JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2020; 3(1): 20-28
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2020.d103
Published by www.bsmiab.org

Age-related development and histomorphological observations of bursa of Fabricius in sonali chicken

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Academic Editor: Dr. Md Jamal Uddin, Ewha Womans University, South Korea.
Received: 13 October 2019; Accepted: 28 November 2019; Published: 07 January 2020.

ABSTRACT: The aim of this research was to investigate the age related histomorphological and involutory changes of bursa of Fabricius of sonali chicken at different postnatal stages in Bangladesh. The present research was carried out on bursa of 25 healthy sonali chicken representing different stage of postnatal life; Day 1, Day 14, Day 28, Day 42 and Day 56. Sample (bursa) was collected after sacrificing the chickens by cervical subluxation method. Harris's Haematoxylin and 1% Eosin Y stain was done to facilitate microscopic study. Grossly, the bursa was smooth, yellowish to milkish white in color. The average weight gain, length, width and thickness of bursa was developed parallel with age and found statistically significant (p<0.001). Age dependent changes were noticed in number of follicle per plicae, length and breadth of plicae, length and breadth of follicle, thickness of cortex and breadth of medulla of follicle, height of lining epithelium and thickness of tunica muscularis and found to be developing significantly (p<0.05) from Day 1 to Day 56. No involutory signs were found during study period. From the present study, it might be concluded that the growth and development of bursa of sonali chicken in Bangladesh was age related. The findings of this experiment would help to give an idea about the immune status of sonali chicken and provide a basis for further immunization research at different postnatal stages of development in sonali chicken.

KEYWORDS: Bursa of Fabricius, Postnatal stage, Histomorphology, Sonali chicken.

INTRODUCTION

In mammals, a large number of lymph nodes form a key feature of a well-developed and rather complex lymphoid system but in birds typical lymph nodes are only present in some aquatic species such as ducks, geese and swans. In other birds, only small lymphoid nodules that are associated with the walls of the lymph vessels are present [1]. The avian lymphoid tissue was represented by a small group of organs and tissues consisting of thymus, spleen, bursa and cecal tonsil. An obvious characteristic of the lymphatic tissues is that they are densely packed with lymphocytes. This is because they are involved with lymphocyte production, immune responses or both of the processes occurring at the same time [2]. Furthermore, lymphoid tissue can be classified into "central" and "peripheral" tissues. The central lymphoid tissue is the primary sites of development of lymphocytes which includes bone marrow, thymus and bursa in bird. The peripheral or secondary lymphoid tissues apparently depend on the primary lymphoid tissue for their origin, development and function. In birds, they include lymphoid tissue in the spleen and in the alimentary tract including the cecal tonsils.

The avian immune system provides an invaluable model for studies on basic immunology. The domestic fowl had been popular with the embryologists and immunobiologists as a suitable experimental animal model due to the occurrence of unique hind gut lymphoid organ, the bursa of Fabricius that regulates humoral antibody production [2]. Also the anatomical separation of primary lymphoid tissue of chicken has

provided useful experimental models for study of immune system [3]. The bursa of Fabricius is an immunological organ that plays a primordial role in the poultry immunity [4]. The different aggressions of the environment (stress, bad hygiene, vaccination, pathologies) undergone by birds, influential on the histo-anatomical and physiological development of the bursa of Fabricius. It can, therefore, lead to an immune depression at certain birds [5, 6].

The bursa of Fabricius is an epithelial and lymphoid organ that is found only in birds and is generally considered to be a central lymphoid organ which is responsible for the development and differentiation of B lymphocytes [7,8]. The bursa of Fabriciusis an asymmetric medial, lymphoepithelial gland unique to class aves and was located dorsal to the cloaca. It is a blind, round to oval, sac like diverticulum of the proctodeum situated on the dorsal aspect of cloaca as reported earlier [4, 9, 10].

The luminal (interior) surface of the bursa is plicated with primary and secondary plicae or folds. These plicae have hundreds of bursal follicles containing follicle-associated epithelial cells, lymphocytes, macrophages, and plasma cells. The bursa is surrounded by a thick, smooth muscle layer like other hollow organs. It is an immunological organ that plays a primordial role in the poultry immunity [11].

The bursa is active in young birds. It atrophies after about six months, reaches its maximum size at 8–10 weeks of age then, like the thymus, it undergoes involution. By 6–7 months most bursa are heavily involuted. In white leghorn chickens of both sexes, the involution of the bursa develops in approximately 8th weeks. In fact, it started at 20th weeks with scattered atrophic or cystic follicles, was obvious at 24th weeks, being essentially complete by 26th weeks, and only cicatrized vestiges of bursa were present at 28th weeks of age [12]. The gross manifestations consisted of decreased weight, bursal atrophy, variable yellowish discoloration of the mucosa, and matting or total loss of identity of the mucosal plicae [1, 13].

Concerning this immunological point of view, the histology of the bursa of the chicken is very important. Although, the development, differentiation, histological observation in native chicken [2,14], frequency of immunocompetent cells in the lymphoid tissues in Vencobb chicken [15] and histomorphological study of the lymphoid tissues of broiler chickens [9] have already been studied, however, regarding sonali chicken, it is yet to be done.

In poultry sector of Bangladesh, Sonali chicken was introduced during 1996–2000 in northern parts of country [16, 17]. The Sonali chicken has better production records and higher disease resistance capability [18]. The most common diseases of Sonali chicken are found to be salmonellosis, mycoplamosis, new castle disease, gumboro, coccidiosis, colibacillosis, gangrenous dermatitis, ascitis and omphalitis at the time of chicks rearing period [19, 20]. For understanding the clinical course of various types of diseases in sonali chicken, a comprehensive study of the lymphoid organs is essential. Furthermore, a clear knowledge of the anatomy of lymphoid organs is a prerequisite for undertaking treatment of diseases including more intelligent planning for any kind of medical or surgical management.

Therefore, the present research was structured to study the age-related development and histomorphological observations of bursa of Fabricius of sonali chicken in Bangladesh.

MATERIALS AND METHODS

Statement of the experiment

The research was conducted in the post-graduate laboratory of Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The samples were also processed in the same laboratory.

Ethical approval

The present study and all experimental procedures were approved and performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh [AWEEC/BAU/2019(30)].

Rearing and care

The experimental sonali chickens (male chickens) were reared in Bangladesh Agricultural University (BAU) Poultry Farm, Mymensingh-2202, Bangladesh. The experimental chickens were reared in proper hygienic conditions, food and water *ad libitum*. Before collecting experimental animals, their feeding history, vaccination schedule and management practices were taken into consideration. The brooding time of chicks was 12 days. The experimental chicken was reared feeding the sonali starter and sonali grower throughout the study period. The vaccination schedule was maintained. During the whole experimental tenure the uniformity of the management practice was maintained as much as possible. Airflow/ventilation of the poultry shed was sufficient. Biosecurity of the poultry shed as well as the

farm was maintained strictly. The collected chickens had neither any developmental disorder nor detectable diseases that may cause any problem in the experiment or affect the result of the experiment.

Developmental age groups

Total number of experimental chickens was twenty five (25). The chickens collected from the Bangladesh Agricultural University (BAU) poultry farm were divided into five (5) groups; Day 1, Day 14, Day 28, Day 42 and Day 56; having five (5) chickens in each groups.

Sample collection

Chickens from each group were sacrificed by cervical sub-luxation method. Just after sacrificing the experimental animals, the samples were immediately collected for gross and microscopic study with the help of scalpel and forceps; and individually weighed (gm) for each individual (electrical weighing measure). All kind of abnormalities were also observed. The color of the samples was recorded by eye estimation. Length, width and thickness of the bursa were measured by slide calipers. Unit of length, width and thickness measurement was millimeter (mm).

Tissue processing and staining

After collecting gross data, the bursa was preserved in Bouin's fluid and was processed for paraffin wax embedding. The sample was dehydrated by using ascending grade of alcohol i.e., 70%, 80%, 90%, 100% (1), 100% (2) and 100% (3) alcohol for 2 hours in each. Clearing was achieved through three changes of xylene for 2hoursin each; and infiltration with three changes of paraffin wax for 30 minutes in each. Sections were cut at 6µm thickness with a rotatory microtome. The sections were stained by Harris's Haematoxylin and 1% Eosin Y (H and E) stain. The sections were protected by a thin cover slip attached to the slide with a mounting medium "DPX".

Photography and illustration

Necessary photography was done during gross morphological and histological investigation for better illustration of the result. The gross anatomical pictures were taken directly from the organs by using digital camera and the histological pictures were taken using Carl-Zeiss photomicroscope. The measurement of histological parameters was done using pre-calibrated ocular micrometer.

Statistical analysis

All the collected data were then analyzed using Statistical Package for the Social Sciences (SPSS; version 22.0) software and disrobe the results in graphical form. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Duncan's test. Results were expressed as mean ± standard error (SE). The differences were considered statistically significant when the *p* values were less than 0.05 [21, 22].

RESULTS

Gross anatomy

The bursa of Fabricius of Sonali chicken was an asymmetric, lympho epithelial organ unique to class aves. The organ was a sac like diverticulum of the proctodeum situated on the dorsal wall of cloaca (Figure. 1 A). In the gross observation of bursa, it was found that the organ appeared as a smooth, globular to oval structure with slight anterior and posterior compression. The color of the organ was yellowish to milkish white (Figure 1 A).

The bursa consists of a wall surrounding a small, axial, main cavity. The central lumen of the organ was found to a great extent obscured by the presence of plicae, long mucosal folds of bursal wall, which resemble villous projections (Figure 1 B). The numbers of mucosal folds were found around 15-20 throughout postnatal stages of development.

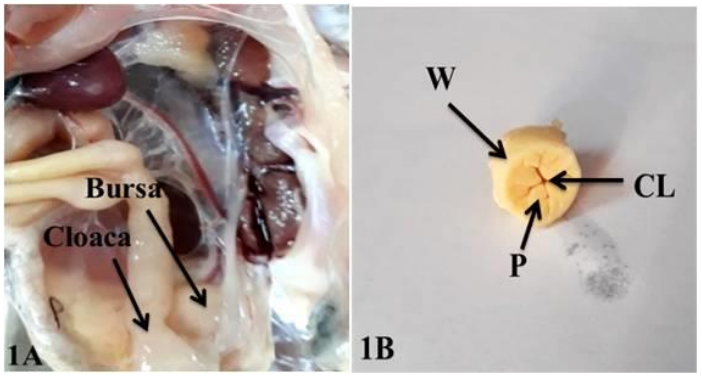

Figure 1. A: Gross photographs of the bursa of Fabricius showing located on dorsal aspect of cloaca; B: central lumen (CL) obscured by plicae (P), producing from the bursal wall (W).

Weight

The changes of average weight of bursa during the experimental period showed that the average weight gain was significantly (p<0.001) increasing according to age. At Day 1, the average weight was measured 0.105 ± 0.005 gm, at Day 14 was 0.205 ± 0.028 gm, at Day 28was 0.205 ± 0.054 gm, at Day 42 was 1.164 ± 0.102 gm and at Day 56 was found 1.550 ± 0.202 gm (Figure 2).

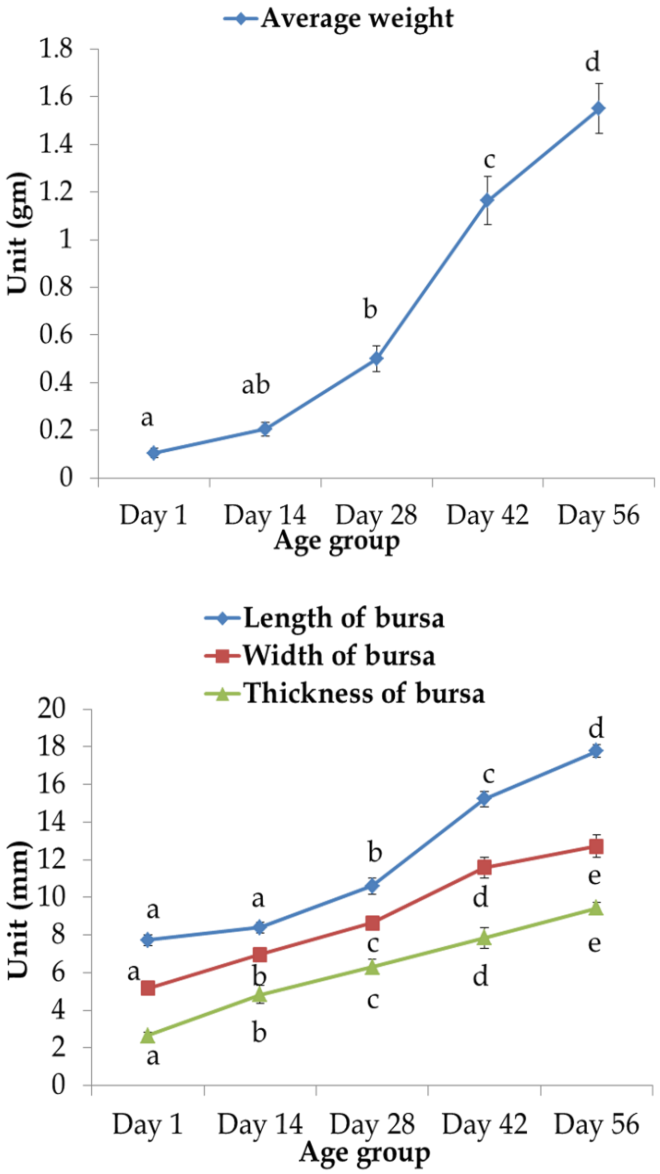

Figure 2: Gross anatomy (average weight; length, width and thickness) of the bursa of Fabricius among different groups, mean ± standard error. **Values with different letter (a,b,c,d,e) within the same line differ significantly (p<0.001).

Length, width and thickness

The length of bursa among different groups were found 7.720 ± 0.102 mm, 8.400 ± 0.293 mm, 10.600 ± 0.443 mm, 15.220 ± 0.292 mm and 17.780 ± 0.250 mm from Day 1 to Day 56, respectively. The maximum length was obtained at Day 56.Like length, the width of bursa was found increasing throughout the study period. It was found that the width was 5.160 ± 0.093 mm at Day 1, 6.960 ± 0.151 mm at Day 14, 8.640 ± 0.181 mm at Day 28, 11.580 ± 0.543 mm at Day 42and 12.720 ± 0.604 mm at Day 56. In sonali chicken the thickness of this organ was measured 2.640 ± 0.186 mm, 0.840 ± 0.470 mm, 6.300 ± 0.401 mm, 7.840 ± 0.549 mm and 9.440 ± 0.291 mm at different postnatal stages. From the statistical analysis, it was obtained that the length, width and thickness of bursa increased significantly (p<0.001) as the age increases (Figure 2).

Histological observations

At the day of hatching, the bursa of sonali chicken was well-developed. Bursa was composed of tunica mucosa, tunica muscularis and tunica serosa. Tunica mucosa was thrown into longitudinal folds (plicae) lined by pseudostratified columnar epithelium and the lumen of bursa was obliterated with these mucosal folds (plicae) of different length and thickness (Figure 3A-a). The result impression suggest that the lamina propria within the plicae had lymphatic follicles of the round, oval or polyhedral shape separated by connective tissue. All the follicles had clear margin and separated from each other by connective tissue. Cortex and medulla was not well differentiated in all the lymphatic follicles. The tunica muscularis layer was thin. Amount of connective tissue was very minimal inside the core of the plicae. Within the tunica mucosa layer, intraepithelial lymphocyte was found at this stage of development (Figure 3A-b).

At Day 14 post hatch, the size of plicae increased along with the size and number of bursal follicle within the plicae (Figure 3B-a). Each follicle had clearly defined cortex and medulla with a marked increase in follicular cell densities. The capillary layer between the cortex and medulla was distinct (Figure 3B-b). At Day 28, the plicae were very tall and the size and shape were not uniform. All the lymphatic follicles were not in same size and shape. Lymphoid follicles were oval, almost round or elongated (Figure 3C-a). The lymphoid follicles had clear cut margin and separated from the adjacent follicles by connective tissue fibers. With high power magnification, it was found that the capillary layer was prominent among cortex and medulla. The connective tissue core among the bursal follicles was thicker than day 1 (Figure 3C-b). There were no significant variations in developmental changes observed for days 14 and 28. The changes found were age related development.

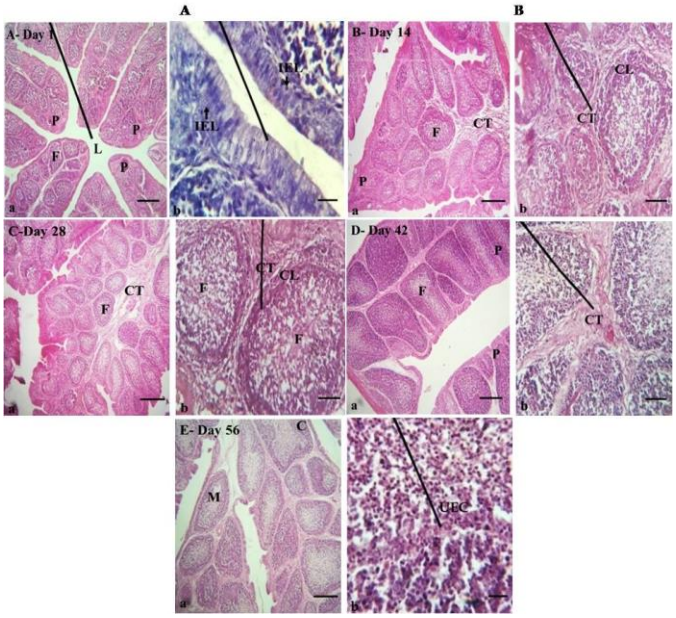

Figure 3: Histological architecture of bursa among different groups of postnatal stages. A-Day 1, a:Plicae with follicles,(X10), b:IEL in lining epithelium,(X100); B-Day 14, a:Size of both plicae and follicle increased,(X10), b:CT between follicles and CL between cortex and medulla of follicle,(X40); C-Day 28, a:Plicae and follicle(X10), b:CT and CL, (X40); D-Day 42, a:Large sized follicle within plicae,(X10), b:CT,(X40); E-Day 56, a:Reduction of medullary lymphocyte,(X10), b:UEC in capillary layer,(X100). C-Cortex, M-Medulla, P-Plicae, F-Follicle, L- Lumen, IEL-Intraepithelial lymphocyte, CL-Capillary layer, CT-Connective tissue, UEC-Undifferentiated epithelial cell. H & E stain. Scale bar: 5 µm (X10), 1 µm (X40) and 0.5 µm (X100)

At Day 42 there was further increase in follicle size and cell densities with the cortex still more densely populated than the medulla. All histological parameters increased than the previous groups (Figure 3D-a). Follicles separated by connective tissue fibers and space become more distinct (Figure 3D-b). At Day 56, the bursa was characterized by the presence of tall and thick plicae. Each plica consisted of large sized and polyhedral, almost square or elongated shaped follicles with dark stained cortex and lighter medulla. At this stage the density of medullary lymphocytes within some bursal follicles found to be decreased than the previous groups (Figure 3E-a). The thickness of the outer tunica muscularis was greatly increased. Undifferentiated epithelial cells at capillary layer were more distinct (Figure 3E-b).

Number of follicles per plicae

The histological architecture of bursa in different groups revealed that the number of follicle per plica was found to be gradually increasing along with the increase of age (p>0.05). It was found 20.20 ± 2.557 at Day 1, 25.60 ± 4.082 at Day 14, 28.20 ± 3.455 at Day 28, 31.00 ± 7.314 at Day 42 and35.80 ± 6.216 at Day 56 (Figure 4).

Length and breadth of plicae

The length of plicae at Day 1 was measured 1391.20 ± 27.383 µm, at Day 14 was 1861.20 ± 88.196 µm, at Day 28 was measured 2662.60 ± 181.065 µm, at Day 42 was 3181.60 ± 132.456 µm and at Day 56, it was increased to 3685.00 ± 160.803 µm. Similarly, the breadth of plicae also increased throughout the whole study period. The breadth of plicae was found 445.40 ± 30.016 µm, 700.00 ± 43.677 µm, 944.28 ± 69.901 µm, 1129.00 ± 63.842 µm and 1194.60 ± 33.591 µm from Day 1 to Day 56, respectively. Both the parameters were developing significantly (p<0.001) (Figure 4).

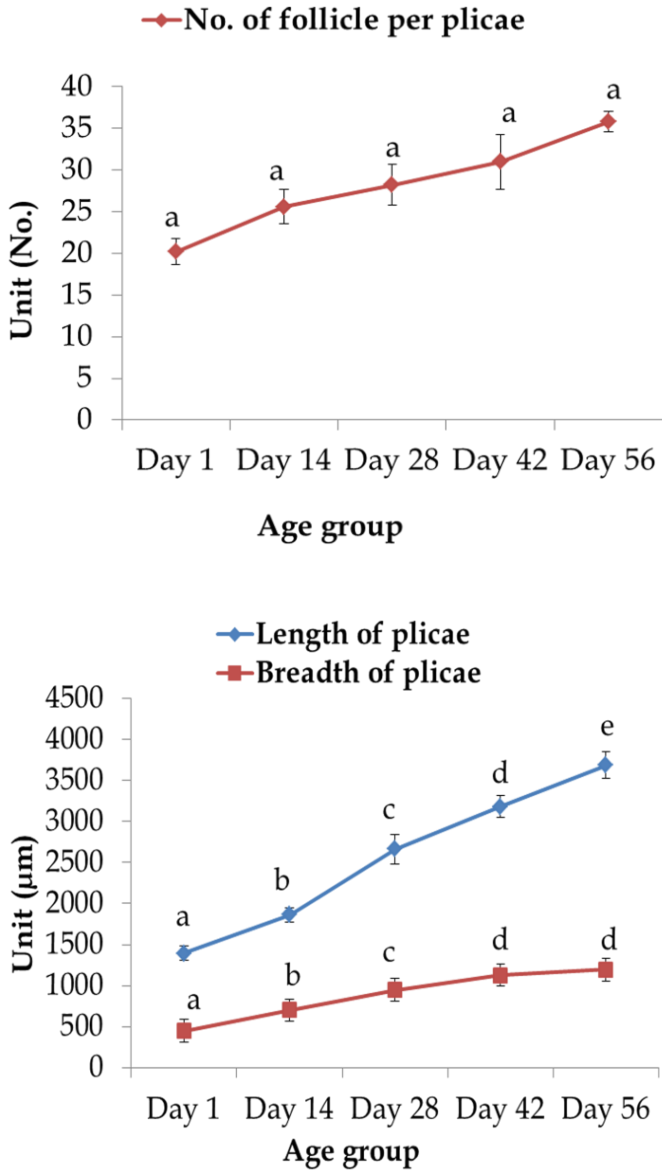

Figure 4: Analysis of no. of follicles per plicae and length and breadth of plicae; mean ± standard error. **values with different letter (a,b,c,d,e) within the same line differ significantly (p<0.001).

Length and breadth of follicle

In case of follicle of bursa of sonali chicken, the length was measured 185.20 ± 14.665 µm at Day 1, 210.10 ± 19.212 µm at Day 14, 337.10 ± 27.434 µm at Day 28, 989.32 ± 39.525 µm at Day 42 and 1194.60 ± 33.591 µm at Day 56. The breadth of plicae at different stages of postnatal development was measured developing gradually: 113.00 ± 12.401 µm, 199.80 ± 19.568 µm,237.60 ± 26.875 µm, 314.80 ± 37.101 µm and 327.90 ± 32.311 µm. (p<0.05) (Figure 5).

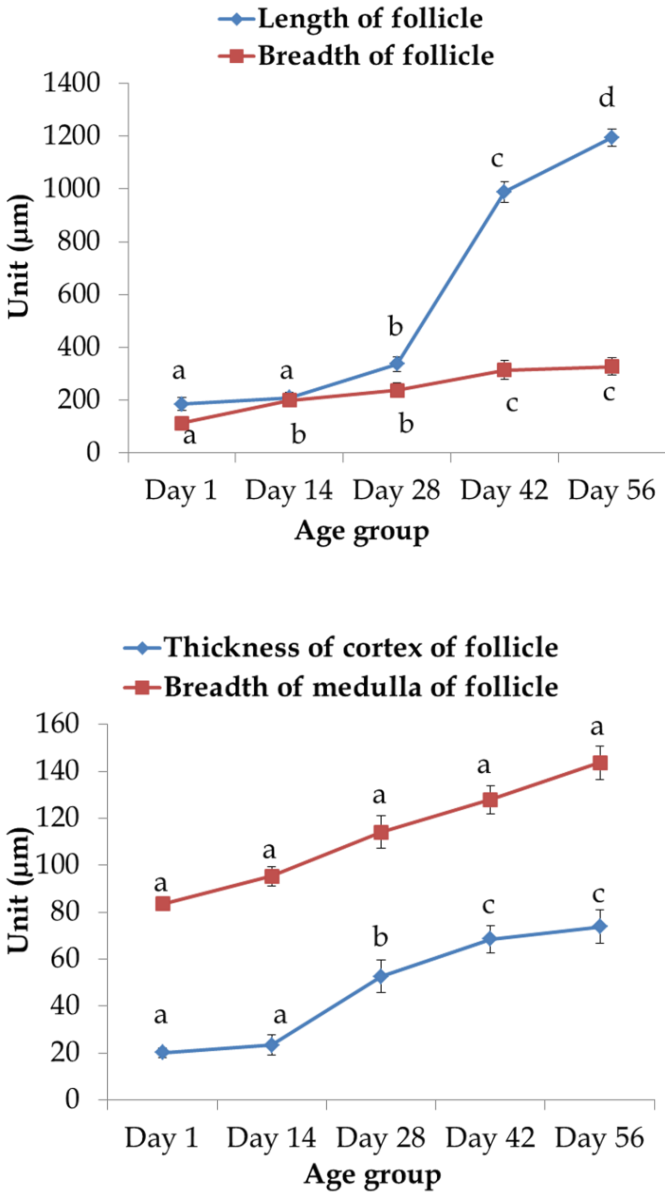

Figure 5: Analysis of length and breadth of follicle and thickness of cortex and breadth of medulla of follicle of bursa; mean ± standard error. **Values with different letters (a,b,c,d) within the same line differ significantly (p<0.05).

Thickness of cortex and breadth of medulla of follicle

The thickness of cortex of bursal follicle gradually increased through the whole study period. The thickness at Day 1 was measured 20.06 ± 1.157 µm, at Day 14was 23.40 ± 4.298 µm, at Day 28was 52.64 ± 6.841 µm, at Day 42 was 68.50 ± 5.877 µm and at Day 56 it was increased 73.82 ± 8.146 µm. In case of breadth of medulla, they were found 83.46 ± 19.108 µm, 95.22 ± 12.954 µm, 114.10 ± 22.906 µm, 127.84 ± 9.235 µm and 143.58 ± 10.145 µm for D_1, D_{14}, D_{28}, D_{42}, D_{56}, respectively. The age related development of these two parameters found to be statistically significant (p<0.05) (Figure 5).

Height of lining epithelium and thickness of tunica muscularis

The height of lining, pseudostratified columnar epithelium was measured 18.94 ± 6.409 µm at Day 1, 21.20 ± 4.705 µm at Day 14, 36.62 ± 7.289 µm at Day 28, 45.24 ± 6.673 µm at Day 42and 56.32 ± 9.608 µm at Day 56. The thickness of tunica muscularis was found to be increasing gradually. It was measured 28.44 ± 2.723 µm, 33.62 ± 2.471 µm, 45.60 ± 7.011 µm, 64.38 ± 7.833 µm and 87.74 ± 6.304 µm at postnatal stages (Figure 6).

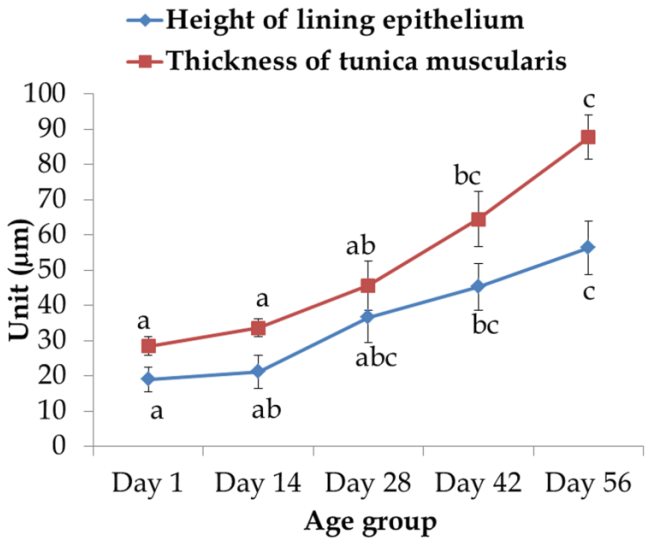

Figure 6: Analysis of height of lining epithelium and thickness of tunica muscularis of bursa; mean ± standard error. **Values with different letter (a,b,c,d) within the same line differ significantly (p<0.05).

DISCUSSION

The current experiment explored that the bursa of Fabricius of sonali chicken appeared as a sac like diverticulum of the proctodeum situated on the dorsal wall of cloaca. The shape of the organ appeared as a smooth, globular to oval with slightly anterior and posterior compression. These findings were in

agreement with the findings of [23] in deshi chicken of Bangladesh, [9] in broiler chicken, [10] in CARI shyama and vanaraja breed of poultry, [11] in broiler chicken, [22] in broiler chicken of kelantan, [4] in quail birds.. The color of bursa of Fabricius of sonali chicken was yellowish to milkish white and it was found similar with the finding of [24] in indigenous ducklings.

The average weight of bursa of sonali chicken was revealed gradually increasing with the increase of age of chicken. So, it can be said that the weight gain of bursa depends on the age. This result was similar to the findings of [23] in deshi chicken. The present experiment also revealed that the length, width and thickness of bursa also increase according to age. This result was in agreement with [23] and [22]. It is revealed [11] that the size and the weight of the Bursa of Fabricius reach their maximum between the 10th and the 11th weekend the complete regression of the Bursa appears clearly to the 27th week and remains in the fibrous state in adult broilers. Previous report [8] stated that the involutory changes started at the age of two months in both the sexes in Guinea Fowl. But in bursa of Fabricius of sonali chicken no sign of involution found in the morphometric development up to day 56. Further study required to detect the age of involution in sonali chicken in Bangladesh.

The microscopic observations on the general structure of bursa of Fabricius of sonali chicken were found similar to the other literatures stated before [8, 25, 26,27]. Bursa of Fabricius was composed of tunica mucosa, tunica muscularis and tunica serosa. Tunica mucosa was thrown into longitudinal folds (Plicae). Each plica containing many lymphatic follicles with distinct cortex and medulla. Follicles were separated from each other by connective tissue fibers and cells. All the histological structures present at day 1 become well-developed and more prominent as age increases.

The lining epithelium of mucosal folds or plicae was pseudostratified columnar epithelium which agree with [9] in broiler chicken, [27] in domestic fowl, [22] in broiler chicken, [28] in turkey, [4] in quail birds, [29] in kedaknath breed. But [25], reported about the surface epithelium of simple columnar in chicks. This variation in lining epithelium may be due to breed variation. Intraepithelial lymphocytes were present at the lining epithelium which indicates that the bursa was functional for defense mechanism at hatching day.

Literature [8] said that the involutary signs appeared in bursa of Fabricius in Guinea Fowl at two months of age in both sexes and the signs reported were depletion of lymphocytic population from periphery of the cortex and medulla, separation of cortex from adjacent follicles, severe fatty changes in the sub epithelial and inter follicular connective tissue and formation of epithelial cysts, in later stages of involution, the height of the surface epithelium was reduced, with only vacuolated structures being visible in the areas of epithelium. But in current experiment up to Day 56 no significant histological involutary signs were present apart from the decrease of medullary lymphocyte in some bursal follicle.

But the histological involution started at 11th week of age in broiler chicken [11]. From the present study result, it can be estimated that the number of follicles per plicae, length of plicae, width of plicae, length of follicle, width of follicle, height of lining epithelium and thickness of tunica muscularis underwent age dependent changes. With the advancement of age, all this histological structures were becoming more developed and increasing in size up to Day 56.

CONCLUSIONS

The growth and development of bursa revealed that histological modifications are well correlated with the morphometrical changes of bursa. In both anatomical and histological observations it was noticed, all the parameters measured were found significantly increased from day 1 to day 56. And no significant involutary signs were present. From these points, it can be concluded that the growth and development of bursa of sonali chicken was age-related. Moreover, further study required to detect the age of involution.

ACKNOWLEDGEMENT

The research work was supported with the grants from the Ministry of Science and Technology (NST fellowship, 2017-18), Bangladesh. Grant number [1005/499].

AUTHOR CONTRIBUTIONS

Shonkor Kumar Das designed the experiment. Ummay Ayman carried out the experiments, analyzed the data and wrote the initial draft of the manuscript. Shonkor Kumar Das and Md. Rafiqul Alam critically revised the manuscript and finalized the manuscript.

CONFLICTS OF INTEREST

The author declares that no conflict of interest exists.

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