that hyalocytes were distributed in the region near the ora serrate with a few cells were located along the pecten oculi, however this result was obtained by using a light microscope. SEM findings in the present work reveal that hyalocytes are numerous toward the base of the pecteneal plates and at the pecteneal surface around the blood capillaries. The cell projects on the surface and decrease gradually toward the apex of the plate and disappears on the pecteneal bridge. In histological sections stained by H&E, crossman's trichrome and toludine blue stains and were examined by light microscope, hyalocytes were seen to adhere to the outer surface of the retino-pecteneal membrane.

By SEM study of the chicken hyalocytes, Uehara *et al*. (1996) showed that hyalocytes were varied in size, shape and number of cytoplasmic processes. The predominant shape of the pecten hyalocyte was a flattened oval. Its surface was mostly covered by ruffles. Some cells exhibited a variety of surface structures such as blebs, filopodia and spreading lamellipodia, these were prominent in most but far less in others in which processes were relatively sparse and short. The ciliary hyalocytes were more irregular in contour and flatter than those on the pecten oculi. These cells frequently displayed a smooth surface and several broad lamellipodia. According to Micali *et al*. (2012), electron microscopical observations of the pecten oculi in the Budgerigar (*Melopsittacus undulatus*), on the vitreal surface of the pecten, between the endothelial basement membrane and the inner limiting membrane, large hyalocytes were present that showed an elliptical nuclei with dispersed chromatin, a thin halo of cytoplasm and many cellular processes. When the inner limiting membrane lacks owing to its loose adherence to the pecteneal surface, long rectilinear processes adherent to the pecteneal capillaries surface can be observed with the SEM, either at low or at higher magnification. SEM observations in the present work revealed that some oval to rounded hyalocytes with numerous cell processes were noticed along the surface of the pecten, some of them were lodged with some traces of the vitreous on the surface of the pores and were covered by their processes. By TEM hyalocyte appears as large oval to elliptical cell located within ambush or depressions along the outer surface of the retino-pecteneal membrane with numerous cytoplasmic vacuoles and large oval nucleus located near the internal part of the cell. There were many cytoplasmic processes join each other as a rete enclosing several vesicles along the external surface of the cell, while along the internal surface of the cell there were numerous filopodia extended and occupied the depressions on the outer surface of the retino-pecteneal membrane.

Despite there are numerous literatures about the presence of hyalocytes in different birds, little data are present about its functions. Braekevelt (1990, 1991 a,b, 1994) stated that hyalocytes are occasionally noted adherent to the outer surface of this membrane, but they do not appear to be a regular feature in all species. However in most species including the great blue heron these hyalocytes were not reported (Braekevelt, 1984, 1986, 1988). Hyalocytes are occasionally noted adherent to the outer surface of the limiting membrane of the pecten of the Australian Galah (*Eolophus roseicapillus*) (Braekevelt and Richardson, 1996). Braekevelt (1990) reported that the function of these hyalocytes (when present) is unknown; they display morphology indicative of phagocytes and appear to be ameboid in nature. Hyalocytes are considered as a subtype of blood-borne macrophages (Liombart *et al*., 2009), originated during embryogenesis from the primitive arteria cupulae opticae (Liebner *et al*., 1997). In the present work it was noticed that in one of the examined

sample by light microscopy, there was numerous number of hyalocytes adherent along the outer surface of the pecteneal fold in a form of continuous sheath (clusters) with high condensation of pigment cells and congestion of pecteneal capillaries. These findings may suggest the role of the hyalocytes as a phagocytic cell. Thorough transmission electron microscopical investigations revealed that hyalocytes can be considered as phagocytic cells, with its processes, which join each other as a rete or a network with numerous cytoplasmic processes that enclose several vesicles or parts of foreign materials. The hyalocytes appear with numerous filopodia and occupying depressions on the outer surface of the pleats. Some cells show clear amoeboid movement along the outer surface of the retino-pecteneal membrane, others are dormant in the depressions either after a phagocytic process or waiting for attaching and engulfing of a foreign body. Our results indicate that phagocytosis is the proper function of the hyalocytes and insure its ability to move along the outer surfaces of the pleat.

Conclusion

The presence of the cytoplasmic processes along the external surface of the cell; numerous cytoplasmic vesicles or ingested foreign materials, localization of the nucleus toward the internal part of the cell and the appearance of filopodia in a moving condition along the internal surface of the cell insure that hyalocytes are considered highly active phagocytic cells.

Acknowledgement

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