Bulgarian Journal of Veterinary Medicine, 2022, **25**, No 3, 372–378
ISSN 1311-1477; DOI: 10.15547/bjvm.2387

Original article

AGE-RELATED HISTOLOGY OF THE BURSA OF FABRICIUS IN BRONZE TURKEYS (*MELEAGRIS MELEAGRIS GALLOPAVO*)

G. PENCHEV

Department of Veterinary Anatomy, Histology and Embryology,
Faculty of Veterinary Medicine. Trakia University, Stara Zagora, Bulgaria

Summary

Penchev, G., 2022. Age-related histology of the bursa of Fabricius in bronze turkeys (*Meleagris meleagris gallopavo*). *Bulg. J. Vet. Med.,* **25**, No 3, 372–378.

The aim of the present study was to establish age-dependent histological changes occurring in the bursa of Fabricius of bronze turkeys. To this end, histological preparations from 60 clinically healthy birds (30 males and 30 females) were examined by light microscopy. The turkeys were divided into ten age groups – at 1, 7, 14, 28, 35, 49, 56, 90, 120 and 240 days of age. Specimens were processed by routine histological techniques. At one day of age, the wall of the bursa of Fabricius comprised 4 tissue layers: tunica mucosa, tela submucosa, tunica muscularis and tunica serosa, and the mucosa formed high fold directed to the lumen. These folds were filled with lymphoid follicles, whose cortex and medulla were indistinguishable. From the 7th to the 120th days if age, the size of follicles increased and the peripherally located dark-staining cortex was clearly distinct from the centrally located lighter staining medulla. On the 240th day, signs of bursa of Fabricius' involution were visible – destruction of follicles, cystic formations, thickening of interfollicular connective tissue septa and fat accumulation within the wall. The obtained results allowed concluding that the physiological involution of the bursa of Fabricius started at the onset of sexual maturity.

Key words: bronze turkey, bursa of Fabricius, histostructure, involution

INTRODUCTION

The bursa of Fabricius is a lymphoepithelial organ found only in birds, belonging to primary haemopoietic organs together with the thymus. Both are the sites where T and B lymphocytes develop and mature. Afterwards, lymphocytes migrate to secondary lymphoid organs – the spleen, tonsils and lymph nodes (Eurell & Frappier, 2006). These organs play a primary role in avian immunity. Innate immunity includes physical barriers as the skin, mucous membranes, the complement, macrophages and granulocytes, whereas acquired immunity comprises immunocompetent cells, like B-cells (humoral response) and T-cells (cell-mediated response) (Ciriaco *et al.* 2003). Bursa of Fabricius is an oval sacciform organ located dorsal to the cloaca and communicating with it by a short duct.

The bronze turkey in the Republic of Bulgaria is well adapted and resistant to a

number of factors, therefore appropriate for rearing in pheasant farms in the southern part of the country until late autumn, then it is resettled and used for hunting (Dimitrov *et al.*, 2017). It is reported that the cloacal bursa in sexually immature birds is well developed and with puberty onset, it undergoes species-specific involution. In Pekin ducks, a full loss of lymphoid follicles was reported to occur, and the bursa remained as a fibrous sac (Hashimoto & Sugimura, 1976), whereas in guinea fowl, the bursa of Fabricius was not completely involuted even at 224 days of age and remained functionally active (Onyeanusi *et al.*, 1993). Age-related changes in avian lymphoid organs were not extensively studied, and available research studies are mainly associated with their weight and some other metric parameters (Yamada, 1966; Ward & Middleton, 1971; Indu *et al.*, 2000; Nabil *et al.*, 2005; Jayachitra *et al.*, 2009; Tamiselvan *et al.*, 2017; Lavanya *et al.*, 2019). Data about the histological features of the bursa of Fabricius in turkeys have been reported, but in relatively young birds (Karadag Sari & Kurtdede, 2007; Jayachitra *et al.*, 2009; Nnadozie *et al.*, 2014).

Therefore, the aim of the present study was to establish age-dependent histological changes associated with the development of the bursa of Fabricius of bronze turkeys from the first to the 240th day post hatch with emphasis on signs of occurring involution of the organ.

MATERIALS AND METHODS

For the study, 60 clinically healthy bronze turkeys (30 females and 30 males) purchased from state hunting enterprise "Masalat", were used. The birds were reared in an aviary. They were divided into 10 age groups (at 1, 7, 14, 28, 35, 49, 56, 90, 120 and 240 days of age). Each group consisted of 3 male and 3 female turkeys. The experiments were carried out in strict compliance with the rules of the Ethical Committee to the Trakia University, Stara Zagora.

Biological materials from bursa of Fabricius of the respective age group were used. Tissue samples were fixed in 10% buffered formalin (Merck KGaA, Darmstadt, Germany). After the fixation they were washed in running water, dehydrated in an ascending ethanol series, cleared in xylene and embedded in paraffin. Cross sections 5 μm thick were cut on rotational microtome YD-335A (J. Y. M. A. Ltd., China). After double deparaffinisation in xylene and rehydration in a descending ethanol series they were stained with haematoxylin (Ehrlich) – eosin to obtain permanent histological preparations (Vitanov *et al.*, 1995). Preparations were observed with a Leica DM 1000 microscope and results documented with Leica DOC 290 digital camera. Data were processed with LAS V 410. 0 2016 software.

RESULTS

The light microscopy of histological preparations from bursas of Fabricius of one-day-old male and female bronze turkeys demonstrated that the bursal wall was made of 4 tissue layers: tunica mucosa, tela submucosa, tunica muscularis and tunica serosa. The mucosa had only 2 sublayers – lamina epithelialis and lamina propria. Lamina muscularis was absent, therefore the boundary between propria and submucosa could be hardly identified. It was assumed that the loose connective tissue among the follicles in plicae was lamina propria, whereas the tissue in the remaining part of the wall – tela submucosa.

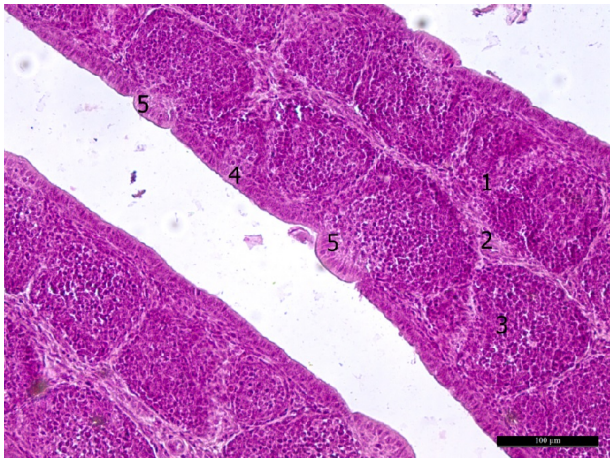

Fig. 1. Bursa of Fabricius at day 1 post hatch: 1 – folds, 2 – septae, 3 – follicles, 4 – interfollicular epithelium, 5 – follicle associated epithelium, bar=100 µm.

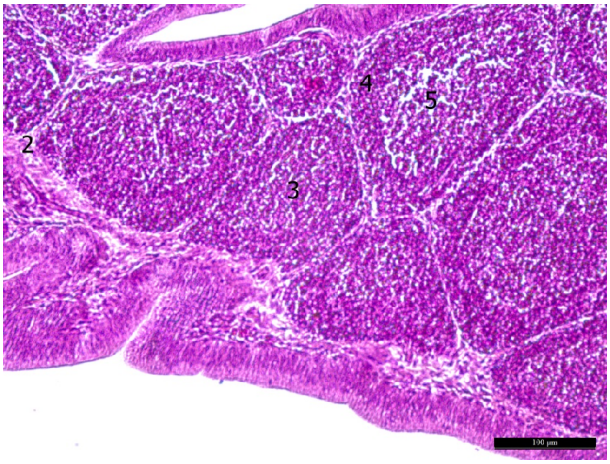

Fig. 2. Bursa of Fabricius at day 7: 1 – fold, 2 – septa, 3 – follicle, 4 – cortex of the follicle, 5 – medulla of the follicle; bar=100 µm.

The mucosa formed 11–13 folds of various shape and size, directed to the lumen. They were filled with oval lymphoid follicles, whose cortex and medulla could not be distinguished. These follicles were divided by relatively thin, well vascularised interfollicular connective tissue septa (Fig. 1). The epithelium lining the folds was of 2 types: pseudostratified interfollicular and simple prismatic, follicle-associated on the top of follicles directly contacting the epithelium. In the other follicles, connective tissue was present between them and the epithelium. The nuclei of epithelial cells were usually ovoid. Tunica muscularis was built of an inner sublayer with circularly oriented smooth muscle cells and an outer sublayer with longitudinally located smooth muscle cells. On the outside, the bursa of Fabricius was lined by tunica serosa, made of loose connective tissue.

At 7 days of age, folds filled with follicles were observed, whose peripherally located dark staining cortex, containing a lot of basophilic lymphocytes could be distinguished from the centrally located lighter staining medulla (Fig. 2).

As age progressed (14, 28, 35, 49, 56, 90 and 120 days), the lymphoid follicles were of larger size and clearly differentiated cortex and medulla. Here, the follicle-associated epithelium, though which the medulla was connected to the bursal lumen, was even more distinct.

At 240 days of age, the signs of involution affecting both male and female turkeys were clearly visible. In some folds, destructed follicles, thickened septa with large blood vessels and rose-stained structures in the medulla, most probably representing vacuolated reticular cells were observed (Fig. 3). Other folds demonstrated cystic formations surrounded by

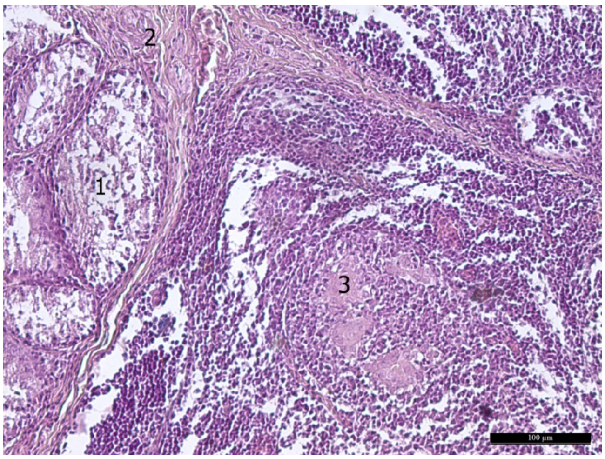

Fig. 3. Bursa of Fabricius at day 240; 1– destroyed follicle, 2 – septa, 3 – eosinophilic structures in the medulla; bar=100 µm.

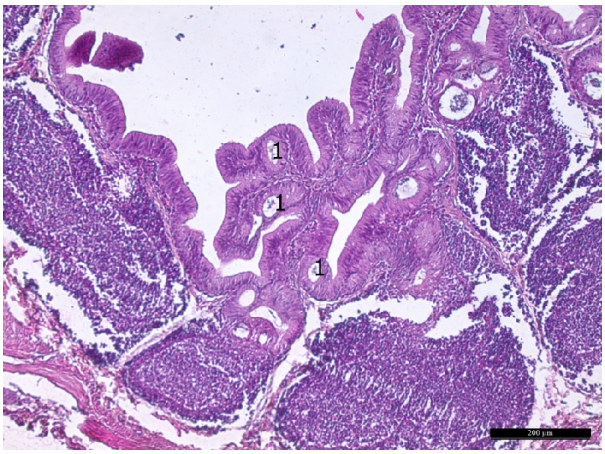

Fig. 4. Bursa of Fabricius at day 240; 1 – cysts; bar=200 µm.

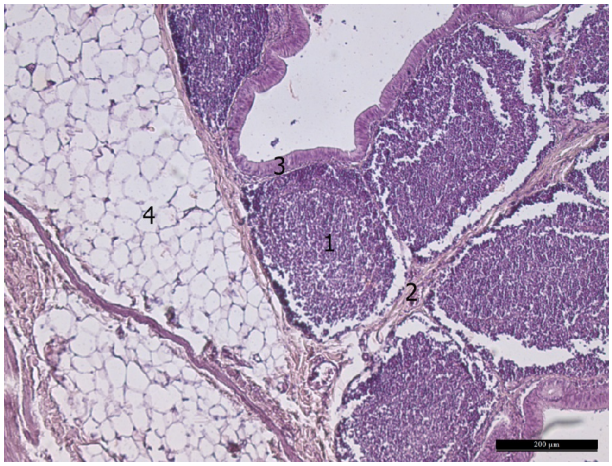

Fig. 5. Bursa of Fabricius at day 240; 1 – follicle, 2 – septa, 3 – epithelium, 4 – adipose tissue in the submucosa, bar=200 µm.

epithelial cells (Fig. 4). At some locations in the wall of the bursa of Fabricius, large amounts of fat was detected (Fig. 5).

DISCUSSION

This study elucidates the histological structure of the bursa of Fabricius in bronze turkeys with respect to age, and found no sexual dimorphism. A special attention was paid to the signs of organ's involution in birds.

The histological examination showed that the bursal wall was made of 4 tissue layers, confirming the findings of Payal *et al.* (2010) and Nnadozie *et al.* (2014). However, other researchers reported only 3 layers, without tela submucosa in geese (Gulmez & Aslan, 1999), quails (Hassan *et al.*, 2011) and chickens (Karadag Sari *et al.*, 2015). Similarly to other bird species, the epithelium was divided into follicle-associated, pseudostratified and interfollicular, simple prismatic (Hashimoto & Sugimura, 1976; Ciriaco *et al.*, 2003; Jayachitra *et al.*, 2009; Hassan *et al.*, 2011). The structure of lymphoid follicles described in this study with an outer dark staining cortex and inner, lighter staining medulla was in line with data reported in other bird species – geese (Gulmez & Aslan, 1999), chickens (Leena *et al.*, 2009), quails (Hassan *et al.*, 2011) and broiler chickens (Khenenou *et al.*, 2012). Our observations on the signs of bursa of Fabricius' involution corresponded to previously described signs such as destructed follicles, indistinct cortex and medulla, thickened interfollicular septa, increased number of cysts (Ward & Middleton, 1971; Hashimoto & Sugimura, 1976; Ciriaco *et al.*, 2003; Hussein *et al.*, 2019). We agree with the hypothesis that involution was most probably due to the activity of sex hormones (Khenenou *et al.*, 2012), manifested with most pronounced changes at 240 days of age, e.g. the onset of sexual maturity in the bronze turkey.

At the background of the described normal age-related histostructure of the bursa of Fabricius, some authors have reported disorders characterised with atrophy of lymphoid follicles, cystic changes and depletion of lymphoid cells in

different diseases states: Marek disease (Bochukov & Semerdjiev, 1992); ochratoxicosis (Elaroussi *et al.*, 2008), Gumboro disease (Khenenou *et al.*, 2017), aflatoxicosis (Groseva *et al.*, 2020).

CONCLUSIONS

The results of this study allowed concluding that the histological changes occurring the bursa of Fabricius were age-related and not dependent on the sex of birds. The involution was associated rather to the age of sexual maturity than to the live weight.

REFERENCES

Bochukov, A. & V. Semerdjiev, 1992. The effect of Marek disease and gamma-irradiation on the histostructure of Fabricius cyst in chickens of various alkaline phosphatase genotype. *Genetics and breeding,* **25**, 363–366.

Ciriaco, E., P. Pinera, B. Dias-Esnal & L. Laura, 2003. Age-related changes in the avian primary lymphoid organs (thymus and bursa of Fabricius). *Microscopy Research & Technics*, **62**, 482–487.

Dimitrov, D., G. Penchev, D. Yovchev & K. Stamatova-Yovcheva, 2017. Age-related light microscopy histometric investigation on bursa of Fabricius in the common bronze turkey (*Meleagris meleagris gallopavo*). *Bulgarian Journal of Veterinary Medicine*, **20**, Suppl. 1, 71–75.

Elaroussi, M., F. Mohamed, M. Elgendy, E. Barkouky, A. Abdou & M. Hatab, 2008. Ochratoxicosis in broiler chickens: Functional and histological changes in target organs. *International Journal of Poultry Science*, **7**, 414–422.

Eurel, J. & B. Frappier, 2006. Delmann's Textbook of Veterinary Histology, 6th edn, Blackwell Publishing, Ames, Iova, USA.

Grozeva, N., I. Valchev, L. Lazarov, Ts. Hristov, D. Kanakov & R. Binev, 2020. Cloacal bursa morphology in turkey broilers challenged with aflatoxin B alone or co-administered with Mycotox NG. *Bulgarian Journal of Veterinary Medicine,* **23**, 121–129.

Gulmez, N. & S. Aslan, 1999. Histological and histometrical investigations on bursa of Fabricius and thymus of native geese. *Turkish Journal of Veterinary and Animal Sciences*, **23**, 163–171.

Hashimoto, Y. & M. Sugimura, 1976. Histological and quantitative studies on the postnatal growth of the thymus and the bursa of Fabricius of White Pekin ducks. *Japanese Journal of Veterinary Research*, **24**, 65–76.

Hassan S., J. Hussein & B. Rasol, 2011. Histological study on bursa of Fabricius of quail birds (*Coturnix coturnix japonica*). *Egyptian Poultry Science*, **31**, 613–620.

Hussein, A. & A. Reshag, 2019. Histological and immunf-histochemical study of postnatal development of bursa of Fabricius in turkeys (*Meleagris gallopavo*). *Online Journal of Veterinary Research,* **23**, 255–261.

Indu, V., J. Chungath, K. Harshan & N. Ashok, 2000. A morphological study on the postnatal development of the bursa of Fabricius in White Pekin duck. *Indian Journal of Poultry Science*, **35**, 124–127.

Jayachitra, S., K. Balasundaram, A. Kumaravel & P. Ramayya, 2009. Histological and histochemical studies on the bursa of Fabricius of turkey (*Meleagris gallopavo). Indian Journal of Veterinary Anatomy*, **21**, 14–18.

Karadag, Sari, E. & N. Kurtdede, 2007. Light and electron microscopic studies of the bursa of Fabricius in turkeys. *Kafkas University Veterinary Fakulty Dergesi*, **13**, 177–184.

Karadag Sari, E. H. Altunay, N. Kurtdede & B. Bakir, 2015. The structure of bursa of Fabricius in the long-legged buzzard (*Buteo rufinus*): Histological and histo-

chemical study. *Acta Veterinaria,* **65**, 510–517.

Khenenou, T., M. Melizi & H. Benzaoui, 2012. Morpho-histological study of the bursa of Fabricius of broiler chickens during post-hashing age. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering,* **6**, 1131–1133.

Khenenou, T., M. Bougherara, M. Melizi, R. Lamraoui, 2017. Histomorphological study of the bursae of Fabricius of broiler chickens during Gumboro disease in Algeria area. *Global Veterinaria*, **18**, 132–136.

Lavanya, C., K. Balasundaram, S. Jayachitra & R. Madheswaran, 2019. Gross morphological study on the bursa of Fabricius in Japanese quail (*Coturnix coturrnix japonica*). *Journal of Entomology and Zoology Studies,* **7**, 67–69.

Leena, C., R. Prasad, K. Kakade & K. Jamuna, 2009. Age-related histochemical changes of the bursa and thymus of domestic fowl. *Journal of Veterinary and Animal Sciences*, **40**, 9–11.

Nabil Alloui, M, Sellaoui & S. Djaaba, 2005. Morphometrical and anatomopathological survey of the bursa of Fabricius in broiler chickens. In: *Proceedings of the XII International Congress of ISAH – Warsaw, Poland*, pp. 52–54.

Nnadozie, O., D. Ezeasor, U. Nlebedum, E. Ikpegbu & I. Agbakwuru, 2014. Assessment of the morphological development of the bursa of Fabricius in turkey (*Meleagris gallopavo*). *International Journal of Poultry Science,* **13**, 1–11.

Onyeanusi, B., C. Ezeokoli, J. Onyeanusi & A. Emma, 1993. The anatomy of the cloacal bursa in the helmeted Guinea fowl. *Anatomia, Histologia, Embryologia*, **22**, 212–221.

Payal, J., S. Ingole & U. Dang, 2010. Gross and histological studies on bursa of Fabricius of CARI Shyama and Vanaraja breeds of poultry. *Haryana Veterina*, **49**, 51–53.

Tamilselvan, S., K. Balasundaram & S. Jayachitra, 2017. Gross anatomy of bursa of Fabricius in Guinea fowl (*Numida meleagris*). *Indian Veterinary Journal*, **1**, 14–16.

Vitanov, S., D. Dimitrov & A. Bochukov, 1995. Practical Guide of Cytology and Histology, Zemizdat, Sofia, Bulgaria, pp. 3–22 (BG).

Ward, J. & A. Middleton, 1971. Weight and histological studies of growth and regression in the bursa of Fabricius in the mallard (*Anas platyrhynchos*). *Canadian Journal of Zoology*, **49**, 11–14.

Yamada, J., 1966. The weight and the histological changes with age of the bursa of Fabricius in chickens. *Japanese Journal of Veterinary Research*, **14**, 136.

Paper received 08.09.2020; accepted for publication 08.10.2020

Correspondence:

G. Penchev
Department of Veterinary Anatomy,
Histology and Embryology,
Faculty of Veterinary Medicine.
Trakia University,
6000 Stara Zagora, Bulgaria,
e-mail: georgi_pnchv@yahoo.com