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Original Research Article

MORPHOLOGICAL EVALUATION OF DEVELOPING BURSA OF FABRICIUS IN PRE- AND POST-HATCH NIGERIAN INDIGENOUS CHICKEN

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ABSTRACT

The pre- and post-hatch development of the bursa of Fabricius in Nigerian indigenous chicken of south eastern region was studied from embryonic incubation day (EID) 10 to day (D) 42 post hatch. The bursa increased significantly in weight between D 0, 14 and 42. The relative weight only increased significantly between D 0, D 7 and D 42. The bursa was oval in shape at EID 10, EID 18 and all through the post hatch periods, but slightly elongated at EID 14. The colour of the bursa was relatively pale during the embryonic and post hatch periods. At EID 10 there was full differentiation of the bursal wall with the mucosa already thrown into longitudinal folds and the submucosa infiltrated by some bursal cells. Increase in bursal cell density was apparent at EID 14 with development of bursal follicles having homogenous cell distribution at EID 18. At hatch (D 0), follicles with distinct cortical and medullary regions emerged. Plasma cells and some mitotic figures were observed at D 7. At D 14, D 28 and D 42 bursa possessed follicles with high concentrations of immunocompetent lymphocytes and plasma cells.

Keywords: bursa of fabricius, indigenous domestic fowl, pre-hatch, post-hatch, morphology, development

INTRODUCTION

The importance of indigenous chicken for rural economy in the developing and underdeveloped countries mostly Asia and Africa cannot be overemphasized. They are indeed part of balanced system that has vital roles in the rural households as source of high quality animal protein and emergency cash income (Mtileni *et al*., 2016). Globally, disease has been identified as one of the major constraints to poultry production (Fathi *et al*., 2016). Although the indigenous chicken ecotypes in the tropics have very low egg and meat production potentials (Desie *et al*., 2011), the predominant indigenous chicken breeds kept by smallholder rural communities are selected naturally or by the farmers who keep them mainly for their adaptive traits rather than their production performance (Moreki *et al*., 2010; Negassa *et al*., 2014). For a long time, genetic selection for disease resistance to major infectious agents in chicken has been recognized as an adjunct to non-genetic means of disease control (Boa-Amponsem *et al*., 1997; El-Safty *et al*., 2006; Chatterjee *et al*., 2007; Fathi *et al*., 2016). The indigenous breeds of chicken have been shown to be more resistant to diseases and also excellent gene reservoirs, particularly for those genes that have adaptive values (Minga *et al*., 2004; Egahi *et al*., 2010; Melesse *et al*., 2011).

The ability of organisms to resist disease is a fundamental responsibility of a defense mechanism controlled by the lymphoid system. This system is composed of several lines of defense to prevent pathogen entry and subsequent infection (Corton *et al*., 1998; Erf, 2004, 2007). The avian lymphoid system is structurally divided into two distinct components namely, the primary or central and the

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secondary or peripheral components (Firth, 1977). The primary or central component comprises the thymus and the bursa of Fabricius in which lymphocytes that are responsible for cell mediated and humoral immune responses respectively develop (Ratcliffe, 1989; Silverstein, 2001; Ciriaco *et al*., 2003). The secondary or peripheral component includes all areas of normal lymphocytic aggregation or proliferation outside the central component. This includes the spleen and all mucosa associated lymphoid tissues (MALT) such as the caecal tonsil (Firth, 1977; Davison *et al*., 2008; Islam *et al.,* 2012). The bursa of Fabricius has been found to be well developed in sexually immature birds, but undergoes regression or involution at the onset of sexual maturity (Glick, 1956; Payne, 1971). However, several studies had shown that the age of commencement and completion of involution varies considerably among avian species.

The developmental processes, gross features and histological structures of the bursa of Fabricius have been widely studied in many avian species, however; there is still insufficient information on the pre- and post-hatch development of this organ in Nigerian indigenous chicken. Hence, this study is aimed at evaluating the age-related morphological changes in the bursa of Fabricius of Nigerian indigenous chicken as it develops in both pre and post-natal phases of development.

MATERIALS AND METHOD

A total of fifteen chick embryos and twenty five post hatch chicks of either sex were used for the study. However, one hundred and twenty indigenous chicken eggs were acquired from apparently healthy laying native chickens raised by free range backyard method in Ovim community, Isuikwuato Local Government Area of Abia State, Nigeria. The eggs were incubated in an electric egg incubator at 37°C and 55 – 60% relative humidity (Yoshimura *et al*., 2009; Oznurlu *et al*., 2010).

Five chick embryos were harvested on Embryonic Incubation Day (EID) 10, 14 and 18 by cracking the gravid egg shell around the vertical midline using a knife, and the transverse diameter was cut using a surgical scissors to expose the embryo. The yolk sac was detached from the embryo by severing the stalk and foetal debris was removed. The weights of embryos were determined on SCOUT PRO – 200 X 0.001g (OHAUS Corporation) analytical balance. The remaining eggs were left to hatch for the post-hatch studies. Following hatching, the chicks were housed in a deep-liter pen in the poultry unit of the College of Veterinary medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The chicks were fed commercially compounded feed (Topfeed^{R}, broiler chick mesh) and water was given *ad libitum.* No medications including vaccination were given throughout the period of study.

Five randomly selected chicks were sacrificed at hatch or day 0 (D 0), D 7, D 14, D 28 and D 42 post-hatch by inhalation anaesthesia using chloroform soaked in cotton wool in a lid plastic container after the live body weights have been determined using either SCOUT PRO – 200 X 0.001g (OHAUS Corporation) analytical balance (for weights < or = 200g) and MEASURE-TECH, MB 2610 triple beam balance (for weights > 200g). The bursa of Fabricius of both embryos and post hatch chicks was collected by ventral abdominal dissection using the approach of Alboghobeish and Mayahi (2003), weighed and examined for gross features.

HISTOLOGICAL INVESTIGATION

Slices of the bursa of different ages were fixed in Bouin’s fluid (75% picric acid, 20% acetic acid and 5% formaldehyde) and transferred to 70% ethanol after 24 hours to avoid excess tissue hardening. The specimens were processed by placing them in ascending grades of ethanol in the following order, 70% for 1 hour, first 95% ethanol for 1 hour and second 95% ethanol for 1¼ hours, first absolute ethanol for 1½ hours, second absolute ethanol for 2 hours and third absolute ethanol for 2 hours to ensure proper dehydration of the tissues. It was then transferred to mixture of equal volumes of ethanol and xylene where it was left overnight. It was later cleared in two changes of xylene for 1 hour each. It was then infiltrated for 1 hour with molten paraffin wax (salsa wax) in the oven at 60°C. The tissues were embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5□m thickness.

The sections were floated in floating-out bath from where it was picked with clean albuminized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene, hydrated by descending grades of ethanol in the following order- absolute ethanol, 95% ethanol and 70% ethanol for 2 minutes each. The sections were stained with Ehrlich hematoxylin for 15 minutes, and then washed in water for 5 minutes, differentiated in 1% acid alcohol for 3 seconds, and blued in running tap water for 10 minutes. It was then counter stained with eosine for 2 minutes. Excess eosine was removed in ascending grades of ethanol in the following order- 75% ethanol, 95% ethanol and absolute ethanol for 2 minutes each. It was then cleared in two changes of xylene and cover-slipped with Depex mountant.

The slides were viewed under a light microscope (BestSco, e, China) and selected images were captured using Moticam 2.0 digital camera attached to a computer.

STATISTICAL ANALYSIS

Data on weight were subjected to one way analysis of variance (ANOVA). Variant means were separated using Duncan's multiple range tests. Significance was accepted at $p < 0.05$.

RESULTS

The body weights of embryos and post hatch chicks increased with age, but only varied significantly ($p<0.05$) between EID 10 and EID 18 (Table I). Post-hatch results on bursal weight showed that bursa of Fabricius progressively increased in weight from D 0 to D 42. During these periods, the mean weights of bursa of Fabricius were 0.03±0.002g and 0.61±0.08g at D 0 and D 42 respectively. The mean bursal weight only increased significantly ($p < 0.05$) between D 0, D 14 and D 42. The mean relative weight of bursa increased between D 0, D 7 and D 14 but decreased slightly at D 28 and increased again by D 42. At D 0 and D 42 the mean relative weights were 0.13±0.01% and 0.36±0.02% respectively, but within this age bracket, the mean relative weights only increased significantly ($p < 0.05$) between D 0, D 7 and D 42 and the maximum mean relative weight of 0.36±0.02% was attained at D 42 (Table II).

Table I: Absolute Body Weight of chicks at various ages

Age of Animal (day)	Weight of Animal (gram)
EID 10	2.82 ± 0.39^{a}
EID 14	7.40 ± 0.51^{a}
EID 18	22.73 ± 1.05^{bc}
0	27.23 ± 0.38^{c}
7	43.98 ± 1.34^{d}
14	66.89 ± 0.06^{e}
28	90.12 ± 5.06^{f}
42	168.59 ± 0.2^{g}

*Results with different superscripts in the column are significantly different at $p< 0.05$

GROSS OBSERVATIONS

At embryonic incubation day (EID) 10, the bursa in-situ was almost inconspicuous due to its small size and poor differentiation from the surrounding tissues. However, following dissection the bursa was observed to be oval in shape and pale in colour (Figure I).

At EID 14, the bursa remained pale in colour but slightly elongated in shape. The size in-situ at this stage of development was fairly significant although, still partly covered by the surrounding tissues (Figure II). At EID 18, there was pronounced increase in size of the bursa as it could be identified without altering the surrounding structures. The shape and colour were oval and pink respectively (Figure III). At hatch (D 0), there was further increase in size of the bursa, but the shape and colour were still oval and pink respectively as observed in embryos of day 18. At D 7 post hatch the bursa still appeared oval in shape but relatively pale in colour. The bursae of D 14, 28 and 42 were morphologically similar to the bursa of D 7 both in shape and colour, but the size varied significantly with age (Figure IV).

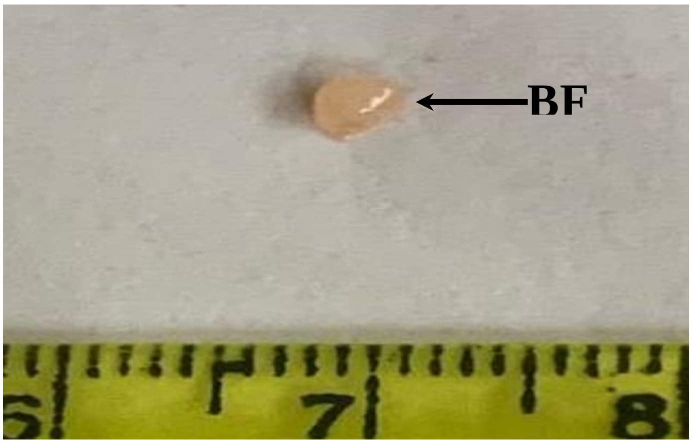

Figure I: Gross photograph of a dissected bursa of Fabricius, BF at embryonic incubation day (EID) 10, Note the shape and colour of the organ.

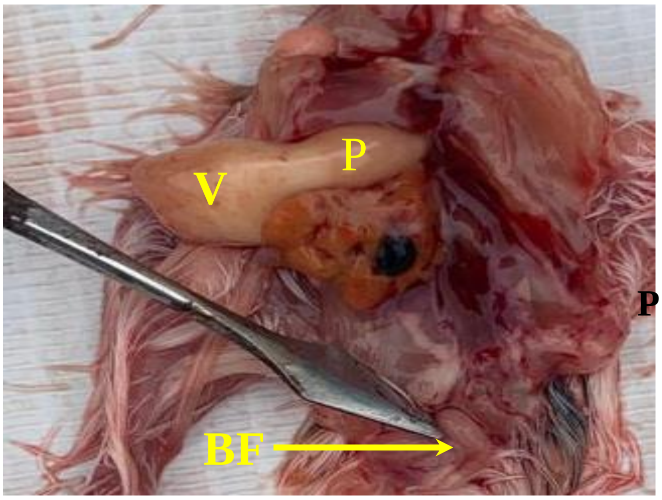

Figure II: Gross photograph of a dissected section of an embryo at embryonic incubation day (EID) 14 showing the abdominal viscera. Note the colour and shape of the bursa of Fabricius (metallic arrow). P: proventriculus; V: ventriculus

HISTOLOGICAL OBSERVATIONS

At Embryonic Incubation Day (EID) 10, the wall of the bursa of Fabricius had apparently differentiated into four layers that comprised an outermost serosa, the tunica muscularis, the submucosa and the innermost tunica mucosa that had already formed irregular longitudinal folds in the bursal lumen (Figure V). The mucosa was composed of pseudostratified columnar epithelium and the cores of its folds contained cells suspected to be predominantly lymphocytes and some plasma cells in a network of loose connective tissue (Figure VI). By EID 14, some mucosal folds which had apparently increased in height and thickness interconnected with opposite folds to form a single fold across the bursal lumen (Figure VII). The submucosal cell density presumed to be predominantly lymphocytes increased conspicuously and in some embryos the bursa possessed spherical shaped aggregates of bursal cells surrounded by a layer of cells resembling the corticomedullary arch forming cells (Figure VIII).

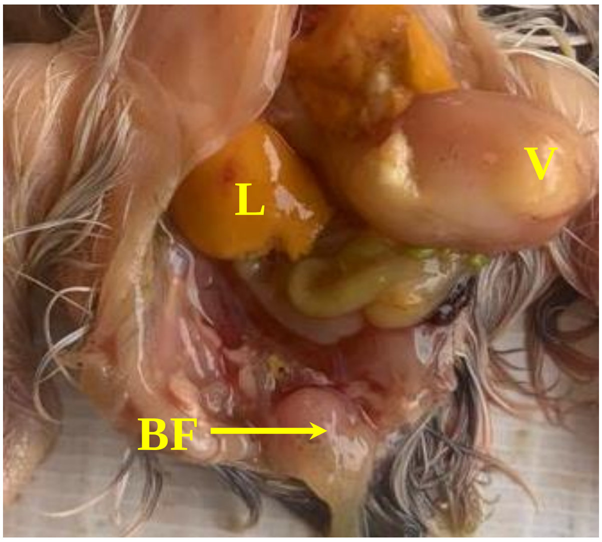

Figure III: Gross photograph of a dissected section of 18 days old embryo showing the abdominal cavity containing some visceral organs. Note the shape and colour of the bursa of Fabricius, BF. V: ventriculus; L: liver

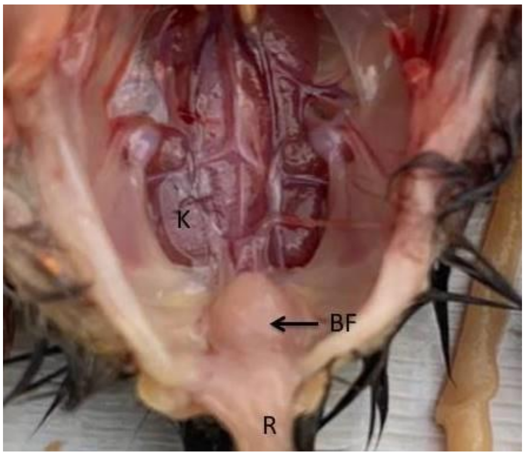

Figure IV: Gross photograph of a dissected section of a chick at D 7 post-hatch showing the abdominal cavity containing some abdominal viscera. Note the bursa of Fabricius, BF. R: rectum; K: kidney.

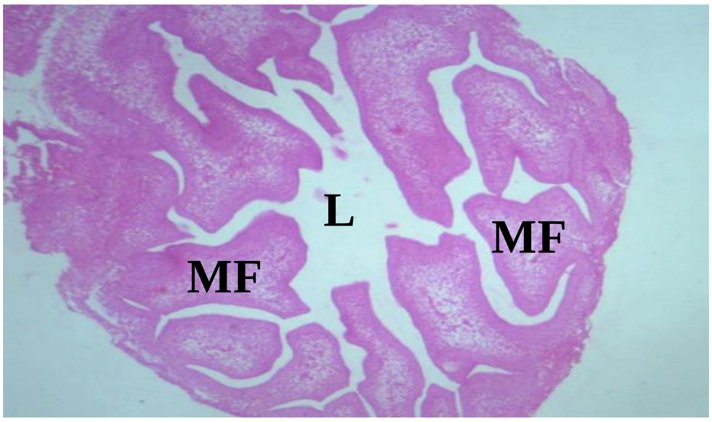

Figure V: Photomicrograph of a transverse section of the bursa of Fabricius at EID 10 showing the differentiated bursal wall. Note the mucosal folds, MF of different dimensions within the bursal lumen, L (Haematoxylin and Eosin stain; X40).

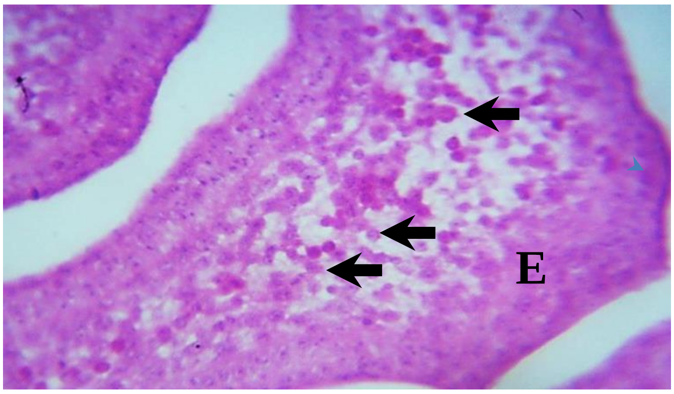

Figure VI: Photomicrograph of a transverse section of the bursa at EID 10 showing a plica. Note the bursal cells (arrow) diffusely distributed in a connective tissue network. E: pseudostratified columnar epithelium (Haematoxylin and Eosin stain; X400).

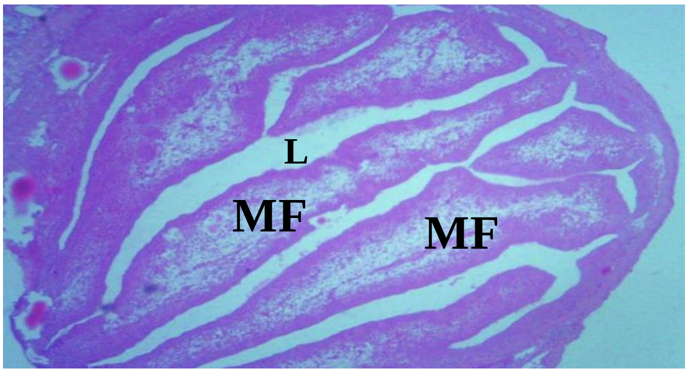

Figure VII: Photomicrograph of a transverse section of the bursa at EID 14 showing the bursal plicae. Note the tall interconnected mucosal folds, MF running across the bursal lumen, L (Haematoxylin and Eosin stain; X40).

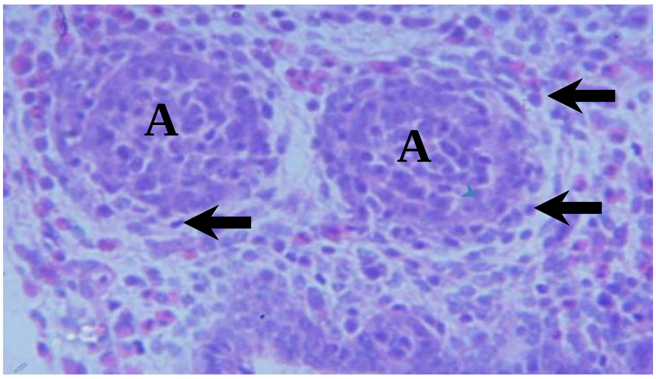

Figure VIII: Photomicrograph of a transverse section of the bursa of fabricius at EID 14 showing the submucosal cell density. Note the aggregates of bursal cells, A surrounded by epithelial-reticular-like cells (arrow) (Haematoxylin and Eosin stain; X400).

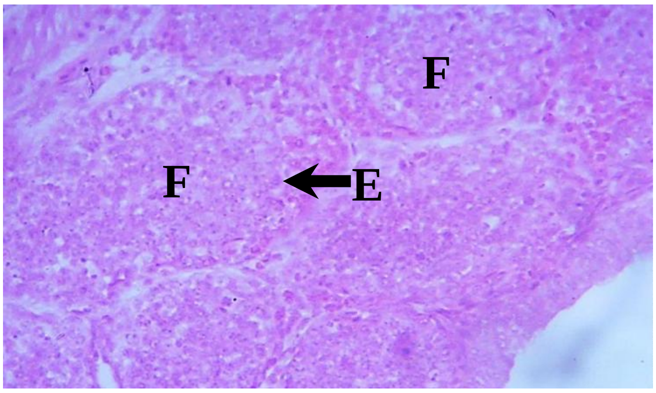

Figure IX: Photomicrograph of a transverse section of the bursa of Fabricius at EID 18 showing a plica. Note the follicles, F containing a population of homogenously distributed lymphocyte. E: pseudostratified columnar epithelium (Haematoxylin and Eosin stain; X400).

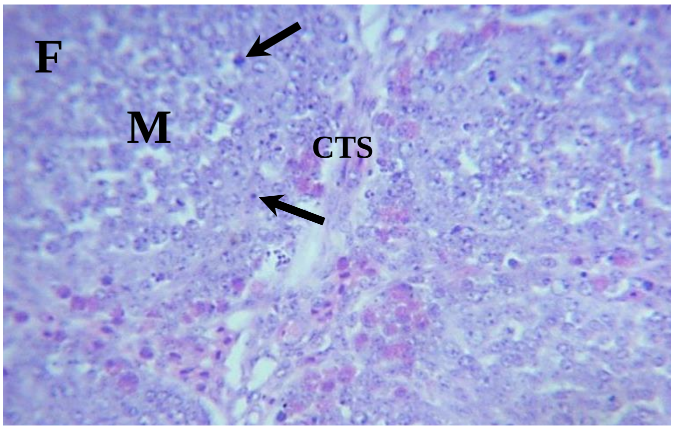

Figure X: Photomicrograph of a transverse section of the bursa of Fabricius at hatch (D 0) showing the parenchyma. Note the lymphocyte density and the follicles, F with fairly distinct cortex, C and medulla, M. CTS: connective tissue septa; ARROW: cortico-medullary border (Haematoxylin and Eosin stain; X400).

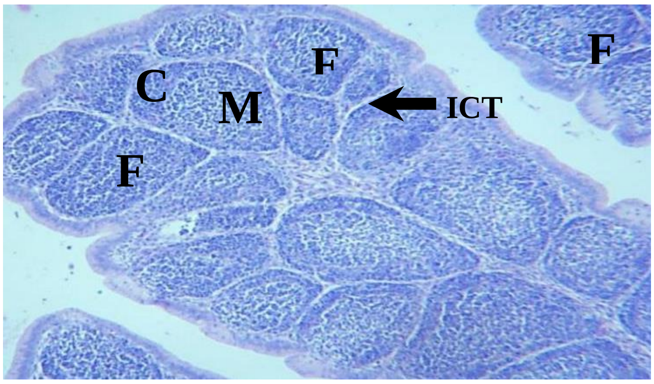

Figure XI: Photomicrograph of a transverse section of the bursa of Fabricius at D 7 post hatch showing a plica filled with follicles, F. Note the cortex, C and medulla, M of each follicle. ICT: interfollicular connective tissue (Haematoxylin and Eosin stain; X100).

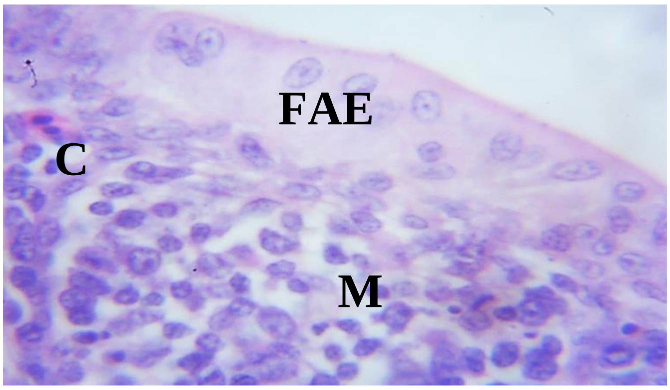

Figure XII: Photomicrograph of a transverse section of a bursal follicle at D 7 post-hatch. Note the follicle associated epithelium, FAE. C: cortex; M: medulla (Haematoxylin and Eosin stain; X1000).

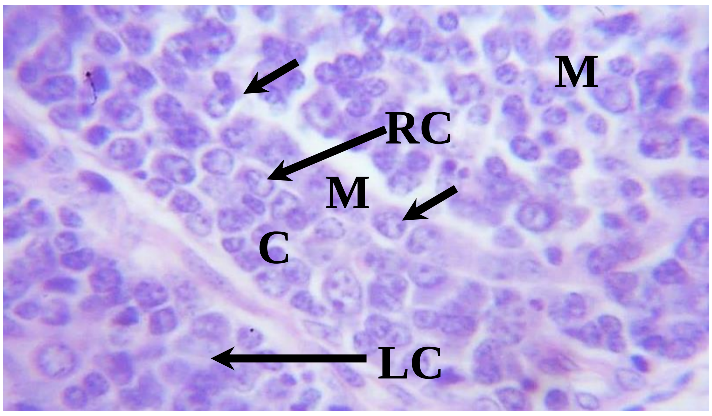

Figure XIII: Photomicrograph of a transverse section of bursa of Fabricius at D 7 post-hatch showing the parenchyma. Note the lymphocytes, LC; reticular cells, RC; corticomedullary arch forming cells (arrow). C: cortex, M: medulla (Haematoxylin and Eosin stain; X1000).

At EID 18, the mucosal folds further increased both in height and thickness. There was also remarkable increase in submucosal cell densities with landmarks of developing follicles apparent, and few registered follicles were composed of homogenously distributed bursal cells (Figure IX).

At hatch (D 0), the bursal cell density increased remarkably with a distinct increase in connective tissue fibre network within the submucosal tissue. Development of follicles with associated interfollicular connective tissue and differentiation of follicular cells into cortical and medullary regions equally improved (Figure X).

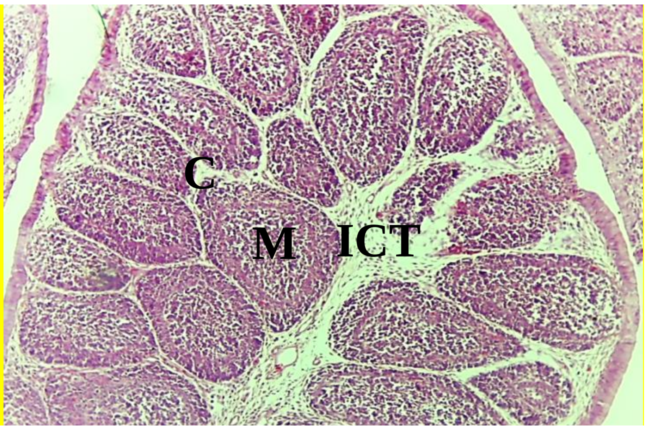

Figure XIV: Photomicrograph of a tranaverse section of bursa of Fabricius at D 14 post-hatch showing follicles, F. in a plica. Observe the morphology of the follicles and the thickness of interfollicular connective tissue, ICT. C: cortex; M: medulla (Haematoxylin and Eosin stain; X100).

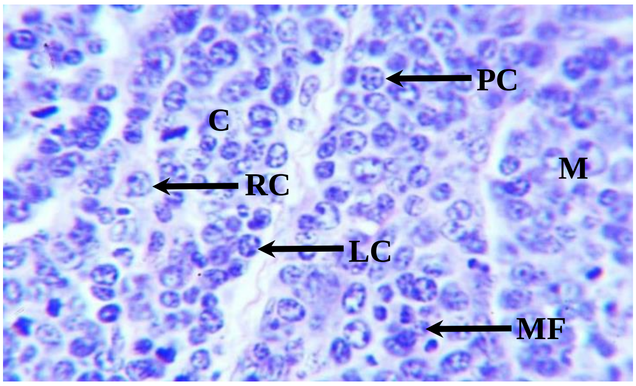

Figure XV: Photomicrograph of a transverse section of the bursa of Fabricius at D 28 post-hatch showing a bursal parenchyma. Note the bursal cell composition. LC: lymphocyte; PC: plasma cells and RC: reticular cells. MF: mitotic figure (Haematoxylin and Eosin stain; X1000).

By day 7 post hatch, bursa had developed follicles with very pronounced cortical and medullary regions and each follicle distinctly surrounded by interfollicular connective tissue (Figure XI). The bursal epithelium remained closely aligned pseudostratified columnar cells with apparent modification at its point of contact with the follicles. At this junction the epithelial cells appeared cuboidal in form with apically placed nuclei and the follicular cortex terminated at the point the follicle made contact with the epithelium, thereby creating a direct link between the follicle medulla and the bursal epithelium (Figure XII)

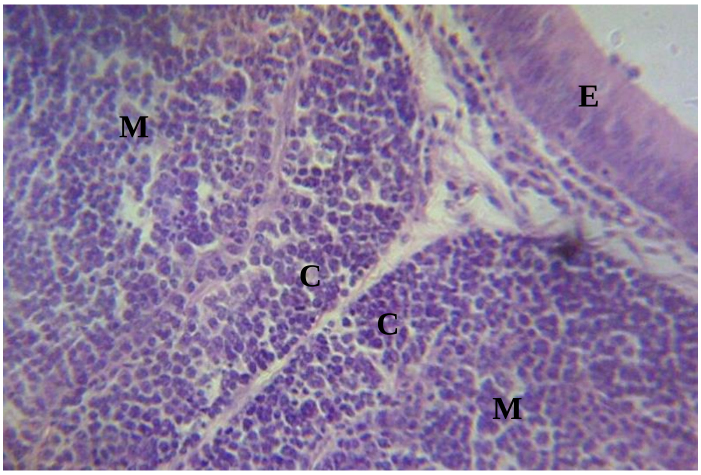

Figure XVI: Transverse section of the bursa of Fabricius at D 42 post hatch showing the follicles. Note the relationship between the cortical and medullary cell densities. C: cortex; M: medulla; E: pseudostratified columnar epithelium (Haematoxylin and Eosin stain; X100).

Table II: Weights (Absolute and Relative) of Bursa of Fabricius at various ages

Age of Animal (day)	Weight of Animal (g)	Weight of bursa (g)	Relative weight of bursa (%)
0	27.23±0.38^{a}	0.03±0.00^{a}	0.13±0.01^{a}
7	43.98±1.34^{b}	0.12±0.02^{ab}	0.28±0.04^{b}
14	66.89±0.06^{c}	0.22±0.01^{c}	0.35±0.02^{bc}
28	90.12±5.06^{d}	0.29±0.05^{c}	0.32±0.02^{bc}
42	168.59±0.72^{e}	0.61±0.08^{d}	0.36±0.02^{c}

Results with different superscripts in a column are significantly different at $p < 0.05$

Cells of both cortex and medulla apparently appeared similar and predominantly lymphocytes, but at the junction between the cortex and medulla were linearly arranged cortico-medullary arch forming cells that appeared to be seated on a basement membrane. Plasma cells were equally observed within the lymphocyte population in some follicles (Figure XIII).

At D 14 post hatch, the mucosal folds together with their associated follicles of different shapes increased conspicuously in size. The follicles with their dense concentration of immunocompetent lymphocytes still possessed apparent cortical and medullary compartments. The connective tissue strands within the epithelial folds especially the axial connective tissue and the muscle layer of the bursal wall remarkably increased in thickness (Figure XIV).

By day 28, the bursa appeared morphologically similar to those of D 14, although the follicles increased further in size and filled the cores of the plicae. Lymphocytes with some plasma cells and reticular cells remained the dominant cells of the follicles (Figure XV).

At day 42 post hatch the bursa relatively resembled those of day 28. However, the follicles further increased in size with their cell densities, but the difference in cell concentration between the cortex and medulla was not as obvious as in D 28 (Figure XVI).

DISCUSSION

The bursa of Fabricius in indigenous chicken appeared as a sac-like diverticulum of the proctodeum on the dorsal wall of the cloaca. The organ had a smooth surface and was oval in shape. These findings are in agreement with the findings of Khalil (2001) in deshi chicken of Bangladesh, Akter *et a*l. (2006) in broiler chicken, Ingole *et al.* (2010) in CARI shyama and vanaraja breed of poultry, Khenenou *et al.* (2012) in broiler chicken, Khan *et al*., (2014) in broiler chicken of Kelantan and Hassan *et al.* (2015) in quail birds. The present finding contrasted the reports ofthe bursa of Fabricius in pekin ducks which is elongated (Glick, 1963), in the goose which is cylindrical (Jolly, 1915), and in the guinea fowl which has varied in shape such that in one group, the bursa was reported to appear as an ovalsac,and in another group,has a pointed cranial blind end with bulging mid-section (Onyeanusi *et at*., 1993).

The colour of bursa of Fabricius in indigenous chicken from the present study was pale to milkfish-white. Sultana *et al.* (2011) and Ayman *et al.* (2020) report similar colour of bursa of Fabricius in indigenous ducklings and Sonali chicken of Bangladesh respectively

Histological investigations revealed that the bursa of Fabricius in indigenous chickens appeared structurally similar to those described for most avian species (Leena *et al*., 2012; Tamilselvan *et al*., 2017. The bursal wall was made up of 4 layers as recorded in other species. The muscular layer of the bursal wall was composed entirely of circularly oriented smooth muscle fibers as was observed in guinea fowl (Onyeanusi *et al*., 1993), but in the duck and goose the tunica muscularis consists of outer circular and inner longitudinal smooth muscle fibers (Gulmez & Aslan, 1998; Indu *et al*., 2005). The innermost layer, the mucosa which was thrown into folds was lined by predominantly pseudostratified columnar epithelium without goblet cells as observed in broiler chicken (Akter *et al*., 2006; Khan *et al*., 2014), domestic fowl (Chandrashekhar *et al*., 2012), quail (Hassan *et al*., 2015), in kedaknath breed of chicken (Kanasiya *et al*., 2018) and White Pekin duck (Indu *et al*., 2005), but contrary to the pseudostratified columnar epithelium with goblet cells in the guinea fowl (Onyeanusi *et al*., 1993), and goose (Gulmez & Aslan, 1999). Payal *et al.* (2010) observed an age variation in form of the bursal epithelium in Vanaraja and Cari breeds of chicken. According to these authors, the epithelium is simple columnar in chicks and layers, but pseudostratified columnar in growers.

The epithelium in indigenous chickens as observed from the study was composed of the more elaborate interfollicular epithelium (IFE) of pseudostratified columnar cells and the modified follicle associated epithelium (FAE) whose cells possess apically placed nuclei. The structural arrangement and the morphology of the follicle-associated epithelial cells suggest a modification to meet the functional requirement of trans-epithelial movement of substances from the follicles into the bursal lumen. However, Olah and Vervelde (2008) described a basal layer of cuboidal cells in the surface epithelium whose function according to the authors is unknown, but could be predestined interfollicular epithelial cells (IFEC), since the surface epithelium does not proliferate and no epithelial stem cell has been identified. In one of his reports, Hodges (1974) stated that there were three definitive types of surface epithelial cells in the chicken, which include some oval, round nucleated cells with PAS positive cytoplasmic granules suspected to be these basal cells, but their functions are as well not mentioned.

Variations were also recorded in the number and structure of the mucosal folds of the bursa. From the study, there were about 11-13 primary folds in the indigenous chickens. However, the immunological significance of the structural design and number of these mucosal folds has not been defined, but there could be a correlation between immune competence of the bursa of Fabricius and the number of folds; since the functional units (follicles) of the bursa are located within these folds, hence the higher the number of folds the more the number of follicles and the immune competent cells. Onyeanusi *et al.* (1993) recorded about 12-13 folds in the guinea fowl, while in the White Pekin duck, Indu *et al.* (2005) observed 2 large well-developed folds on the ventral aspect and about 5-6 smaller folds round the circumference, and in the goose, there are about 11-12 folds (Gulmez & Aslan, 1999).

The bursal follicles in Nigerian indigenous chickens consisted of a lymphocyte-dominated stroma that was differentiated into a peripheral cortex and central medulla within a connective tissue envelop. Some of these bursal follicles were in direct contact with the bursal epithelium, while others were completely surrounded by connective tissue. Gulmez & Aslan (1999) reported similar occurrence in the goose, but Olah & Velverde (2008) stated that each bursal follicle is attached to the epithelium through the follicle associated epithelium (FAE), such that the number of follicle associated epithelial attachments is identical to the number of follicles in the bursa. Therefore, the observation that some follicles are completely surrounded by connective tissue may have resulted from the plain of sectioning which might have excluded the points of attachment of these follicles to the epithelium. Honjo and Hirota (1993), Onyeanusi *et al.* (1993) and Khan *et al* (1998) also observed bursal follicles with cortical and medullary regions in other avian species.

The bursal weight from the study increased progressively with age during the 6 weeks under investigation. Within this period the maximum mean absolute weight was attained at day 42 post-hatch. Generally, increase in organ weight may involve the growth of both the primary functional tissues and their supportive components. In the case of bursa, increase in density of lymphocytes and other immune related cells may be considered as a growth of the functional components of the bursa, while development of structures like the connective tissue septae which do not impact directly on the immunological efficiency of the bursa of fabricius may be regarded as growth of supportive components. Therefore, the effect of variation in absolute weight of the bursa on immunocompetence is relative, but can be determined from the histological and immunohistochemical status of the bursa at the respective ages of variance. Nevertheless, connective tissue growth within the studied age limit may not be the source of weight increase since the bursa only develops substantial amount of connective tissue during bursal involution which usually begins at the onset of sexul maturity (Onyeanusi & Onyeanusi, 1990). The progressive increase in weight of developing bursa of Fabricius is equally observed in Deshi chickens of Bangladesh (Khalil, 2001), Sonali chickens of Bangladesh (Ayman *et al*., 2020) and indigenous guinea fowl of Nigeria (Onyeanusi & Onyanusi, 1990), where there is continuous increase in bursal weight with age even beyond 6 weeks of age. Therefore, further investigations beyond 6 weeks may be necessary in the Nigerian indigenous chickens as there still could be further growth changes of the organ. In fact, previous reports show that the chicken attains maximum mean bursal weight between days 56 and 70 (Ciriaco *et al*., 2003), while in Nigerian cockerels, maximum bursal weight is reached at 98 days post-hatch with involution setting in only after 140 days (Aire, 1974). In the white Pekin ducks, the bursa attains maximum size at 63 days of age (Hashimoto & Sugimura, 1976); while in guinea fowl, Onyeanusi *et al.* (1993) found that the bursa of Fabricius reaches maximum mean weight at 126 days.

Analysis of relative weights of bursa indicates that the indigenous chickens attain mean maximum relative weight at D 42 post-hatch. The relative weight of an organ is a function of the absolute weight of the organ, and its relevance to immunocompetence may depend on the structural components that primarily influence the weight of the organ. Therefore, appreciable immunocompetence is expected to be exhibited by the indigenous chicken if the immune related stuctures contributed a greater percentage of the absolute weight of the bursa, because the larger the organ, the higher the relative weight and the number of immunocompetent cells.

Evaluations in indigenous guinea fowl of Nigeria revealed that the bursa attains maximum relative weight at 4 weeks of age (Onyanusi & Onyeanusi. 1990). Glick (1956) analyzed the growth pattern of the bursa of Fabricius in chicken with interest in sex variations. His observations then show that both male and female chickens attain different values of maximum relative weights at 6 weeks of age. Glick (1956) also observed that the bursae of White Leghorns, prior to their regression, are heavier than the bursae of Rhode Island Reds. It was suggested that this may account for the greater degree of resistance of White Leghorns to S. pullorum infection.

During the early embryonic stages of development, precisely EID 10, the present study shows that the bursae in indigenous chickens possess well-developed tunics with mucosa already thrown into tall longitudinal folds and its cores filled with developing immunological and other supporting cells. Also at EID 14, organization of bursal cells into follicles was very pronounced which suggests an early establishment of bursa endowed with immunological potentials. The early differentiation of the bursal wall and development of the mucosal folds together with organization of bursal cells into follicles as observed in indigenous chickens are in agreement with the findings of Islam *et al.* (2012) in native chickens of Bangladesh. Furthermore, it was observed that the cell densities of the bursa increased rapidly at EID 18.This rapid increase in immunological cell density may have arisen to fortify the immune potential of the chicks prior to hatching and exposure to the hazard environmental influences. Hyun Ko *et al.* (2018) made similar observation in chicken and reported that the bursal B cells substantially increased in the late embryonic stage, especially at EID 17–EID 18, suggesting that it is a critical time for the development and homeostatic proliferation-like expansion of B cells in the bursa of Fabricius.

At hatch (D 0), follicle formation was still in progress as compartmentalization of follicular cells was yet inconclusive, although evidence of future cortical and medullary regions was obvious in some follicles. This indicates that the bursa in indigenous chickens did not conclude the establishment of the basic structural components embryonically. The marked increase in bursal lymphocyte density at hatch as observed in the indigenous chickens may be a transformation to further boost the immunological strength of the bursa to accommodate the challenges that may arise from the exposure of the chicks to the new environment. Riddle, (1982) stated that the bursa of Fabricius is fully developed in sexually immature birds, but undergoes regression at the onset of sexual maturity; and the age of attainment of maximum bursal size varies with the strain, sex and rearing method of bird (Onyeanusi and Onyeanusi, 1990). Ayman *et al*. (2020) also observed bursa with incomplete compartmentalized follicular cells in Solani chicken of Bangladesh at hatch. Pike *et al*. (2004) and Ratcliffe (2006) explained that the first cortical cells in the chicken appear around hatching, but the cortex only fully developed by day 14 post-hatch. Onyeanusi *et al*. (1990) observed that in guinea fowl the plicae are filled with follicles by day 1 post-hatch, although the status of the follicles was not clarified.

The development of bursa with large follicles that are separated by thick connective tissue and composed of varied cortical and medullary cell densities at about day 28 post hatch as observed from the study indicates that the bursa may be approaching full development. Similar observation was made by Hyun Ko *et al*. (2018) in chicken. However, with advancement in age, it was discovered that the bursa in indigenous chickens at day 42 possessed follicles with almost similar cortical and medullary cell densities. This suggests two possible cell activities within the follicles: (1) there could be an unparalleled cell proliferation between the cortex and medulla or (2) there was unidirectional cell migration from the cortex to the medulla. The significance of the observed identical cortical and medullary cell densities at D 42 post-hatch is not clear, but it could be related to morphological change indicative of attainment of peak bursal development. Hyun Ko *et al*., (2018) however,made a different observation in chicken at day 42. They reported that the follicles are composed of cortex that is more densely populated with cells than the medulla.

CONCLUSION

The gross morphology and histology of the embryonic and post-hatch bursa of Fabricius of Nigerian indigenous chickens are relatively similar to those of other studied avian species. The weight index of the bursa indicates that the indigenous chickens possess large-sized bursa of Fabricius. Histological studies also revealed that the bursa developed quite early in the indigenous chickens. There was marked increase in immunological cells proliferation during the late embryonic and early post-hatch periods of development, and growth increase in bursal cell density appeared to peak at about D 42 post-hatch.

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