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RESEARCH ARTICLE

Scanning electron and light microscopic investigation of Bursa fabricius in turkey *(Meleagris gallopavo)*

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Özet

Gültiken ME, Yıldız D, Karahan S, Bolat D. Hindi *(Meleagris gallopavo)* Bursa fabricius'unun taramalı elektron ve ışık mikroskobu ile incelenmesi. ***Eurasian J Vet Sci, 2010, 26, 2, 69-73***

Amaç: Hindide Bursa fabricius'un morfolojisinin belirli dönemlerde incelenmesi ve dönemlere göre morfometrik analizinin taramalı elektron mikroskobu ve ışık mikroskobu kullanılarak yapılmasıdır.

Gereç ve Yöntem: Çalışmada 1-24 haftalığa kadar toplam 50 hindiye ait Bursa fabricius'lar kullanıldı. Hayvanların ve nekropsi sonrası Bursa fabricius'ların ağırlıkları belirlendi. Taramalı elektron mikroskobu ile yapılacak çalışma öncesi dokular gluteraldehit ile tespit edildikten sonra mikroskop kullanılarak görüntüler elde edildi. Histolojik çalışma için dokular rutin histolojik takip sonrası Mallory's triple ve Haematoxylen-eosin ile boyanarak ışık mikroskobu ile incelendi.

Bulgular: Morfometrik veriler Bursa fabricius'un maksimum büyüklüğüne 9. haftada ulaştığını gösterdi. Dokuzuncu haftayı takiben Bursa fabricius ağırlığındaki azalma hindide involusyonun bu dönemi takiben şekillenmeye başladığını gösterdi. Taramalı elektron mikroskop ile yapılan incelemelerde Bursa fabricius lumenine uzanan plica yüzeylerindeki kubbe şeklindeki epitel görüntüsünün 5. ve 9. haftalarda en belirgin ve düzgün olarak tespit edildi. İlerleyen haftalarda (13. ve 24.) Bu manzarada dikkat çekici bir düzensizlik gözlendi.

Öneri: Çalışma sonucunda elde edilen bulguların, ilerde yapılacak çalışmalara ve hindi aşılama programlarına katkı yapacağı düşünülmektedir.

Abstract

Gultiken ME, Yildiz D, Karahan S, Bolat D. Scanning electron and light microscopic investigation of Bursa fabricius in turkey *(Meleagris gallopavo)*. ***Eurasian J Vet Sci, 2010, 26, 2, 69-73***

Aim: The aim of this study was postnatal investigation of morphometric features of Bursa fabricius in Turkey by using scanning electron microscope and light microscope.

Materials and Methods: One 1-24 week old 50 turkeys (meleagris gallopavo) were used. Their Bursa fabriciuses were taken out after the necropsy and weighted. Tissues were fixed with glutaraldehyde, examined and photographed under scanning electron microscope. For histological examination, tissues were prepared using routine histologic methods and stained with Mallory's triple and Hematoxylen-Eosine.

Results: The morphometric data concerning turkey ducklings used in the study showed that Bursa fabricius reaches its maximum size at the 9th week. The decrease in the weight of Bursa fabricius following the week of 9 proved that involution session in the turkey begins after that period. On the investigation performed by means of scanning electron microscopy, dome shaped surface epithelium which covers subepithelial lymph follicles on the surface was determined to be most clear in the 5th and 9th weeks. There was a distinct irregularity of this appearance in the following weeks (13th and 24th).

Conclusion: It was concluded that the results may contribute to the researches which relate to humoral immunity formation and effectiveness of vaccination programme.

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Keywords: Bursa fabricius, scanning electron microscope, turkey

► Introduction

Bursa fabricius is a pouch organ peculiar to avian. It is located on the dorsal wall of the urodeum and its lumen is connected with proctodeum by a vertical groove. It attains its maximum size in the 14th and 16th weeks. Rapid involution is performed with puberty. Intra-epithelial and intra-follicular cysts which are the first histological signs of involution are formed from the 12th week. These cysts progressively include the whole medulla of lymph follicle. It is rudimentary as a nodule of approximately 0.5 g in the month of 20 in the chicken and finally it disappears (Nickel 1977).

The wall of Bursa fabricius consists of tunica mucosa, tunica muscularis and tunica serosa (Glick 1988). There are 11-13 plicas on tunica mucosa which form protrusions towards lumen in longitudinal pattern. The rest of the surface area of the plicae is covered by cylindrical shaped epithelium which produces mucus, secreted into the bursal lumen and this epithelium is named as inter-folliculer epithelia (IFE) (Olah and Glick 1992, Davenport and Allen 1995, Nagy et al 2001). Lamina propria consists of polyhedral lymph follicles between the numbers of 8000-12000 (Olah and Glick 1978). Epithelial cells are related to follicles and acquired a character of its own (Follicle-associated-epithelial, FAE) locate among IFE cells on follicles (Bockman and Cooper 1973). Each follicle has its own cortex and medulla which are independent from each other. Cortex seems darker than medulla because it has more lymphocyte concentration (Glick 1988).

There is no literature data about the morphogenesis of Bursa fabricius in the turkey despite it has been investigated in different avian species (Onyeanusi et al 1993, Gülmez and Aslan 1999). In the present study, morphogenesis of Bursa fabricius in the turkey an important organ of avian immune system, was researched by means of sub-gross, scanning electron microscopy (SEM) and light microscopy techniques and the result were compared with other studies.

Table 1. Morphometric measurements concerning turkey ducklings (mean ± SE).

Week	Body weight (W) (g)	Bursa fabricius		
		Weight (g)	Length (mm)	Diameter (mm)
1	90.9 ± 2.98^{e}	0.566±0.09^{c}	11.95 ± 1.18	8.25 ± 1.26
2	181±2.55^{e}	0.765±0.04^{c}	14.31 ± 1.24	10.88 ± 1.84
5	702±44.1^{d}	1.418±0.11^{c}	17.66 ± 2.44	13.51 ± 2.04
9	2608±29.3^{c}	3.846±0.56^{ab}	23.06 ± 1.96	17.32 ± 0.36
13	3112±107^{b}	3.046±0.18^{b}	24.57 ± 4.11	17.76 ± 3.28
24	6929±35.8^{a}	4.536±0.48^{a}	22.81 ± 2.81	17.2 ± 2.9

a, b, c, d, e; different letters in the same column are statistically significant (Duncan test, p<0.05)

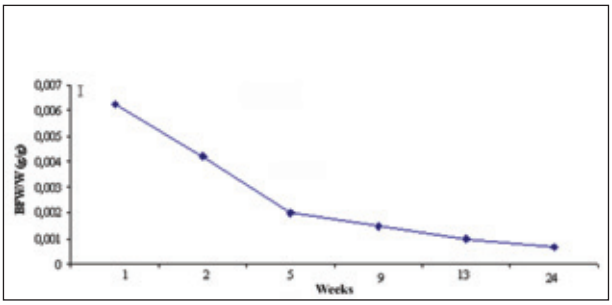
► Materials and Methods

Fifty turkeys (Meleagris gallopavo) of 1-24 weeks were taken from slaughterhouse of Keskin turkey breeding center. Their Bursa fabricius were taken out after the necropsy and weigh by Ohaus Explorer Pro analytical scale (code no: EP214C, Switzerland). The length and diameters of Bursa fabricius was measured using a Mitutoyo Digimatic Vernier Scale (150 mm, code no: 500-311, Model CD-15D, serial no: 7175731, Mitutoyo Corporation, Japan). Bursa fabricius were then washed with sodium phosphate buffer solution (PBS) and were cut immediately into small pieces in the same solution. The pieces were immersed in 3% glutaraldehyde phosphate (pH: 7.2) at +4 °C for prefixation during two hours. They were then washed with 1% osmiumtetraoxide- PBS for two hours. The pieces were washed again in PBS overnight and dehydrated through serial ethanol dilutions and air-dried overnight. The pieces were mounted onto stubs as the bursal mucosa can be analysed (Kiami et al 1994). They were sputter-coated with gold by Polaran SC-500 (VG Microtech, Sussex, UK), and finally examined with a JSM 5600 JEOL Scanning Electron Microscope (Tokyo, Japan). Histological sections were prepared, stained with the Mallory's triple and Haematoxylen-Eosin staining techniques (Bancroft JD, Stevens A 1983) and analyzed with light microscopy (Olympus CX31, Tokyo, Japan).

Body and Bursa fabricius' weights were analyzed ANOVA and Duncan multiple range test (SPSS 10.0). $p<0.05$ was accepted as statistically significance.

► Results

The morphometric data (Table 1) concerning turkey ducklings used in the study showed that Bursa fabricius reaches its maximum size at the 9th week. The decrease in the weight of Bursa fabricius (BFW) following the week of 9 proved that involution in the turkey begins after that period. It was observed that proportion of BFW and alive weight (Graphic 1) decrease rapidly following hatching and reaches its minimum value. This situation showed that BFW declines relatively after hatching.



Graphic 1 . The proportion of live weight (W) and the weight of Bursa fabricius (BFW).

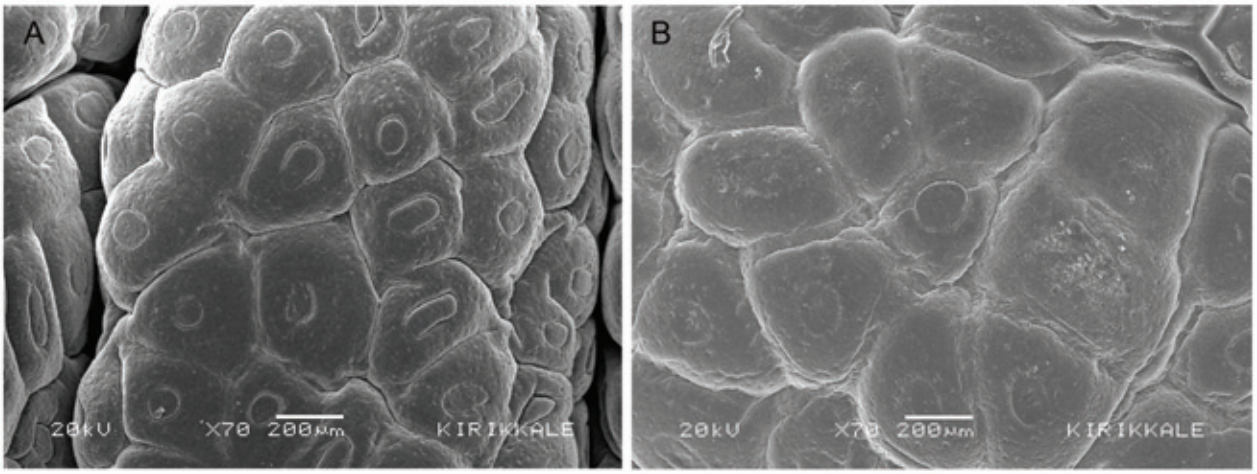


Figure 1. Scanning electron micrograph of the Bursa fabricius in turkey (Meleagris gallopavo) of 5 weeks (A) and 24 weeks (B).

On the investigation performed by means of SEM, dome shaped surface epithelium which covers sub-epithelial lymph follicles on the surface extending lumen of Bursa fabricius. The appearance of this epithelium of lumen surface was like dome shaped projection in the middle of generally pentagonal and sometimes tetragonal areas (Figure 1A). This dome shaped appearance of epithelium was determined to be most clear in the 5th and 9th weeks. In the samples of 13th and 24th week irregularity of surface epithelia was defined and this showed that changes were occurred in the surface epithelia during involution process (Figure 1B).

It was determined that sub-epithelial lymph follicles are characterized by pores and these pores are filled with lymphocytes (Figure 2) in the samples in which the surface epithelium was removed.

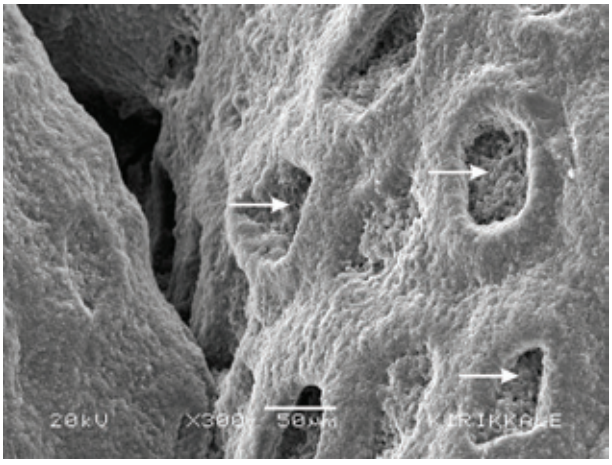


Figure 2. The pores filled with lymphocytes (arrows) in the lumen of Bursa fabricius of one week turkey that the surface epithelium was removed.

Bursa fabricius was determined to be performed by 15-17 plicae extending to lumen in the histological preparations investigated. It was observed that a thin connective tissue was parted plica into two sections in the middle of it and extended to the area between follicles.

It was determined that border between cortex and medulla in follicles was very regular and they were parted into two zones with two lines of epithelial cells which one of them is very distinct. Besides, capillary vessels were observed to have reached that border. There was tone difference of color between cortex and medulla meanwhile cortex was darker (Figure 3A). This situation was due to the fact that lymphocytes in the cortex were more intensive than the medulla. In medulla, there were a few plasma cells and reticular cells among lymphocytes.

Lamellar structures which are the projection of epithelial related to follicle (FAE) were observed from the first week and their incidences were high in the following weeks. This showed that FAE epithelia invasion of follicles fills gradually the medulla of certain follicles and plays an important role in involution.

It was seen that vacuoler degeneration in medulla starts from the 5th week and these vacuoles form one or two big vacuole in a follicle in the 9th week rather than lots of little vacuoles. This explains that small vacuoles form big vacuoles by uniting each other. Besides, shortness on length of the plica, increase of inter-follicular connective tissue and irregularity in the border of medulla and cortex were observed from the 9th week. Shortness of length of the plica was more distinct in the 13th week. It was determined that vacuoler degeneration was enveloped by FAE cells and also occurred in lamellar structures. Certain follicles were observed to have been come smaller surrounding by connective tissue and substituted with follicles in the following weeks.

In the 24th week, in which the shortness of length of the plica was very clear, follicle number in plicae decreased and fat tissue was observed from the baseline of follicles to terminal part. Connective tissue ratio was high and filled some follicles completely (Figure 3B). It has been considered that absence of lamellar structures was due to the fact that many lamellar structures disappeared until the 24th week.

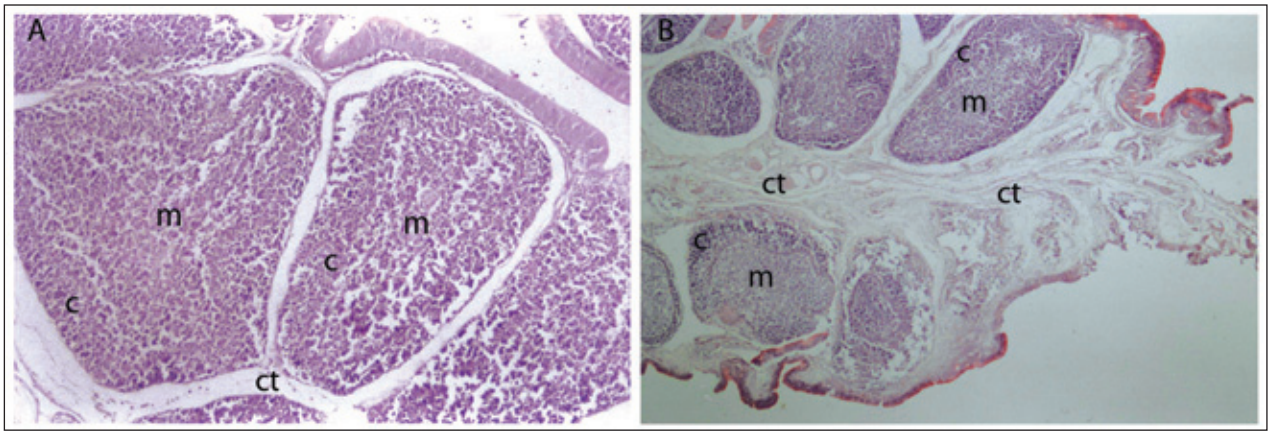


Figure 3. Photomicrographs of cortex (c) and medulla (m) of the bursa fabricius in turkey of 5 (A) and 24 weeks (B). Gradually rising connective tissue (ct) during involution (HEx10).

► Discussion

Location of Bursa fabricius in turkey, general macroscopic features and development process of it were found to be similar with other avian species (Onyeanusi et al 1993, Gülmez and Aslan 1999), though there are certain differences.

There are longitudinal plicae having projections to lumen in number of 11-13. Number of these plicae, located in the lumen of bursa fabricius, was reported as 11-13 in goose (Gülmez and Aslan 1999), 12-14 in Guine chicken (Numida meleagris galeata) (Onyeanusi at al 1993) and 11-13 in chicken (Davenport and Allen 1995). In the present study 15-17 plicae were determined in Turkey.

Development of Bursa fabricius in male chicks that increase in bursa fabricius' weight was highest between the days of 1 to 10 and reaches its maximum weight around the days of 110-120 (Dinç et al 1998). The length of the plica increased sharply during the development of Bursa fabricius in the pigeon and pointed out those long plicae were lobular structure when Bursa fabricius reached its maximum size at day 75 (Ciriaco et al 1985). Our results show that the weight of Bursa fabricius has declined following a rapid increase since the week of 9. The fact that increase in inter-follicular connective tissue and beginning of the length of the plica to shorten from the week of 9 determined under light microscope support the fact that involution period starts in this period.

The changes in the structure of Bursa fabricius depending on age in the pigeon that involution period starts on epithelium, follicular medulla is affected and then all structure is affected and this period is completed by mucoid degeneration following an intensive fat degeneration, besides there are big cystic structures in intercellular region (Ciriaco et al 1989). In our study, it has been determined in the turkey vacuolar degeneration surrounded by FAE cells occurred following the 13th week, in the week of 24 numbers of follicles decreased and fat tissue deposited in the follicles.

It has been determined in the scenes obtain via SEM in the turkey, Bursa fabricius was formed by dome-shaped surface epithelium which covered the surface of lymph follicles and this result was similar to the result, found in Bursa fabricius of chicken by Davenport and Allen (1995). Besides, pores observed in the samples in which surface epithelium was removed and lymphocyte formations that filled these pores were determined to be similar results of the aforementioned literature.

► Conclusion

Consequently, macroscopic and microscopic findings showed that a general feature of Bursa fabricius in the turkey was similar with that of other avian. Changes, observed during the development and involution process, showed that Bursa fabricius in turkey reaches its maximum size at week 9 and involution occurred following that week. In the present study, morphogenesis of Bursa fabricius in turkey was investigated and evaluated by using SEM and it was thought that the results may contribute to the researches relates to humoral immunity formation and effectiveness of vaccination programme.

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