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# Age related histomorphological study of bursa of fabricius in Japanese quail (*Coturnix coturnix Japonica*)

**SD Kadam, JY Waghaye and PN Thakur**

**Abstract**
The present study was carried out to observe histomorphology of bursa of fabricius from one to four weeks of age in Japanese quail birds. Bursa of fabricius was composed of tunica mucosa, tunica muscularis and tunica serosa in all age group of birds. The mucosal folds were lined by interfollicular epithelium and follicle associated epithelium. The interfollicular epithelium was composed of pseudostratified columnar epithelium. The lamina propria formed the core of bursal mucosal fold and was consisted of lymphoid follicles with few other cells. Bursal follicle was composed of outer darker cortex and inner light medulla. The cortical cellular population was found to be increase with the advancement of the age. The medulla was consisted loosely arranged lymphocytes, lymphoblasts, epithelial reticular cells with few dendritic cells, macrophages and plasma cells.

**Keywords:** Histomorphology, bursa of fabricius, Japanese quail, cortex, medulla

## 1. Introduction
Japanese quail has been used extensively as laboratory animal as well as a commercial bird for egg and meat purpose. They are fast growing bird as compared to other poultry birds as Japanese quail start laying as early as six weeks of age with production of about 288 eggs per year and can be dressed at 5 weeks of age. Besides this, low maintenance cost in rearing of quails along with its small body size, short generation interval, considerable resistance to disease and high egg production rendered quail an excellent laboratory animal [11]. The immune system comprised of primary and secondary lymphatic organs. The bursa of fabricius is the primary lymphoid organ in avians. The bursa of fabricius, which is unique to birds, is the dorsal diverticulum of the cloacas proctodeal wall. It is accountable for humoral immunity [6].
Bursa of fabricius plays an important role in maturation of B lymphocytes [4]. It has a separate anatomical structure and controls the complete number of leukocytes and lymphocytes by differentiating and proliferating B lymphocytes [5]. The lymphocytes generated in the bursa of fabricius consist of antibody producing B cells. The mature B cells are transferred to the secondary lymphoid organs through the circulating blood, where they meet and react to foreign antigen, thereby regulating the humoral immune response. The bursa of fabricius in quail exhibited structural modifications and atrophied with age progress [12].
The histoarchitecture of normal bursa of fabricius may serve as a reference for pathological condition, guideline for control strategies against biotic and abiotic diseases, diagnosis of diseases with immunodeficiency and to maintain optimal health. Through knowledge of micro anatomical structures and frequency of immune competent cells of bursa of fabricius is need of the present study, which could be further useful in immunological and immunohistochemical study.
Considering the importance and histoarchitectural alterations of bursa of fabricius with advancement of age, the present study is undertaken.

## 2. Materials and Methods
### a) Place of research work
The present study was conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Parbhani.

**b) Collection of samples**

For the present study 48 Japanese quail birds (*Coturnix coturnix japonica*) irrespective of sex reared on poultry farm of College of Veterinary and Animal Sciences, Parbhani under standard managemental quail rearing practices. The bursa of fabricius was collected from 12 birds each at end of first week, second week, third week and fourth week. These birds are sacrificed by cranial subluxation. The bursa was collected from dorsal side of cloaca by excising the large intestine.

**c) Processing of samples**

The collected organ was washed with normal saline and fixed in 10% neutral buffered formalin, 10% formal saline, Bouin's fluid and Baker's Formal Calcium. Then the tissue was processed for routine paraffin embedding [2]. Sections of 5 µm thickness were taken and processed for following staining procedures for histomorphological studies:

1. Haematoxylin and Eosin for normal histoarchitectural study [10]
2. Masson's trichrome method for collagen and muscle fibers [2]
3. Gomori's reticulin method for reticular fibers [10]
4. Verhoeff's elastic stain for elastic fibers [10]

**3. Results and Discussion**

Histomorhologically, bursa of fabricius was composed of tunica mucosa, tunica muscularis and tunica serosa in all four age group of birds. Tunica mucosa was thrown in to folds of varying number, sizes and height in all age group of birds. (Fig. 1). This observation is in agreement with the findings reported by Ebru and Nevin [3] in Turkey, Davison *et al.* [1] in birds, Gulmez and Aslan [6] in Geese, Tamilselvan *et al.* [13] in Guinea fowl and Leena *et al.* [9] in domestic fowl.

In all age group of birds during present study, it was observed that the mucosal folds were lined by two types of epithelium namely interfollicular epithelium and follicle associated epithelium. The interfollicular epithelium was composed of pseudostratified columnar epithelium. The Follicular associated epithelium was formed at the point of contact of bursal follicle and surface epithelium and was composed tuft of pseudostratified epithelium. Epithelial cells of lining epithelium usually had oval to rounded nuclei, however three types of cells were observed in interfollicular epithelium and follicle associated epithelium. Type I cells were with large oval to rounded euchromatic nuclei with prominent nucleoli. Type II cell were with oval elongated to rounded pale stained nuclei. The third type of cells were small with rounded more heterochromatic nuclei (Fig. 2 and 3).

In agreement with the observations recorded in the present study, Davison *et al*. [1] in birds and Gulmez and Aslan [6] in Geese reported psudostratified columnar bursal mucosal epithelium.

The sections stained with Gomori's reticulin stain showed the distinct reticular lamina of the basement membrane of interfollicular epithelium. The basement was found absent in the follicular associated epithelium. The follicular associated epithelium was observed to be supported by flattened cells in all age group of birds during the present study. This finding is similar to those reported by Davison *et al.* [1] in birds and Ebru and Nevin [3] in turkey.

During the present study, it was observed that the lamina propria formed the core of bursal mucosal fold and was almost occupied by clearly defined and closely packed lymphoid follicles with few connective tissues. The central vascularised connective tissue strand of each mucosal fold radiated from the tunica muscularis, which branched to form the vascularised interfollicular connective tissue septae (Fig. 1 and 4). The connective tissue of lamina propria was composed of collagen fibres, reticular fibres, fibroblasts with diffused lymphatic cells.

In agreement with the observations of the present study, Leena *et al.* [9] in fowl, Tamilselvan *et al.* [13] in guinea fowl and Gulmez and Aslan [6] in Geese reported that lamina propria of mucosal folds consisted closely packed lymphoid follicles separated by interfollicular connective tissue with rare presence of eosinophilic granulocyte in the subepithelial connective tissue. The observation of the present study is in agreement with finding reported by Hashimoto and Sugimura [7]. They reported that the interfolicular connective tissue in bursa of duck was composed of vessels, nerves, few lymphocytes, plasma cells and granular leucocytes.

The collagen fibres and reticular fibres were present at the bases of each bursal mucosal fold, central vascularised connective tissue strand, interfollicular septae and subepithelial region except the follicle associated region. However, in group IV, the amount of both fibres type was found more in the subepithelial region (Fig. 5, 6 and 7).

The bursal lymphoid follicles of varied shapes and sizes were observed in all age group of birds in the present study. Each follicle showed outer darkly stained cortex and inner lightly stained medulla (Fig. 1, 2, 3 and 4). This finding of the present study is similar to those reported by Jain *et al.* [8], Leena *et al.* [9], Tamilselvan *et al.* [13], Ebru and Nevin [3], Davison *et al.* [1] and Hashimoto and Sugimura [7]. They mentioned that the bursal lymphoid follicles consisted of outer cortex and inner medulla. Gulmez and Aslan [6] reported bursal follicles of different form and sizes in Geese.

It was observed from the present study that the cortex and medulla were separated by a distinct capillary. However, capillary between cortex and medulla was found to be less distinct in group I age of birds (Fig. 8 and 9). This is in accordance with the reports made by Jain *et al.* [8] in Shyama and Vanaraja poultry breeds.

During the present study, it was observed that the interfollicular epithelial basement membrane along with the basal cells of the follicular associated basement membrane invaded the follicle to continue with the corticomedullary border (Fig. 2 and 3). The pale stained epithelial reticular cells were noticed along the corticomedullary border, which were more distinct from group II age of birds.

In agreement with these observations Leena *et al.* [9] reported layer of pale stained reticuloepithelial cells at corticomedulary border. Davison *et al.* [1] in avian species, while agreeing with the present findings stated that the basal lamina of the interfollicular epithelium continues with the corticomedullary border.

During the present investigation, the cortex of lymphoid follicles close to the surface epithelium was cup shaped, whereas in deeper follicle cortex was observed to be completely surrounded the medulla. The sections stained by Gomori's reticulin showed the dense network fibres in the cortex. The reticular fibre network was observed to be more obvious from Group II age of birds (Fig. 6 and 7. The cellular population of cortex was consisted of densely packed small lymphocytes, lymphoblasts, with few epithelial reticular cells,

macrophages and plasma cells (Fig. 10 and 11). The cortical cellular population was found to be increase with the advancement of the age during the present study.

The observations of the present study are in agreement with Davison *et al.* [1] in avian. They reported chalice shaped cortex contained supporting reticular cells. Jain *et al.* [8] reported the network of reticular fibres in the cortex of bursal follicles. Hashimoto and Sugimura [7] reported darkly stained bursal follicular cortex consisted of closely packed lymphocytes.

The increase in cortical cellular population with the age during the present study indicated the continuation in growth of bursa till fourth week of age in quail. Similarly, increase in number of macrophages and plasma cells with the advancement of age during present work may attributed to the increased immunity status of organ with age necessary for increasing phagocytic activity.

The medulla was lightly stained. In lymphoid follicles close to the surface epithelium, medulla was in direct contact with the follicular associated epithelium in all age group of birds during the present study. At the point of contact between medulla and follicular associated epithelium, the cortex was absent (Fig. 8 and 9).

In accordance with the present observations, Gulmez and Aslan [6] in Geese reported the absence of cortex at the connection area of bursal follicle with surface epithelium. Davison *et al.* [1] while agreeing with the present observation stated that follicular associated epithelium provides direct connection between follicular medulla and bursal lumen.

The sections stained by Gomori's reticulin stain showed that the medulla was devoid of reticular fibres in all age group of birds (Fig. 6 and 7). The cellular population of medulla was composed of loosely arranged lymphocytes, lymphoblasts, epithelial reticular cells with few dendritic cells, macrophages and plasma cells (Fig. 12 and 13).

These observations are in line with reports made by Ebru and Nevin [3], in turkey, Davison *et al.* [1] in avian and Leena *et al.* [8] in fowl. These workers reported the similar cellular population in bursal follicular medulla.

The no. of macrophages and plasma cells were found to be increase with the age during the present study. The increase in number of macrophages and plasma cells with the advancement of age during present study may be attributed to the increased immunity status of organ.

The tunica muscularis was composed of two layers of smooth muscle fibres. The variation in orientation of muscle fibres of two layers was observed in all age groups of birds during the present study.

The inner layer of tunica muscularis was composed of circularly arranged and outer layer with longitudinally arranged smooth muscle fibres (Fig. 14). However, the reverse arrangement of these muscle fibres in two layers was observed at some places. The present observation is in partial agreement with Tamilselvan *et al.* [13] in guinea fowl, Ebru and Nevin [3] in turkey and Gulmez and Aslan [6] in Geese. They reported two layers of smooth muscle fibres in the tunica muscularis. However, they stated the inner layer with circularly arranged and outer layer with longitudinally arranged smooth muscle fibres.

The tunica serosa was thin layer composed of loose connective tissue. This observation is in line with the reports made by Ebru and Nevin [3] in turkey, Tamilselvan *et al.* [13] in guinea fowl.

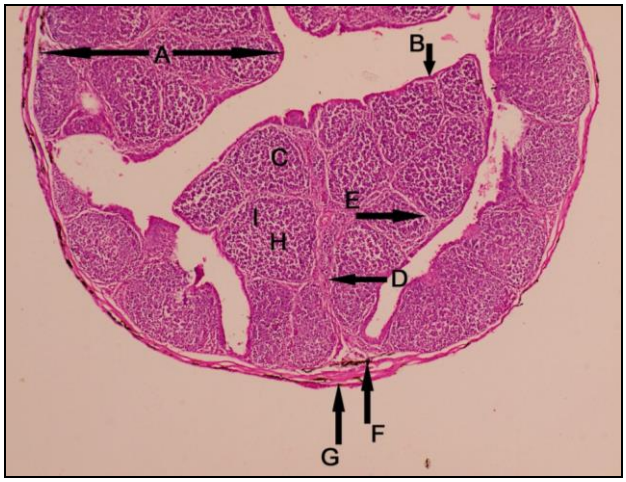


**Fig 1:** Photomicrograph of bursa of fabricius at one week of age showing. A. Mucosal fold/plicae B. Mucosal epithelium C. Lymphoid follicle D. Central connective tissue strand E. Interfollicular connective tissue septa F. Tunica muscularis G. Tunica serosa H. Cortex I. Medulla (Haematoxylin and Eosin, X 40)

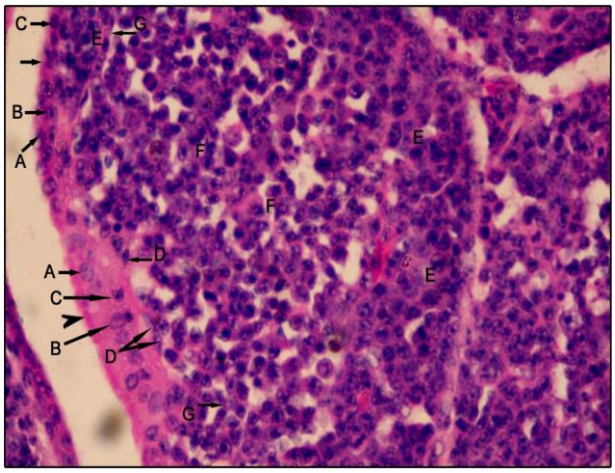


**Fig 2:** Photomicrograph of bursa of fabricius at two week of age showing follicular associated epithelium (Arrowhead) and interfollicular epithelium (Arrow)**:** A. Type I cell B. Type II cell C. Type III cell D. Supporting cells of FAE E. Cortex F. Medulla G. Corticomedullary border (Haematoxylin and Eosin, X 1000)

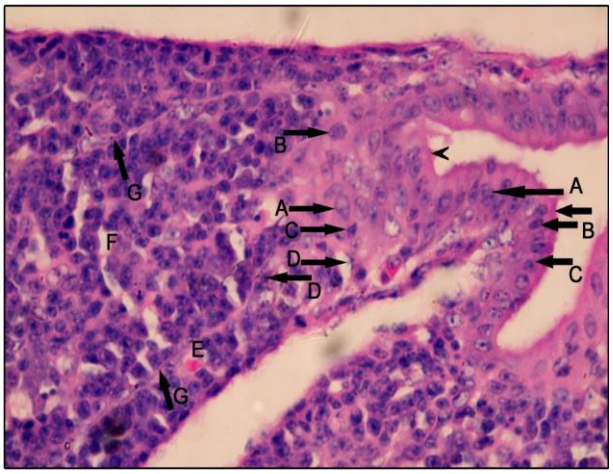


**Fig 3:** Photomicrograph of bursa of fabricius at three week of age showing follicular associated epithelium (Arrowhead) and interfollicular epithelium (Arrow): A. Type I cell B. Type II cell C. Type III cell D. Supporting cells of FAE E. Cortex F. Medulla G. Corticomedullary border (Haematoxylin and Eosin, X 1000)

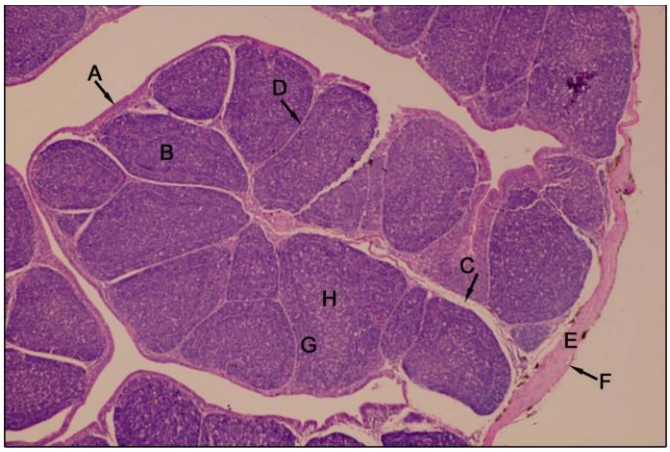


**Fig 4:** Photomicrograph of bursa of fabricius at three week of age showing bursal mucosal fold/plicae: A. Mucosal epithelium B. Lymphoid follicle C. Central connective tissue strand D. Interfollicular connective tissue septa E. Tunica muscularis F. Tunica serosa G. Cortex H. Medulla (Haematoxylin and Eosin, X 100)

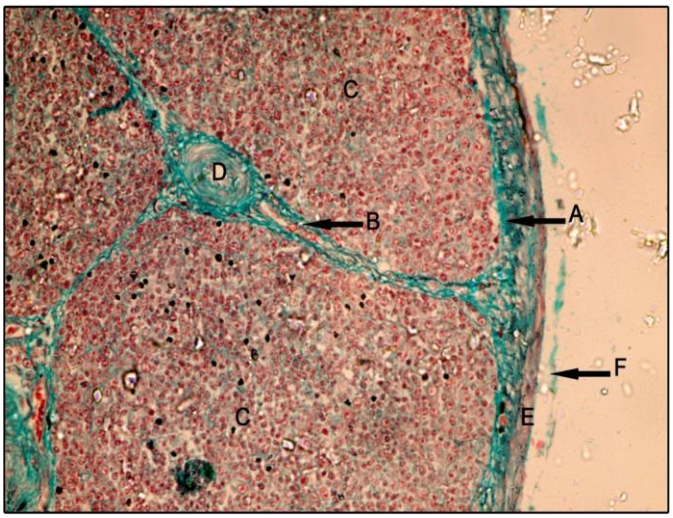


**Fig 5:** Photomicrograph of bursa of fabricius at two week of age showing: A. Collagen fiber layer at base of bursal mucosal folds B. Interfollicular septa C. Lymphoid follicle D. Blood vessel E. Tunica muscularis F. Tunica serosa (Masson's Trichrome, X 400)

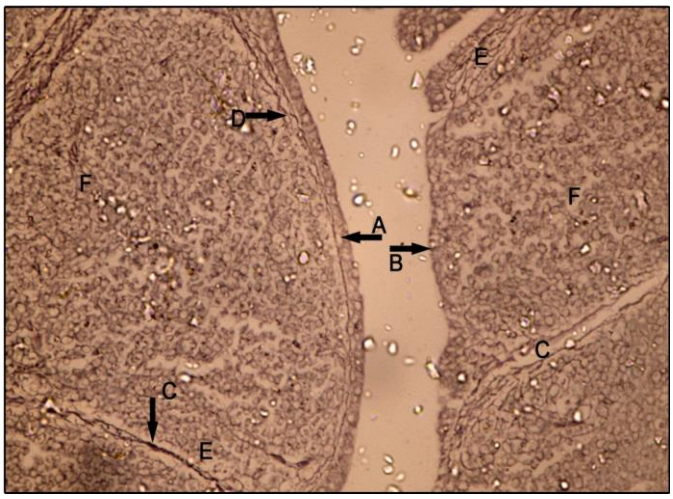


**Fig 6:** Photomicrograph of bursa of fabricius at one week of age showing: A. Interfollicular epithelium B. Follicle associated epithelium C. Interfollicular septa D. Subepithelial region E. Cortex of lymphoid follicle F. Medulla of lymphoid follicle (Gomori's reticulin, X 400)

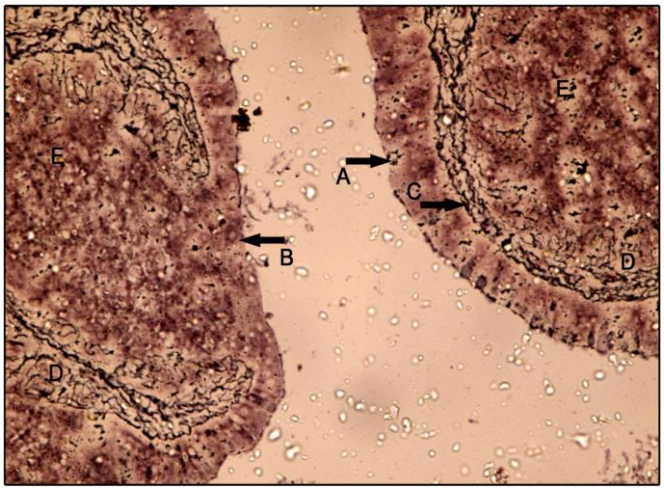


**Fig 7:** Photomicrograph of bursa of fabricius at three week of age showing: A. Interfollicular epithelium B. Follicle associated epithelium C. Interfollicular septa D. Subepithelial region E. Cortex of lymphoid follicle F. Medulla of lymphoid follicle (Gomori's reticulin, X 400)

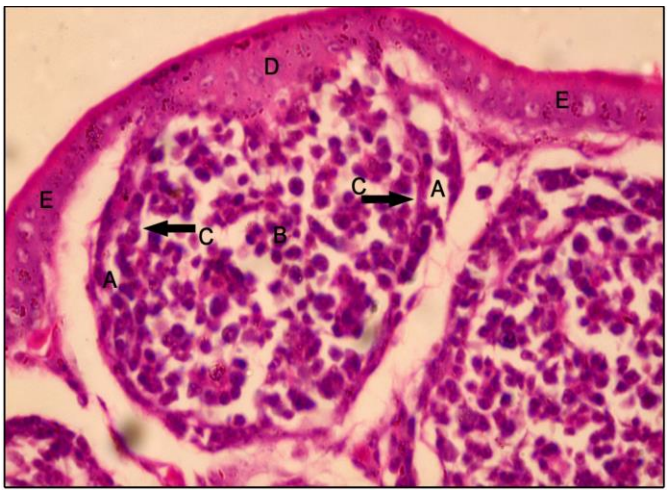


**Fig 8:** Photomicrograph of bursa lymphoid follicle at one week of age showing: A. Cortex B. Medulla C. Corticomedullary capillary D. Follicle associated epithelium E. Interfollicular epithelium (Haematoxylin and Eosin, X 1000)

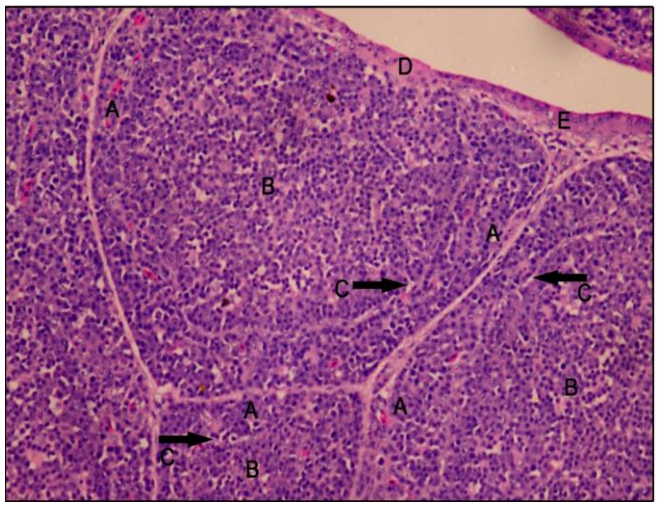


**Fig 9:** Photomicrograph of bursa lymphoid follicle at three week of age showing: A. Cortex B. Medulla C. Corticomedullary vessel D. Follicle associated epithelium E. Interfollicular epithelium (Haematoxylin and Eosin, X 400)

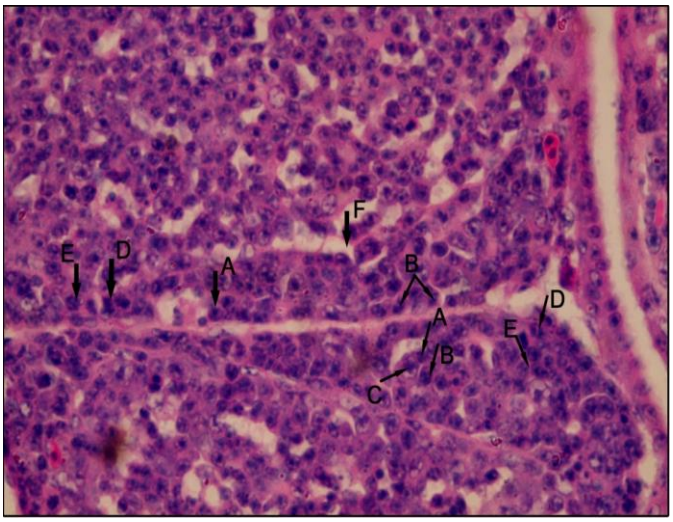


**Fig 10:** Photomicrograph of bursa of fabricius showing cellular population of cortex at two week of age: A. Lymphocytes B. Lymphoblasts C. Reticular cells D. Macrophages E. Plasma cells F. Corticomedullary border (Haematoxylin and Eosin, X 1000)

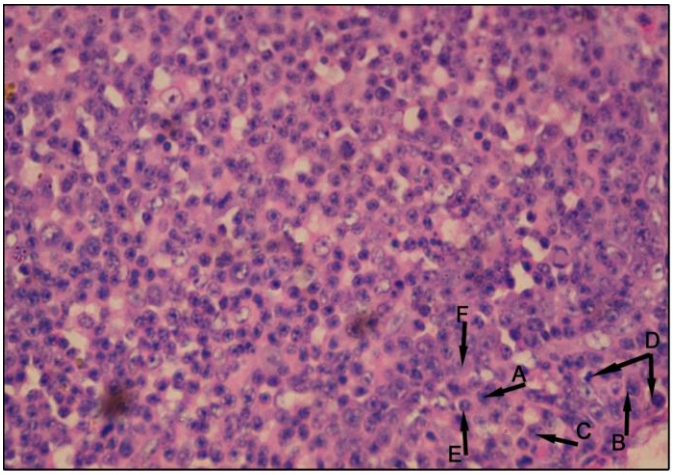


**Fig 11:** Photomicrograph of bursa of fabricius showing cellular population of cortex at three week of age: A. Lymphocytes B. Lymphoblasts C. Reticular cells D. Macrophages E. Plasma cells F. Corticomedullary border (Haematoxylin and Eosin, X 1000)

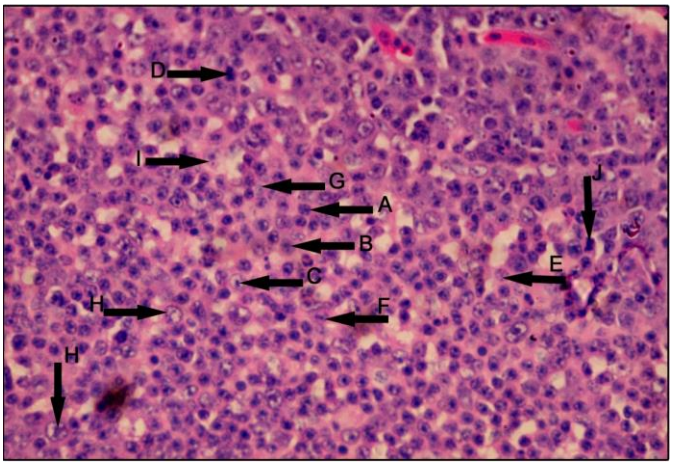


**Fig 12:** Photomicrograph of bursa of fabricius showing cellular population of medulla at three week of age: A. Lymphocytes B. Lymphoblasts C. Reticular cells D. Macrophages E. Plasma cells F. Dendritic cells G. Cells with highly euchromatic nucleus (Haematoxylin and Eosin, X 1000) H. Htpertrophied reticular cell I. Vacuolated cell J. Macrophage in vacuole

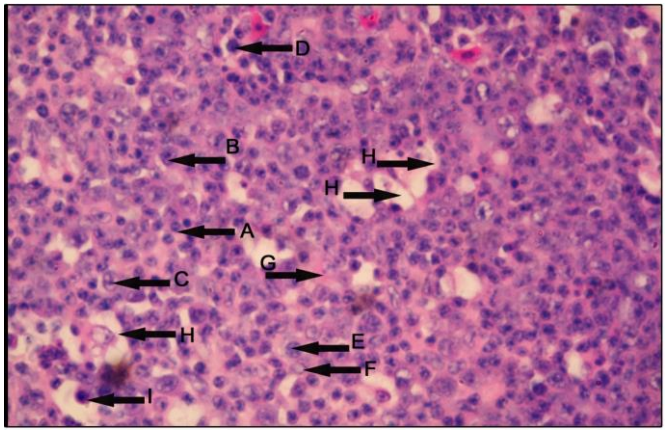


**Fig 13:** Photomicrograph of bursa of fabricius showing cellular population of medulla at four week of age: A. LymphocytesB. Lymphoblasts C. Reticular cells D. Macrophages E. Plasma cells F. Cells with highly euchromatic nucleus (Haematoxylin and Eosin, X 1000) G. Htpertrophied reticular cell H. Vacuolated cell I. Macrophage in vacuole

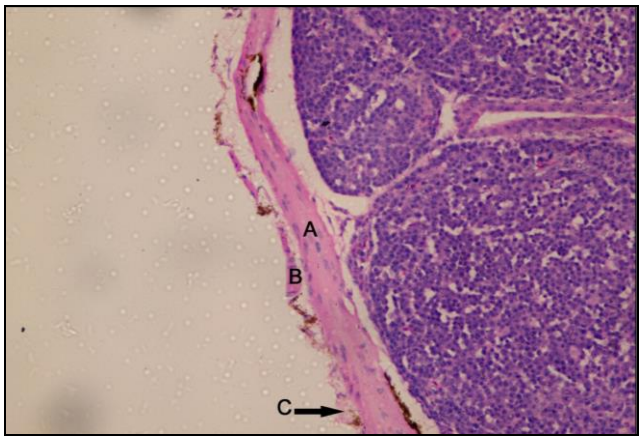


**Fig 14:** Photomicrograph of bursa of fabricius at two week of age showing tunica muscularis: A: Inner circular smooth muscle fiber layer B: Outer longitudinal smooth muscle fiber layer C: Tunica serosa (Haematoxylin and Eosin, X 400)

## 4. Conclusion

The bursa of fabricius of Japanese quail was oval, elongated organ located on dorsal aspect of cloaca. It was composed of tunica mucosa, tunica muscularis and tunica serosa in all age group of birds. Tunica mucosa was thrown in to folds of varying sizes and height. The height of bursal mucosal folds increased with the advancement of age.

The mucosal folds were lined by interfollicular epithelium and follicle associated epithelium. The interfollicular epithelium was composed of pseudostratified columnar epithelium. The Follicular associated epithelium was formed at point of contact of bursal follicle and surface epithelium and was composed tuft of pseudostratified epithelium. The lining epithelium showed three types of cells.

The lamina propria formed the core of bursal mucosal fold and was consisted of lymphoid follicles with few interfollicular connective tissue septa of collagen fibres, reticular fibres, fibroblasts with diffused lymphatic cells. The macrophages, plasma cells and occasionally granular leucocytes were located in the subepithelial region.

Each bursal follicle was composed of outer darker cortex and inner light medulla. The cellular population of cortex was consisted of densely packed small lymphocytes,

lymphoblasts, with few epithelial reticular cells, macrophages and plasma cells. The cortical cellular population was found to be increase with the advancement of the age during the present study.

The increase in cortical cellular population and number of macrophages and plasma cells with the age indicated the continuation in growth of bursa till fourth week of age in quail and attributed to the increased immunity status of organ with age necessary for increasing phagocytic activity.

The medulla was consisted loosely arranged lymphocytes, lymphoblasts, epithelial reticular cells with few dendritic cells, macrophages and plasma cells with increase in macrophages and plasma cells with advancement of the age.

The tunica muscularis was composed of two layers of smooth muscle fibres with variation in orientation of muscle fibres in all age groups of birds. The tunica serosa was thin layer composed of loose connective tissue.

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