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Anatomy of Bursa of Fabricius of Pati duck (*Anas platyrhynchos domesticus*) of Assam at Different Stages of Development

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Abstract

The present investigation was conducted on the histomorphological, histochemical and scanning electron microscopical observation on the Bursa of Pati duck of Assam at different stages of development. The experiment was conducted from 2017 to 2019. In current study, the Bursa of Fabricius was observed at 1st week of age of Pati duck and underwent atrophy at 24th week of age. The Bursa of Fabricius was found at the dorsal aspect of the Proctodeum. The length, breadth, thickness and weight of Bursa of Fabricius showed increasing trend from 1st week to 16th week of age of Pati duck. Histological, the folds of Bursa of Fabricius contained numerous polyhedral shaped lymphoid follicles at the lamina propria and it was composed of outer cortex and inner medulla. A layer of undifferentiated epithelial cells occupied at the periphery of the medulla, which was separated from the cortex by a capillary layer. Histochemicaly, the undifferentiated epithelial cell of lymphoid follicle of Bursa of Fabricius was showing strong reaction for acid phosphatase and adenosine triphosphatase. In scanning electron microscope, the Bursal folds of Bursa of Fabricius contained lining epithelium and lymphoid follicle. This follicle contained numerous lymphocytes along with connective tissue fibers. The Bursal follicle was situated in lamina propria of Bursal folds. Lamina propria and interfollicular area also contained connective tissue fibers.

Keywords: Anatomy, bursa of fabricius, pati duck, postnatal, development

1. Introduction

Duck husbandry provides an additional source of income to the rural women of these states. Ducks are one of the excellent converters of low quality waste products into high quality animal protein in the form of egg and meat. Duck eggs have great demand in the states of Assam as it has high biological value and considered to be a delicacy food item. The Pati duck population constitutes a major indigenous duck variety in the state of Assam. The lymph nodes play an important role in defense mechanism of Pati duck by secreting IgA. Similar studies were conducted in other poultry species in other organs (Abdalla et al., 2011; AbuAli et al., 2019; Madkour et al., 2019; Rabbani et al., 2019; Udoumoh et al., 2019). Bursa of Fabricius is covered by thin serosal layer and the inner surface was made up of several mucosal folds which are projected into lumen. Similar type of work was reported in other avian species such as Long-Legged Buzzard (Ebru et al., 2015), Ostrich (Peng et al., 2012) Turkey (Gultiken et al., 2010) chicken (Bacha and Wood, 1990)

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and duckling of Bangladesh (Sultan et al., 2011). Bacha and Wood (1990) in Chicken, Indu et al. (2005) in White Pekin duck and Akter et al. (2006) in broiler Chicken reported that Bursal follicle was the component of inner medulla and outer cortex and an undifferentiated epithelial cell layer occupied at the margin of medulla and separated by a thin capillary layer. Cortex is tightly packed with small lymphocytes and medulla is loosely packed with fewer lymphocytes (King et al., 1977; King and Mclelland, 1975). Being an indigenous variety of Assam and very scanty literature is available on the micro anatomical study on the Bursa of Fabricius of Pati duck. Hence, the present study was designed to establish anatomical norms on Bursa of Fabricius of Pati duck of Assam at different stages of development. The result of the present study will be helpful to the veterinarian and health experts to select the suitable duck for breeding purpose and useful for characterization of the duck.

2. Materials and Methods

The present studies were conducted on 45 numbers of Pati duck of Assam of irrespective of sex at different stages of development. The experiment was conducted from 2017 to 2019. The ducks were divided into five group viz., 1st week, 4th week, 16th week, 24th week and 42nd weeks of age. The ducks were procured from Pathsala and nearby area of Barpeta district of Assam. The experimental duck were brought to the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati and were sacrificed according to the standard method (Gracy, 1986).The duck of each age group were utilized for histological and micrometrical observation. The samples were collected from lymph node of all age groups of duck. These samples were fixed in 10% neutral buffered formalin solution and were processed as per the standard technique (Luna, 1968). The paraffin blocks were sectioned in Shandon Finesse microtome at 5µm thickness and the sections were stained with Mayer's Haematoxylin and Eosin staining technique for cellular details, Van Gieson's method for collagen fibres, Gomori's method for reticular fibres, Hart's method for elastic fibres and Bielchowsky's method for axis cylinder and dendrite. For Histochemical studies, they were sacrificed and immediately collected the lymph nodes (both cervical and lumbar).The samples were then preserved at liquid nitrogen (-196°C). Samples were made cryosections (-20°C) at 10µm in thickness and were temporally stored at (-22°C). They were than treated for histochemical staining with the following methods:

a. Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1978)

b. Gomori's method for acid phosphatase (Singh and Sulochana, 1978)

c. Lead method for ATPase (Bancroft, 2008)

d. Gomori's method for non-specific esterase (Bancroft, 2008)

For Ultra-structural studies, the tissue samples were collected from lymph node and were processed as per the technique of Parsons et al. (1991). The samples were cut into small pieces of 2 mm size and were fixed in 2% gluteraldehyde solution for 4 hours at 4 °C. The samples were subjected to the following steps:

a. Washing: The tissue sections were washed in 0.1M sodium cacodylate buffer. 3 changes of 15 minutes each at 4 °C.

b. Post-fixation: The tissues were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer at 4 °C.

c. Washing: The tissue samples were washed in 0.1M sodium cacodylate buffer 3 changes of 15 min each at 4 °C.

d. Dehydration: By ascending grades of acetone.

e. Drying: By tetra methyl saline method (Dey et al., 1989)

f. Mounting: The dry specimens were mounted on aluminium stubs.

g. Coating: Gold coating was applied in the tissue samples in a JFC-1100 (Joel) ion sputter coater.

h. The stubs with the tissue samples were loaded in the JMS-35CF (Joel) scanning electron microscope operated at 20KV.

The statistical analysis of the data was performed as per standard procedures. Means were analyzed by one way analysis of variance (ANOVA), followed by the Tukey's post hoc test to determine significant differences among the different experimental groups (Statistical Analysis System for Windows, SAS Version 9.3; SAS Institute, Inc., Cary, NC, 2001).Differences with values of $p<0.05$ were considered to be statistically significant.

3. Results and Discussion

Grossly, the Bursa of Fabricius of Pati duck was observed from 1st week to 16th week of age on the dorsal aspect of cloaca which opened at the dorsal surface of the proctodeum (Figure 1). The Bursa of Fabricius was cylindrical to elongated

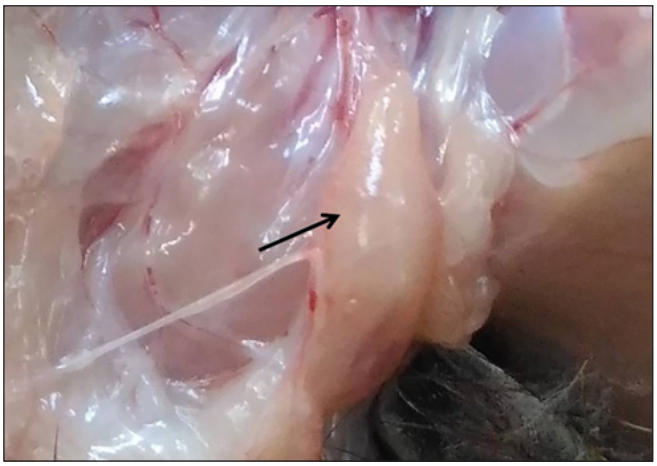

Figure 1: Photograph showing the in-situ position of Bursa of Fabricius (black arrow) of 1st week old Pati duck

structure and off white in colour (Figure 2). The Bursa of Fabricius underwent atrophy at 24th week of age of Pati duck. Similar findings were observed by King (1977) in duck, Sultan et al. (2011) in duckling of Bangladesh and Kumar et al. (2014) in Khaki Campbell duck.

Figure 2: Photograph showing the atrophied Bursa of Fabricius on 24th week old of Pati duck

The mean biometrical value of length of Bursa of Fabricius was 18.79±0.64, 23.79±0.73 and 28.33±1.93 during 1st week, 4th week and 16th week of age of Pati duck, respectively (Table 1). The average value of breadth of Bursa of Fabricius was 3.24±0.28, 3.94±0.31 and 5.10±1.16 during 1st week, 4th week, 16th week of age of Pati duck, respectively (Table 1). The mean value of thickness was 0.43±0.07, 0.70±0.04 and 0.92±0.01 during 1st week, 4th week and 16th week of age of Pati duck, respectively (Table 1, 2).The mean value of weight was 0.20±0.02, 0.38±0.02 and 1.77±0.02 g during 1st week, 4th week and 16th week of age of Pati duck, respectively (Table 1, 2). The length, breath, thickness and weight of Bursa of Fabricius was highly significant (p<0.01) between the various age groups. The present value of length of Bursa of Fabricius found in Pati duck was lower (27.01±0.062) than the earlier reports in Khaki Campbell duck at 4th week of age (Kumar *e*t al., 2013). Similarly, Kumar et al. (2014) reported the lower value of breadth (3.34±0.011 mm) and weight (0.22±0.003 g) in Khaki Campbell duck at 4th week of age. However, Sultan et al. (2011) recorded that the length, breadth and weight of the Bursa of Fabricius of duckling of Bangladesh were 1.87±0.15 cm, 0.53±0.07 cm and 0.22±0.069 g, respectively which corroborated the present findings.

Histologically, in the present study, the Bursa of Fabricius was covered by thin serosal layer. The inner surface of the Bursa of Fabricius was consisting of several mucosal folds (plicae) which were projected into the lumen. These findings were in accordance with the findings of Ebru et al. (2015) in Long-Legged Buzzard, Peng et al. (2012) in Ostrich, Gultiken et al. (2010) in Turkey. Adjacent to the follicles the epithelium lining of the fold become simple columnar where as other part of fold contained pseudostratified (Figure 3). These present study findings were similarly reported in chicken (Bacha and Wood, 1990) and in duckling of Bangladesh (Sultan et al., 2011) and in Ostrich (Song et al., 2012). Numerous polyhedral shaped follicles were found in the lamina propria of each fold. Each Bursal follicle was composed of outer cortex and inner medulla. A layer of undifferentiated epithelial cells occupied at the periphery of the medulla, which was separated from the cortex by a capillary layer (Figure 4). Similar findings were reported by Bacha and Wood (1990) in Chicken, Indu et al. (2005) in White Pekin duck and Akter et al. (2006) in broiler Chicken. This capillary layer was more distinct according to advancement of age. Similar findings were supported in Khaki Campbell duck (Kumar et al., 2013). The colour of the cortex was dark compared to medulla. Cortex contained closely packed small lymphocytes. The paler medulla contained

Table 1: Value of Bursa of Fabricius of Pati duck at different age group (Mean±SEM)

Experimental group	Age in week	Length (mm)	Breath (mm)	Thickness (mm)	Weight (g)
I	1st	18.79±0.64^{c}	3.24±0.28^{b}	0.43±0.07^{c}	0.20±0.02^{c}
II	4th	23.79±0.73^{b}	3.94±0.31^{b}	0.70±0.04^{b}	0.38±0.02^{b}
III	16th	28.33±1.93^{a}	5.10±1.16^{a}	0.92±0.0^{a}	1.77±0.02^{a}
IV	24th	35.31 ± 2.46^{a}	7.32±2.47^{a}	0.96±0.78^{a}	1.99±0.34^{a}

Mean with different superscripts are significantly different from each other

Table 2: Histochemical characterization of bursa of fabricius of Pati duck

Histoenzymes	Follicle associated epithelium	Undifferentiated epithelial cells	Cortex	Medulla	Lamina Propria
Alkaline Phosphatase	+	++++	+	++	+
Acid Phosphatase	++	++++	++	+++	++
Adenosine triphosphatase	+++	+++	-	+	-
Non specific esterase	+	+	-	-	-

Gradation for intensity of histochemical reaction: -=Negative; +=Weak;++=Moderate; +++=Strong; ++++=Intense

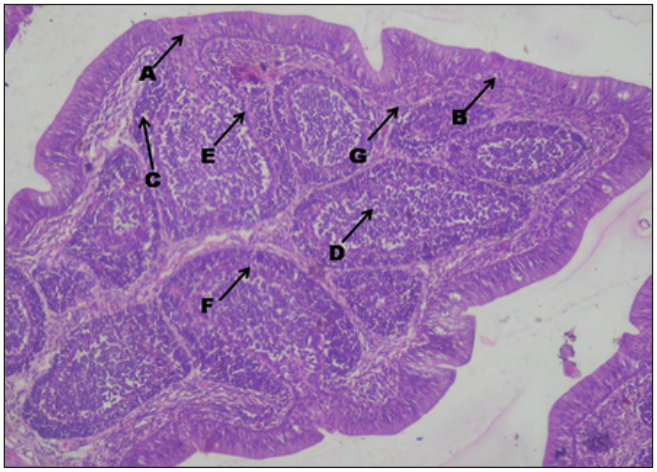

Figure 3: Photomicrograph showing the simple columnar epithelial tuft (a), pseudostratified columnar epithelium, cortex (c), medulla (d), undifferentiated epithelial layer (e), capillary layer (f) and lamina propria (g) of Bursa of Fabricius of 1st week old Pati duck (H& E, X10)

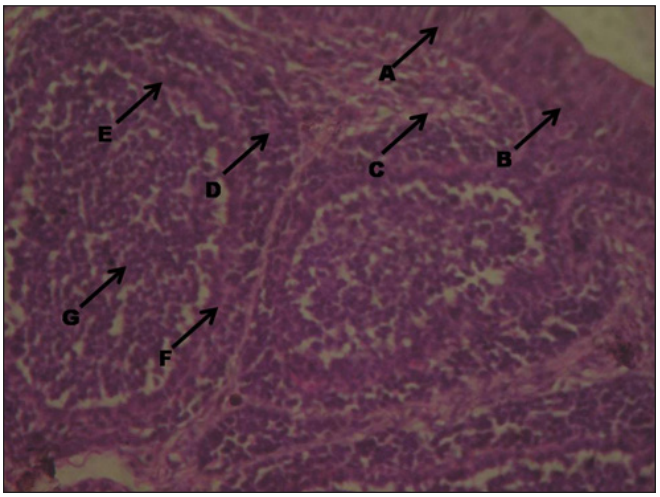

Figure 4: Photomicrograph showing pseudostratified columnar epithelium (a), simple columnar epithelial tuft (b), lamina propria (c), cortex (d), undifferentiated epithelial cell layer (e), capillary layer (f) and medulla (g) of Bursa of Fabricius of 16th week old Pati duck (H& E, X40)

fewer lymphocytes of various sizes. These statements were supported by King et al. (1977) in duck and King and Mclelland (1975) in fowl. Some vacuoles were also observed in the medulla of follicles at 16th weeks of age of Pati duck. These lymphoid follicles were surrounded by abundant reticular fibers (Figure 5, Figure 6 and Figure 7) and collagen fibers, few elastic fibers, nerve fibers (Figure 8). Similar findings were reported in Ostrich chicks (Song et al., 2012) and in Kadaknath birds (Kanasiya et al., 2018). The Bursa of Fabricius was devoid of lamina muscularis mucosae as well as Tunica sub mucosa layer.

Histochemically, in the present study, the follicle associated epithelium of Bursa of Fabricius showed weak reaction while the undifferentiated epithelial cell or cortico-medullary

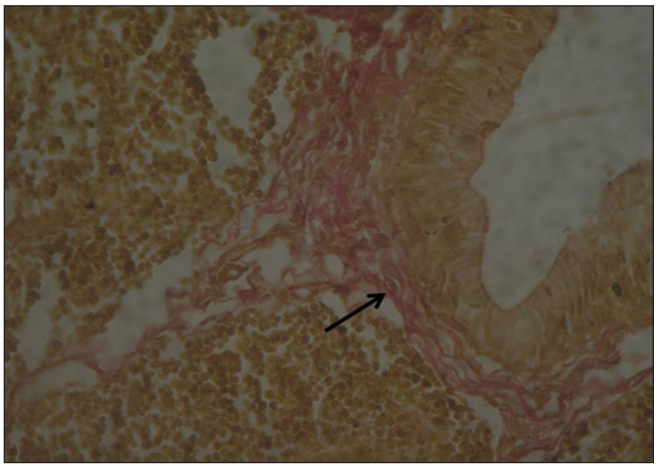

Figure 5: Photomicrograph showing the collagen fibers (black arrow) of Bursa of Fabricius of 1st week old Pati duck (Van Gieson's method, X40)

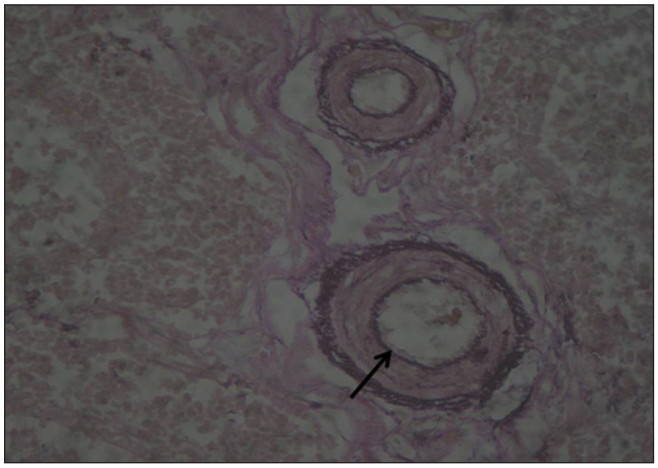

Figure 6: Photomicrograph showing the elastic fibers (black arrow) of Bursa of Fabricius of 4th week old Pati duck (Hart's method, X40)

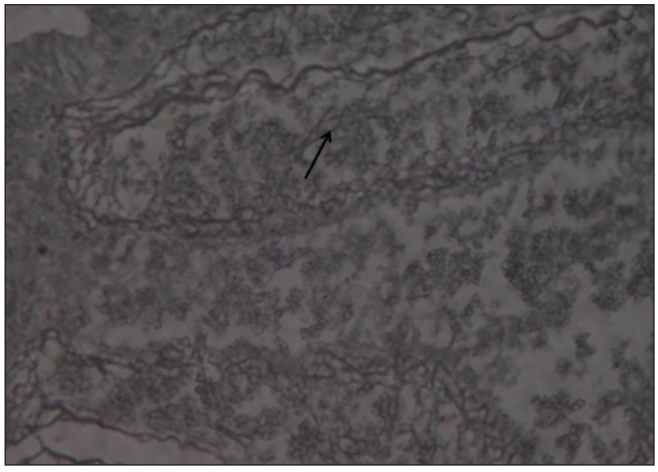

Figure 7: Photomicrograph showing the reticular fibers (black arrow) of Bursa of Fabricius of 16th week old Pati duck (Gomori's method, X40)

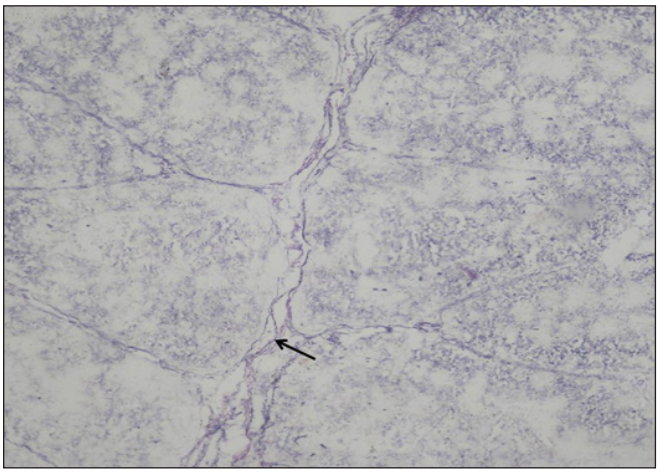

Figure 8: Photomicrograph showing the nerve fibers (black arrow) of Bursa of Fabricius of 4th week old Pati duck (Bielschowsky's method, X10)

junction of lymphoid follicle of Bursa of Fabricius showed intense reaction of alkaline phosphatase at lamina propria (Figure 9). The cortex and medulla of lymphoid follicle of Bursa of Fabricius showed weak and moderate reaction of alkaline phosphatase activity (Table 2). Contrary to the present finding, Kempashi et al. (2017) found that the epithelium covering the Bursal plicae of chicken was moderate reaction and boreal follicles showed mild reaction of alkaline phosphatase. In day old and two week-old birds, there was moderate reaction and there after the Bursal reaction to alkaline phosphatase enzyme decreased whereas Ackerman and Knouff (1959) reported that during the first week after hatching of Fowl epithelial cells of Bursa of Fabricius exhibit a very strong alkaline phosphatase activity but thereafter this was almost completely lost. It might be due to species variation of birds and agro climatic condition of the birds.

There was moderate reaction for follicle associated epithelium and intense reaction for undifferentiated epithelial cell of

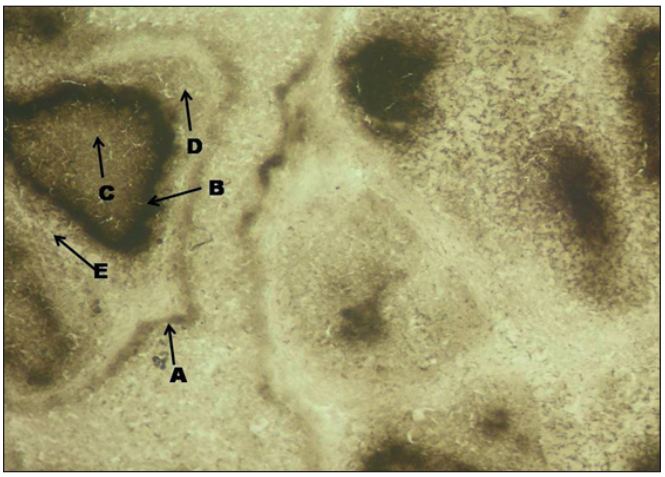

Figure 9: Photomicrograph showing the alkaline phosphatase activity in follicle associated epithelium (a), undifferentiated epithelial cells (b), medulla (c), cortex (d) and lamina propria (e) of Bursa of Fabricius on 4th week old Pati duck (Gomori's, X10)

lymphoid follicle of Bursa of Fabricius for acid phosphatase (Figure 10 and Table 2). Similar findings were reported by Kempashi et al. (2017) in Chicken. Lamina propria of Bursa of Fabricius and cortex of lymphoid follicle showed moderate reaction for acid phosphatase however medulla of lymphoid follicle of Bursa of Fabricius showed strong reaction for acid phosphatase.

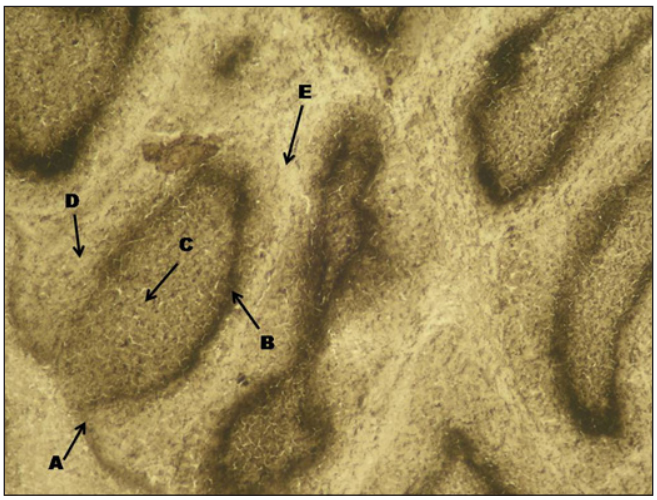

Figure 10: Photomicrograph showing the acid phosphatase activity in follicle associated epithelium (a), undifferentiated epithelial cells (b), medulla (c), cortex (d) and lamina propria (e) of Bursa of Fabricius on 16th week old Pati duck (Gomori's, X10)

There was strong reaction in follicle associated epithelium and undifferentiated epithelial cell of lymphoid follicle of Bursa of Fabricius for ATPase (Figure 11). Lamina propria of Bursa of Fabricius and cortex of lymphoid follicle showed negative reaction for ATPase on the other hand medulla of lymphoid follicle showed weak reaction for all the age groups of Pati duck (Table 2). However, Mazzone et al. (2003) showed that

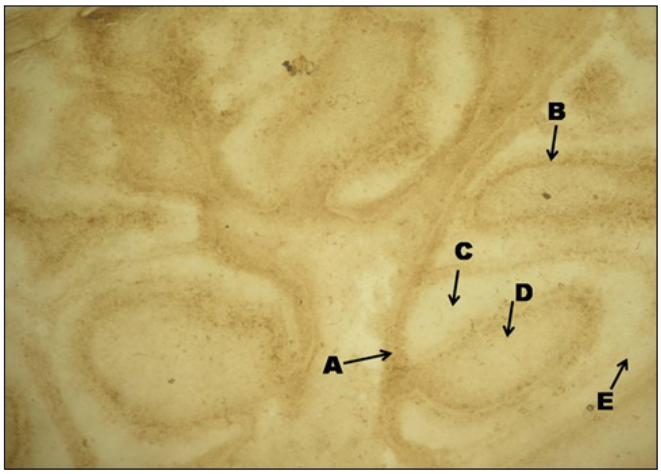

Figure 11: Photomicrograph showing the adenosine tri phosphatase activity in follicle associated epithelium (a), undifferentiated epithelial cells (b), medulla (d), cortex (c) and lamina propria (e) of Bursa of Fabricius on 16th week old Pati duck (Lead method, X10)

the epithelial covering of Bursal plicae and muscular layer showed positive reaction for ATPase, whereas cytoplasm of cortical and medullary lymphocytes showed negative reaction to ATPase activity.

There was weak reaction for follicle associated epithelium and undifferentiated epithelial cell of lymphoid follicle of Bursa of Fabricius fornon specific esterase (Figure 12). Lamina propria of Bursa of Fabricius and cortex of lymphoid follicle showed negative reaction for nonspecific esterase while medulla of lymphoid follicle showed negative reaction for enzyme in all the age groups of Pati duck (Table 1). These findings were in agreement with the findings of Kempashi et al. (2017) in

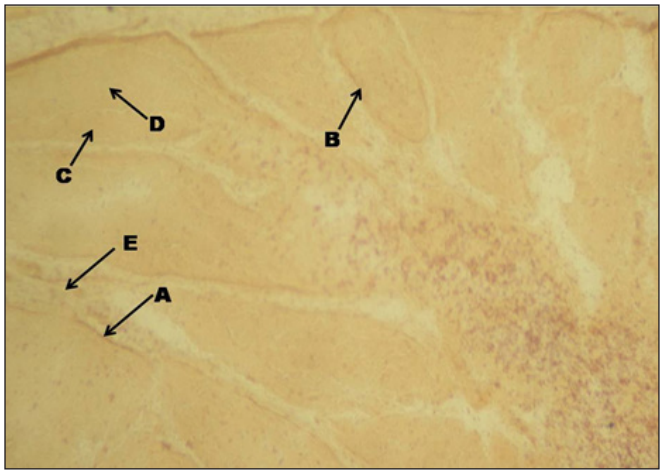

Figure 12: Photomicrograph showing the non specific esterase activity in follicle associated epithelium (a), undifferentiated epithelial cells (b), medulla (d), cortex (c) and lamina propria (e) of Bursa of Fabricius on 1st week old Pati duck (1 Naphthyl acetate method, X10)

chicken and Ebru et al. (2015) in Long –Legged Buzzard.

In Scanning Electron microscopy, the Bursal folds of Bursa of Fabricius contained lining epithelium and lymphoid follicle (Figure 13). These findings were in accordance with

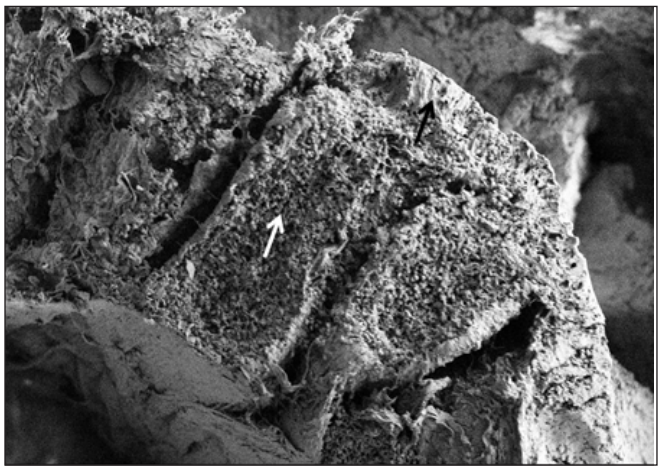

Figure 13: Scanning electron microphotograph showing the lymphoid follicle (white arrow) and epithelium (black arrow) of Bursa of Fabricius bar=10µM, 555X

the findings of Gultiken et al. (2010) in Turkey. This follicle contained numerous lymphocytes along with connective tissue fibers (Figure 14). The Bursal follicle was situated in lamina propria of Bursal folds. Lamina propria and interfollicular area also contained connective tissue fibers.

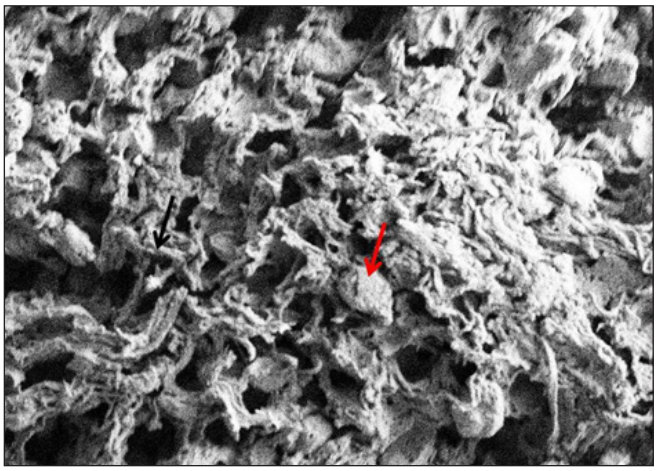

Figure 14: Scanning electron microphotograph showing the lymphocytes and connective tissue fibers of lymph node BAR=2µm, 6.01KX

4. Conclusion

Bursa of Fabricius was observed at 1st week of age and underwent atrophy at 24th week of age. The length, breadth, thickness and weight of Bursa of Fabricius showed increasing trend from 1st week to 16th week of age of Pati duck. Histochemicaly, the undifferentiated epithelial cell of lymphoid follicle of Bursa of Fabricius was showing strong reaction for acid phosphatase and adenosine triphosphatase. In scanning electron microscope, the Bursal folds of Bursa of Fabricius contained lining epithelium and lymphoid follicle.

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