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Comparison of the Development and Involution Periods of Bursa of Fabricius with Histological and Histochemical Methods

Hatice Yaren Kuloğlu[1,a,*], Murat Boydak[2,b]

[1]*Aksaray University, Faculty of Veterinary, Department of Histology and Embryology, Central Campus, Türkiye*
[2]*Selcuk University, Faculty of Veterinary, Department of Histology and Embryology, Alaaddin Campus, Türkiye*
[*]*Corresponding author*

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ABSTRACT

The aim of this study is to histologically and histochemically determine the developmental and involutional stages of bursa of Fabricius of henna partridge (*Alectoris chukar*). In the study, bursa of Fabricius of 12 3-month-old (6 males, 6 females), 12 6-month-old (6 males, 6 females) henna partridges purchased from a private farm were used. It was observed that bursa of Fabricius was surrounded by a connective tissue capsule and consisted of tunica serosa, tunica muscularis, and tunica mucosa layers from the outside to the inside. It was seen that the tunica muscularis consisted of outer longitudinal and inner circular smooth muscle fibers. It was observed that the tunica mucosa made plicae towards the lumen of the organ and consisted of 10-15 plicae. It was seen that lamina epithelialis and lymph follicles were present in each plica. It was determined that the lamina epithelialis consisted of two parts called FAE (Follicle-Associated Epithelium) and IFE (İnter Follicular Epithelium). It was noted that the lymph follicles contained cortex and medulla sections and were separated locally by capillaries together with cortical medullary boundry cells. In the Methyl Green-pyronin staining method, plasma cells were found in the bursa of Fabricius of the henna partridge, in the connective tissue surrounding the organ, around the blood vessels and inside the follicles. In AB pH=2.5 staining, AB-positive reaction was seen only in the apical part of the epithelial cells forming FAE and IFE in the pre- and post-involution period. In PAS staining, PAS-positive reaction was observed only in the apical part of the epithelial cells forming FAE and IFE in the pre- and post-involution period. In PAS/AB pH=2.5 combined staining method, AB-positive reaction was observed only in the apical part of epithelial cells in the pre- and post-involution period. As a result of this study, it was found that bursa of Fabricius of the henna partridge did not undergo any histochemical changes despite its histologically large differences after involution.

[a] haticeyyaren@gmail.com https://orcid.org/0000-0003-0435-4677 | [b] yigitharput@gmail.com https://orcid.org/0000-0002-3251-1177

Introduction

The cloacal bursa of avian, which was first described by Hieronymus Fabricii in the 17th century and named after this researcher, bursa of Fabricius, is an organ of endo mesodermal origin (Le Douarin et al. 1984). Bursa of Fabricius is a sac-shaped diverticulum extending dorsally from the proctodeum of the cloaca (Glick 1956, Hodges 1974, Ciriaco et al 2003).

The average time to reach the maximum size of bursa of Fabricius in chickens is the first 4 months after hatching (Glick 1956, Hodges 1974). Involution of bursa of Fabricius in chickens begins approximately at the age of 8-12 weeks and continues until the 20-week period. It weighs 0.5 g at the age of 20 weeks and is present as a nodular remnant in adults from this week onwards (McLelland 1990). In a six-month-old duck, bursa of Fabricius reaches a maximum size of 5 cm in length and 7 mm in diameter. Later, organ involution begins in all avian species, especially with sexual maturity. Involution begins a little later and develops more slowly in ducks and geese compared to chickens. In geese that do not reach sexual maturity before 2 years, the involution of bursa of Fabricius develops naturally and remains large for up to 2 years. Involution develops more slowly in waterfowls and faster in pigeons (Doğuer and Erençin 1964).

There are many studies on the developmental periods of the bursa of Fabricius in the literature. However, there is no study on the determination of the histochemical features of the bursa of Fabricius after involution. In addition, no information was found in the literature about the development and involution periods of bursa of Fabricius in the henna partridge (*Alectoris chukar*). In this study, it was aimed to determine the histological and histochemical features of bursa of Fabricius in the henna partridge (*Alectoris chukar*) during the development and involution stages and to compare these periodic features.

Materials and Method

Animals

In this study, bursa of Fabricius of 12 3-month-old (6 males, 6 females), 12 6-month-old (6 males, 6 females) henna partridges purchased from a private farm (Turkey/Antalya) were used. The approval was obtained from the Ethics Committee of Selcuk University, Faculty of Veterinary Medicine (SUVFEK) before the study. The animals were kept in conditions of natural light, humidity and temperature, and allowed to freely use water and food.

Histology and Histochemistry

Totally removed bursa of Fabricius tissue samples were fixed by keeping them in 10% neutral formol for 36 hours. Tissue pieces were blocked in paraffin following washing, dehydration and polishing processes with known histological techniques. The following procedures were applied to the 5 µm thick sections taken from the blocks.

- Triple staining technique of Mallory (Mallory 1900) modified by Crosman for general histological examination.
- 2-Methyl Green-pyronin (MGP) staining method for demonstration of plasma cells (Pappenheim 1899).
- In order to investigate the quality of the secretion material in the gland tissue;
  - Periodic acid Schiff (PAS) (McManus 1946) method for the demonstration of neutral mucosubstance.
  - Alcian Blue (AB) pH= 2.5 (Scott and Dorling 1965) method for demonstration of acidic mucosubstance.
  - Periodic acid Schiff/Alcian Blue (PAS/ AB) pH= 2.5 combined staining method for the combined demonstration of neutral and acidic mucosubstance (Mowry 1956).
- Gordon Sweet (GS) staining method for demonstration of reticular fibers (Lillie 1965).

The obtained preparations were examined at the light microscopic (Leica DM2500, Switzerland) level and the shapes of the regions deemed necessary were taken with the Leica DFC 320 (Switzerland) camera attachment of the same microscope.

Result and Discussion

As in other avian species, it was observed that the bursa of Fabricius in the henna partridge was located in the dorsal part of the cloaca and opened into the cloaca with a channel. It was observed that bursa of Fabricius was surrounded by a connective tissue capsule and consisted of tunica serosa, tunica muscularis, and tunica mucosa layers from the outside to the inside. The tunica muscularis consisted of outer longitudinal and inner circular smooth muscle fibers. It was seen that the tunica mucosa also made plicae towards the lumen of the organ and consisted of 10-15 plicae (Figure 1). Lamina epithelialis and lymph follicles were present in each plica (Figure 2a). It was determined that the lamina epithelialis consisted of two parts called FAE and IFE (Figure 2b, 2c). It was noted that lymph follicles contain cortex and medulla sections and are locally separated by capillary vessels together with Cortico medilar nerve cells (CMSH) (Figure 2d).

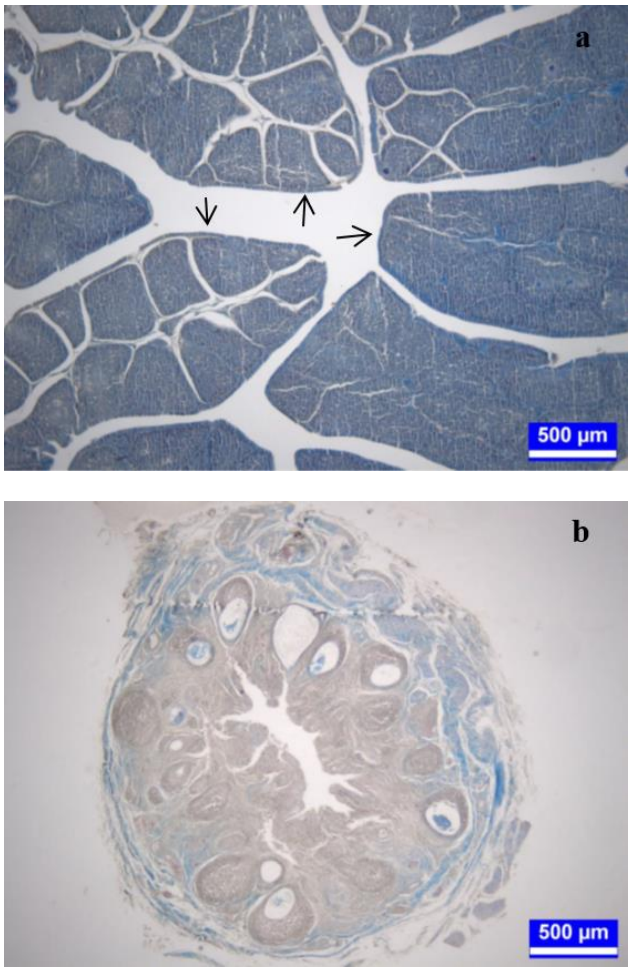

Figure 1. General view of 3- and 6-month old bursa of fabricius

a: General view of the 3-month-old bursa of of Fabricius, pilicae (arrows), Triple, X4. 4b: General view of the 6-month-old bursa of Fabricius. Triple, X4.

Histology

In the bursa of Fabricius of three-month-old henna partridges, the first involution-related changes started in IFE and locally in FAE (Figure 3). It was observed that degenerative changes were mostly formed in the medulla, but similar changes occurred in the cortex. In this period, cysts surrounded by epithelium were found within the corticomedullary border of some lymph follicles. The sizes of these cysts were much smaller than the cysts in the bursa of Fabricius of 6-month-old henna partridges (Figure 4).

It was observed that deep collapses were formed in the IFE of partridge bursa of Fabricius in the six-month-old henna and the number of goblet cells increased in these regions. It was noted that in most sections the plicae disappeared completely (Figure 1b). It was observed that intrafollicular cysts were formed especially in the bottom parts of the follicles and in the areas close to the KMSH when some cells in the medulla of the lymph follicles lose their structure and functions and dissolve (Figure 4b). It was observed that the epithelial cells covering the lumen of the cysts, which were much more in number and larger in volume compared to the 3-month-old henna partridge bursa of Fabricius, transitioned from cubic to prismatic.

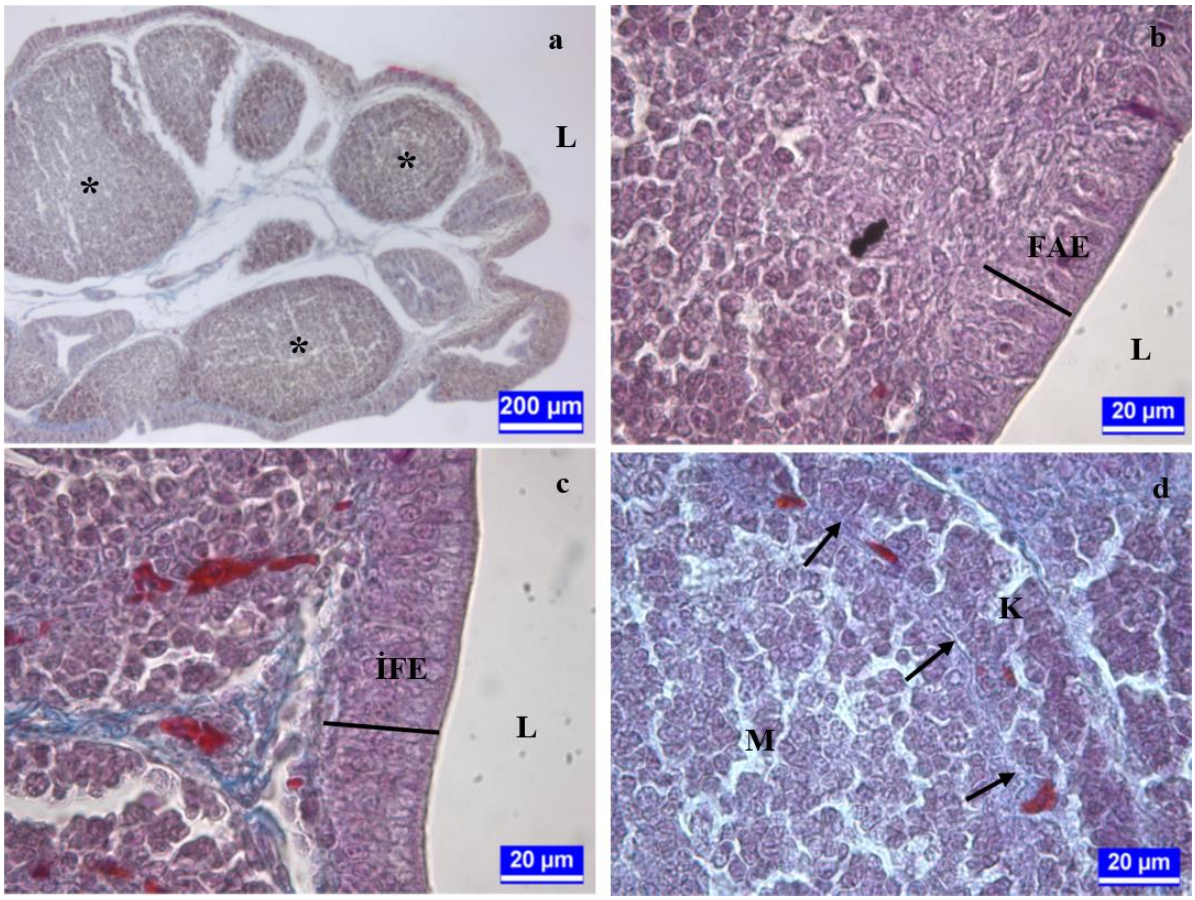

Figure 2. General view of 3-month-old bursa of Fabricius,
a) Lymph follicles within the plica are seen in 3-month-old henna partridge bursa of Fabricius (*). Lumen (L). Triple staining, X10. b) FAE is seen in three-month-old henna partridge bursa of Fabricius. Lumen (L). Triple staining, X100. c) IFE is seen in the three-month-old henna partridge bursa of Fabricius. Lumen (L). Triple staining, X100. d) Cortex (C), medulla (M), and corticomedullary border cells are seen in lymph follicles located in the three-month-old henna partridge bursa of Fabricius (arrows). Triple staining, X100.

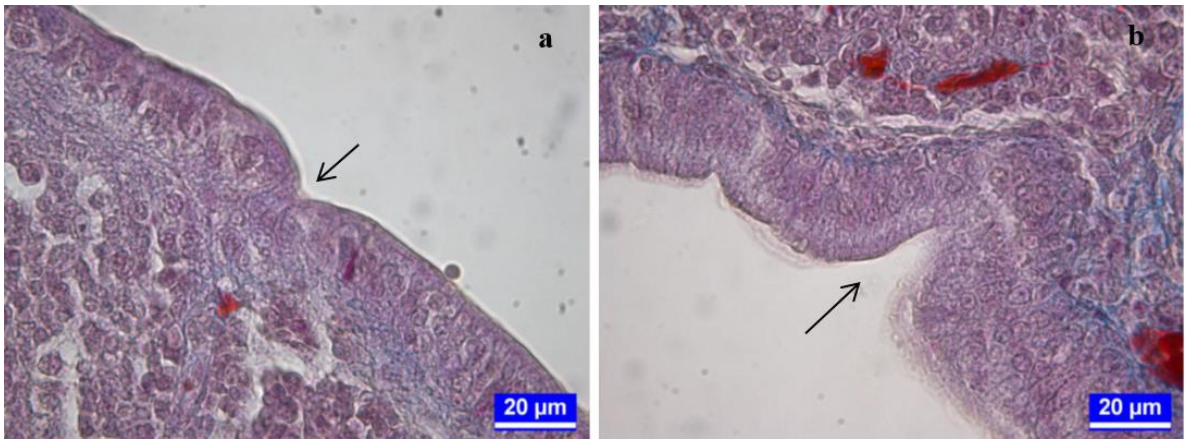

Figure 3. First signs of involution in the bursa of Fabricius in a 3-month-old henna partridge (arrows). Triple, X100.
a: First signs of involution seen in IFE, b: First signs of involution seen in FAE. Triple, X100.

Cysts formed in lymph follicles were also found in FAE. In some sections of bursa of Fabricius in the six-month-old henna partridge, it was observed that the histological organization of the lymph follicles was disrupted or disappeared, and a large part of the organ was covered by connective tissue (Figure 4b).

In the methyl Green-pyronin staining method, plasma cells were detected in the connective tissue surrounding the organ, around the blood vessels and inside the follicles in the bursa of Fabricius of the henna partridge (Figs. 5a and 5b).

In Gordon-Sweet reticular fiber staining, it was observed that the reticular fibers condensed at the base of the epithelial cells forming the IFE and created a border, revealing the medulla and cortex border of the lymph follicles even more clearly. It was seen that the lymph follicles in the henna partridge bursa of Fabricius were surrounded by reticulum fibers and sent extensions to the cortex. In this way, the reticular cells and reticular fibers formed the roof of the lymph follicles. It was observed that the reticular fibers were thinned at the base of the epithelial cells forming the FAE and condensed around the blood vessels and between the follicles (Figure 5).

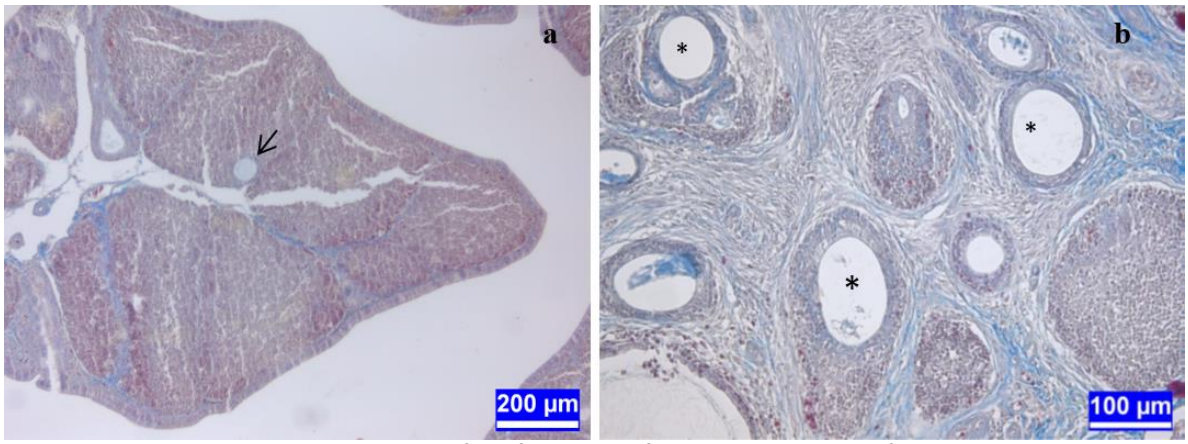

Figure 4 a: The cyst in the medulla, one of the first signs of involution, in bursa of Fabricius in the 3-month-old henna partridge (arrow). Triple, X10. b: Cysts in bursa of Fabricius in the 6-month-old henna partridge (*). Triple, X20.

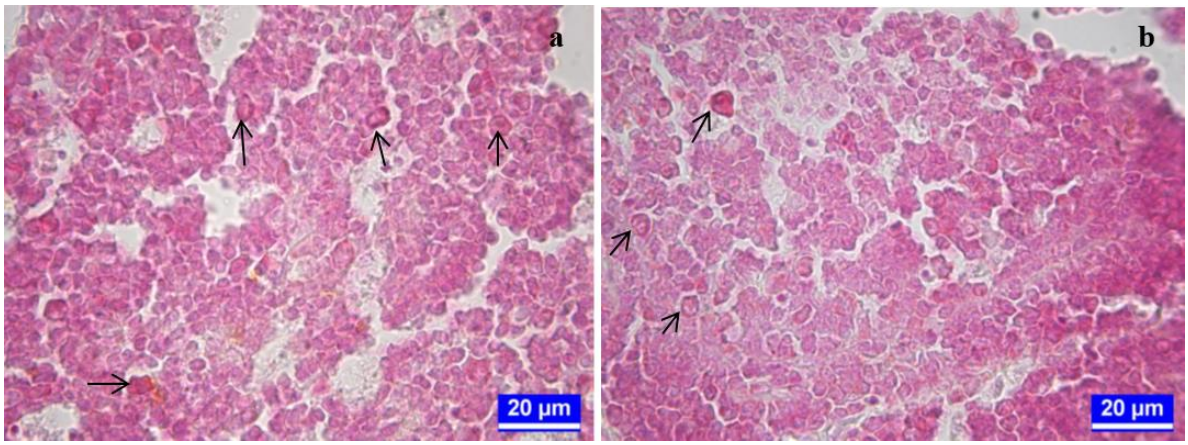

Figure 5. a: Plasma cells in the bursa of Fabricius in a 3-month-old henna partridge (arrows). MGP, X100. b: Plasma cells in a 6-month-old bursa of Fabricius (arrows). MGP, X100

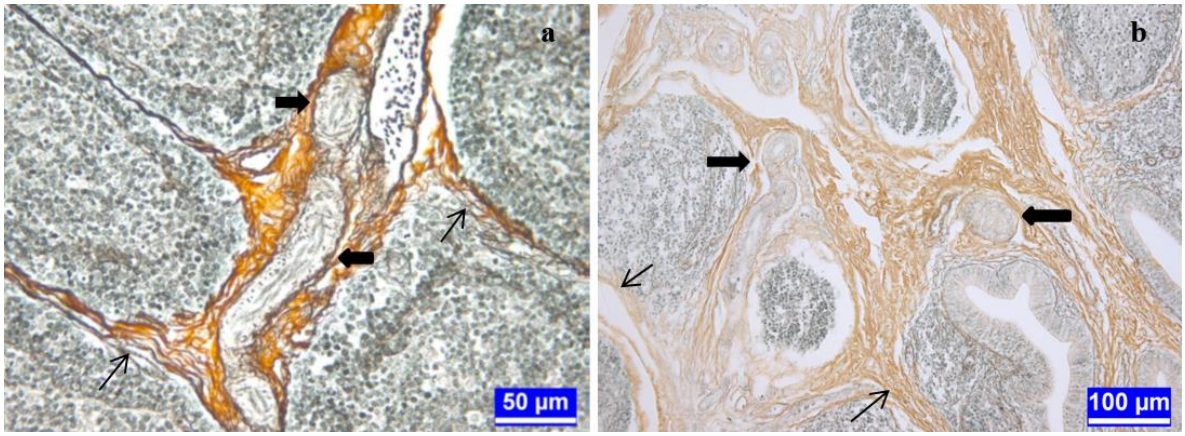

Figure 6. a: Reticular fibers around blood vessels (thick arrows) and between follicles (thin arrows) in bursa of Fabricius in the 3-month-old henna partridge. GS, X100. b: Reticular fibers around blood vessels (thick arrows) and between follicles (thin arrows) in bursa of Fabricius in the 6-month-old henna partridge. GS, X20.

Histochemistry

AB pH=2.5 staining for determination of acidic mucosubstance in bursa of Fabrcius showed AB-positive reaction only in the apical part of the epithelial cells forming FAE and IFE in the pre- and post-involution period (Figure 7). In the PAS staining performed to determine the neutral mucosubstance of the henna partridge bursa of Fabricius, PAS-positive reaction was observed only in the apical part of the epithelial cells forming FAE and IFE in the pre- and post-involution period (Figure 8). In the PAS/AB pH=2.5 combined staining method performed on henna partridge bursa of Fabricius, AB-positive reaction was observed only in the apical part of the epithelial cells before involution (Figure9).

It was reported that the wall structure of bursa of Fabricius consists of the tunica mucosa, tunica muscularis and tunica serosa layers by Ciriaco et al (1985) in pigeon, by Kocaöz (1993) in chicken, by Onyeanusi et al (1993) in guinea fowl, by Gulmez and Aslan (1999) in domestic

geese, by Sari and Kurtdede (2007) in turkey, and by Dirik (2011) in rock partridge. In this study, tunica mucosa, tunica muscularis and tunica serosa layers were observed in the wall structure of the henna partridge bursa of Fabricius. It was seen that the tunica muscularis consisted of outer longitudinal and inner circular smooth muscle fibers.

It was reported that the tunica mucosa forms plicae extending towards the lumen in the bursa of Fabricius by Ciriaco et al (1985) in pigeon, by Kocaöz (1993) in chicken, by Onyeanusi et al (1993) in guinea fowl, by Gülmez and Aslan (1999) in domestic geese, by Sarı and Kurtdede (2007) in turkey, by Dirik (2011) in rock partridge, by Khenenou et al. (2012) in broiler. In this study, it was observed that the tunica mucosa layer extended towards the lumen of the organ and formed plicae in the henna partridge bursa of Fabricius.

It was reported that the lamina epithelial in the bursa of Fabricius consists of two different epithelium, namely IFE and FAE by Ciriaco et al (1985) in pigeon, by Kocaöz (1993) in chicken, by Onyeanusi et al (1993) in guinea fowl, by Hupaya (1995) in broiler, by Gülmez and Aslan (1999) in domestic geese, by Sarı and Kurtdede (2007) in turkey, by Dirik (2011) in rock partridge, by Khenenou et al. (2012) in broiler. In this study, it was observed that the lamina epithelium in the henna partridge bursa of Fabricius consisted of two different epithelium as IFE and FAE.

It was determined that there are lymph follicles consisting of corkex and medulla sections within the plicae in the bursa of Fabricius by Hashimoto and Sugimura (1976) in Peking duck, by Ciriaco et al (1985) in pigeon, by Onyeanusi et al (1993) in guinea fowl, by Kocaöz (1993) in chicken, by Hupaya (1995) in broiler, by Gülmez and Aslan (1999) in domestic geese, by Sarı and Kurtdede (2007) in turkey, by Dirik (2011) in rock partridge, by Khenenou et al. (2012) in broiler. In this study, it was observed that there are parts of cortex and medulla in the lymph follicles located in the plicae of the henna partridge bursa of Fabricius.

Sarı and Kurtdede (2007) detected lymphocytes, lymphoblasts, reticular epithelial cells (REC) and macrophages in the medulla and cortex of the turkey bursa of Fabricius. In this study, lymphocytes, lymphoblasts and macrophages were observed in the cortex and medulla of the henna partridge bursa of Fabricius.

Researchers (Kocaöz et al., 1997) reported that the histological changes that indicate involution begin in the bursa of Fabricius in the 10-12th weeks after hatching, and that the involution resulting in atrophy of the organ was completed in three phases: early involution stage (90-150 days), late involution stage (up to 165 days), and residual stage (165-180 days). They pointed out that there were significant differences both between individuals and in the degree of involutive changes observed in the lymph follicles in the same bursa of Fabricius, considering the time of appearance of the first histological signs of this regression event in the organ. In this study, some three-month-old (90-120 days) henna partridge bursa of Fabricius showed involution-related changes in IFE and locally in FAE.

Butcher et al. (1989) reported that involution-related changes observed in the bursa of Fabricius in White Leghorn chickens are different and generally not age-related but at the beginning of egg production. In a study conducted in chickens (Milicevic et al. 1986), it was reported that the first involution-related changes in the bursa of Fabricius was a decrease in the weight of the bursa of Fabricius, and this situation was more apparent in males. Mercer-Oltjen and Woodard (1987) stated that there was no difference between male and female animals in terms of weight loss of the bursa of Fabricius or other involution-related changes in their study in partridges and pheasants. Ciriaco et al (2003) reported that involution-related changes in chickens started at approximately 8th week, atrophic or cystic follicles, which are among the prominent involutive changes, began to appear at 20th week, very significant changes occurred at 24th week, and involution was completed at approximately 26th week. Similarly, in this study, it was observed that the first involution-related changes in 3-month-old henna partridges started with localized collapses in IFE and FAE epithelial cells. On the other hand, prominent cystic follicles surrounded by epithelium were found in six-month-old henna partridges. It was also observed that the plicae disappeared, the lymph follicles decreased in number and volume, the lumen was narrowed and the connective tissue mass increased.

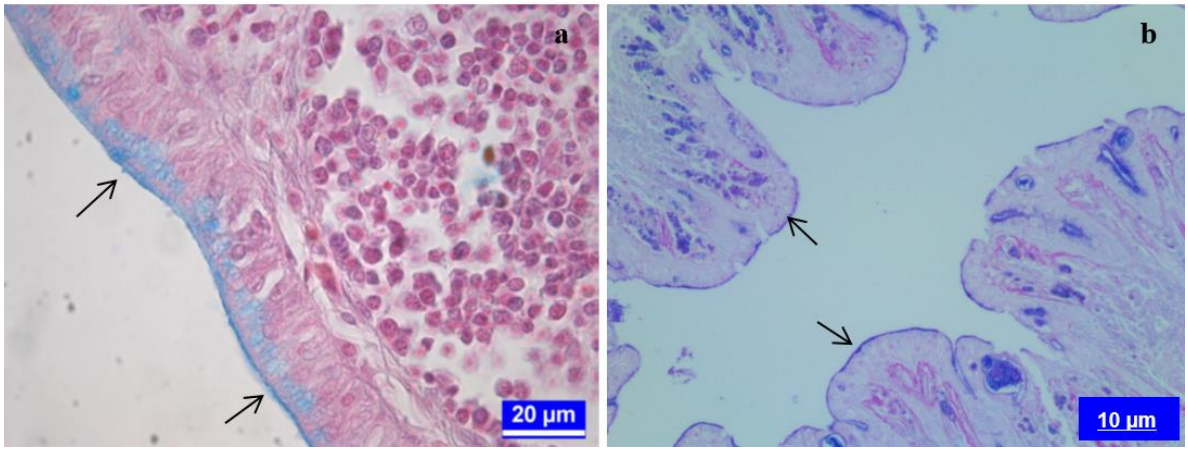

Figure 7. AB-positive reaction (arrows) in the apical part of epithelial cells in bursa of Fabricius in the 3-(a, X100) and 6-(b, X10) month-old henna partridge. AB pH=2.5.

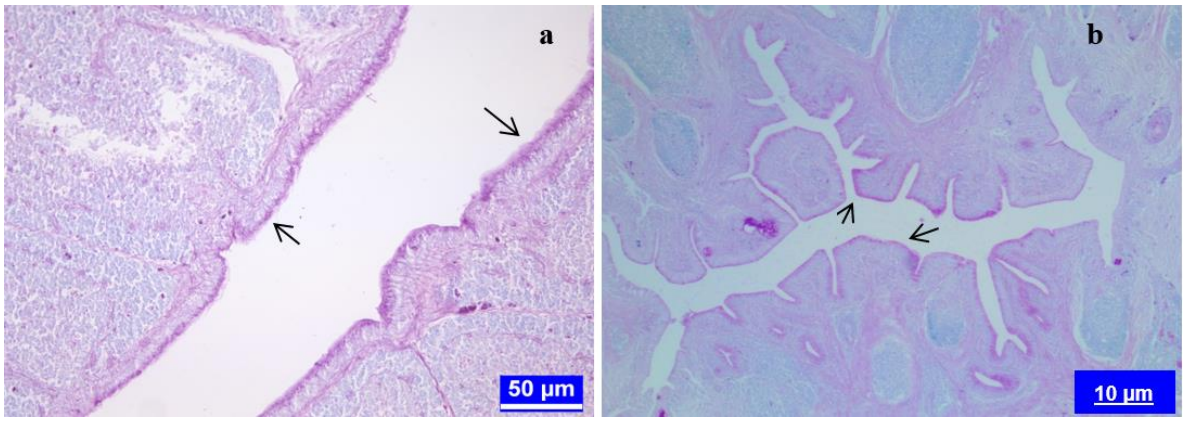

Figure 8: PAS-positive reaction in the apical part of epithelial cells in bursa of Fabricius in the 3-(a, X40) and 6-(b, X10) month-old henna partridge (arrows). PAS

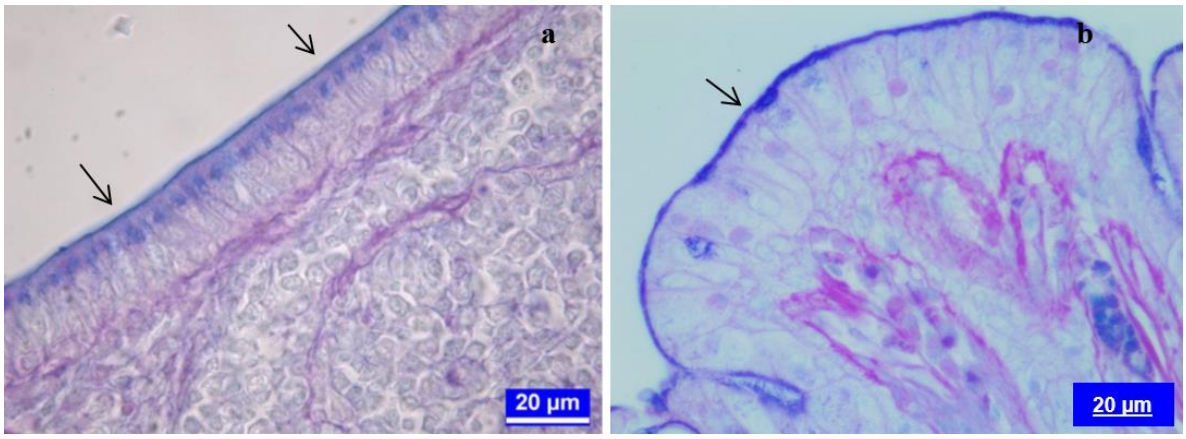

Figure 9. AB-positive reaction in the apical part of epithelial cells in the bursa of Fabricius in 3- (a, X100) and 6- (b, X20) month-old henna partridge (arrows). PAS/AB Ph=2,5.

Conclusions

It was seen that the mucosa of the henna partridge bursa of Fabricius was surrounded by a capsule and consisted of 10-15 plicae. It was observed that the muscularis layer consisted of outer longitudinal and inner circular smooth muscle fibers. It was noted that lymph follicles in the plicae contain cortex and medulla sections and are separated from each other by KMSH. The first signs of involution in three-month-old Henna partridge bursa of Fabricius were observed in IFE and FAE. In six-month-old Henna partridge bursa of Fabricius, it was observed that the plicae disappeared, the lumen narrowed considerably, the lymph follicles shrank and cysts surrounded by epithelium were present in them, and the connective tissue mass increased. In AB pH=2.5 staining method, AB-positive reaction was observed only in the apical part of the epithelial cells in the Henna partridge bursa of Fabricius before and after involution. In PAS staining method, PAS positive reaction was observed only in goblet cells located between epithelial cells. In the PAS/AB pH=2.5 combined staining method, AB-positive reaction was observed only in the apical part of the epithelial cells. As a result of this study, it was found that bursa of Fabricius of the henna partridge did not undergo any histochemical changes despite its histologically large differences after involution.

Acknowledgements

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