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Age Related Histological Changes in the Epithelium of Bursa of Fabricius in Chabro Bird

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ABSTRACT

The study aimed to provide basic findings about the age-related epithelial changes in the lymphoid follicle of bursa of Fabricius, which was conducted on the bursa of Fabricius of thirty Chabro birds. The birds were selected after hatching irrespective of sex and divided into five groups (0, 30, 60, 90 and 150 days of age) each group comprised of 6 birds. The bursa of Fabricius was a sac-like structure connected to the dorsal wall of the proctodeum of the cloaca by a small stalk. As the age increases, there was increase and decrease in the height and width of surface epithelium and formation of an epithelial cyst.

Keywords: Bursa of Fabricius, Chabro Bird, Epithelium.

INTRODUCTION

The Chabro bird is a rural meat type bird of India and it is a cross breed of Barred Plymouth Rock and red Cornish birds. The bursa of Fabricius plays an important role in the immune system, the primary site for B- lymphocytes which is responsible for humoral immunity. The wall of the bursa of Fabricius was comprised of three basic layers; innermost tunica mucosa, middle tunica muscularis and outermost tunica serosa. The tunica mucosa consisted of lining epithelium and lamina propria filled with lymphoid follicles. The tunica muscularis consisted of an outer circular and inner longitudinal smooth muscle layers. The outermost tunica serosa was thin and enclosed the whole organ in all the groups.

MATERIALS AND METHODS

Study was conducted on the bursa of Fabricius of thirty apparently healthy Chabro birds irrespective of the sex. All the birds for the study were selected at the time of hatching and were grown in separate portions of the same poultry farm. These were divided into five groups consisting of six birds each at 0, 30, 60, 90 and 150 days after hatching. Body weight of each bird was recorded. The abdominal cavities of six birds were opened through the careful dissection and the bursa of Fabricius of each bird was exposed and the location, shape, size and colour of bursa and its relations with the adjacent organs were recorded. Biometrical observations on the weight of bursa, length, width, thickness and number of the plicae, length, width and height of plicae were recorded.

Statistical Analysis

The data generated were analyzed using analysis of variance of Statistical package for Social science (SPSS for window, V20.0; SPSS Inc., Chicago, IL USA). Significance was determined at $P<0.05$ and the value are presented in the table.

RESULTS AND DISCUSSION

Tunica Mucosa

The tunica mucosa of the bursa of Fabricius was comprised of two parts, the lining epithelium and the connective tissue framework (lamina propria) filled with different size of follicles. Similar observations were made by Hassan *et al*. (2011) in Quail and Kempashi *et al*. (2017) in Nandnam chicken. There was presence of larger and smaller plical folds. Each plica consisted of surface epithelium and lamina propria filled with numerous lymphoid follicles. The connective tissue core began from base of each plica and ran towards the apex of the plica. The connective tissue septae arose from the connective tissue core and divided the plica into different compartments, each was filled with lymphoid follicles. This is in total agreement with the observations of Indu *et al*. (2005) in White Pekin ducks; Jayachitra *et al*. (2009)

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in Turkey; Jain *et al*. (2010) in CARI Shyama and Vanraja breeds of poultry; Kumar (2014) in Khaki Campbell ducks; Tamilselvan *et al*. (2017) in Guinea fowl and Kanasiya *et al*. (2018) in Kadaknath birds.

The maximum mean thickness of the tunica mucosa of bursa of Fabricius was 2004.89 ± 20.82 µm in Chabro bird at 3rd month (in 4th group). The thickness of the tunica mucosa increased significantly upto 4th group and then the height started decreasing from the group 4th to 5th (Table 1). Jain *et al*. (2010) reported height of mucosal folds was 800 ± 20.50 µm at day 0, 2280 ± 45.1 µm at 8 weeks and 1700 ± 20.50 µm in CARI Shyama whereas these value for Vanraja breed of poultry were 850 ± 25 µm, at day of hatch and 2200 ± 40.60 µm at 8 weeks old bird. The value in present study at day old and 8 weeks old Chabro bird was lower. It might be due to the breed and strain difference.

Epithelium

The tunica mucosa was thrown into folds called primary plicae which branched out to give rise to secondary plicae. Similar findings were made by Sabiha *et al*. (1999) in Japanese quail and Kempashi *et al*. (2017) in Nandnam chicken. Plica comprised of numerous collagen fibres, reticular fibres and very few elastic fibers. As mentioned by Hodges (1974) in domestic fowl; Indu *et al*. (2005) in White Pekin ducks, Jayachitra *et al*. (2009) in Turkey and Jain *et al*. (2010) in CARI Shyama and Vanraja breeds of poultry. Large blood vessels were present in the connective tissue core of plica, which was similar to the findings of Indu *et al*. (2005) in White Pekin ducks.

The maximum mean length of the primary plicae of bursa of Fabricius was 1782.28 ± 15.73 µm in Chabro bird at 3rd month (in 4th group). The length of the primary plicae increased significantly upto 4th group and then the length started decreasing from the group 4th to 5th (Table 2). Tamilselvan *et al*. (2017) reported the indistinct length of primary plicae at day 1 in Guinea fowl.

The maximum mean length of the secondary plicae of bursa of Fabricius was 1255.71 ± 7.22 µm in Chabro bird at 3rd month (in 4th group). The length of the secondary plicae increased significantly upto 4th group and then the length started decreasing from the group 4th to 5th (Table 2). The maximum mean width of the secondary plicae of bursa of Fabricius was 586.71 ± 13.68 µm in Chabro bird at 3rd month (in 4th group). The width of the secondary plicae increased significantly upto 4th group and then the width started decreasing from the group 4th to 5th (Table 2).

The lining epithelium was made up of pseudostratified columnar epithelium while it was simple columnar in crypts. As mentioned by Ackerman and Knauff (1959) in chicken; Hodges (1974) in domestic fowl and Dellmann (1993) in chicken; Khenenou *et al*. (2012) in broiler chicken; Ebru *et al*. (2015) in Long Legged Buzzard and Kanasiya *et al*. (2018) in Kadaknath bird. Contrary to this, Jayachitra *et al*. (2009) in turkey and Tamilselvan *et al*. (2017) in Guinea fowl reported that stratified cuboidal epithelium was found at the apex of the follicles. The epithelium consisted of three types of cells. Type I cells were columnar cells with round or oval nucleus. Type II cells were basally placed with round nucleus. Type III cells were goblet cells found among the columnar cells (Fig. 2). Similar observations were made by Sari and Kurtdede (2007) in turkey; Jayachitra *et al*. (2009) in turkey and Tamilselvan *et al*. (2017) in Guinea fowl. The columnar cells had moderately eosinophilic cytoplasm and a tuft of apical cilia indicated by the hazy luminal border of epithelium. The goblet cells were tall columnar having hyper chromatic basally placed nucleus. The basal cells were located at the level of basement membrane with oval centrally placed nucleus (Fig.2). Present study resembled with those reported by Ackerman and Knouff (1959) in chicken; Hodges (1974) in domestic fowl; Sabiha *et al*. (1999) in Japanese Quail; Indu *et al*. (2005) in White Pekin duck; Kumar (2014) in Khaki Campbell duck; Villanueva and Bernardo (2015) in Game fowl and Kempashi *et al*. (2017) in Nandanam chicken. Kanasiya *et al*. (2018) in Kadaknath bird observed four type of cells as type I or principal cells which were columnar type having basal nucleus and prominent nucleolus, type II cells with basal cells resting on basement membrane, type III cells located on apical portion of epithelium (these cells were round in shape with large nucleus and had scanty cytoplasm) and type IV cells were also columnar type which were found on interfollicular epithelium and contained mucin in their cytoplasm.

The epithelium covering the plicae were divided into two types viz., follicle associated epithelium (FAE) or epithelial tuft with pale columnar cells which was in direct contact with medulla of the lymphoid follicles (Fig. 3) and the interfollicular epithelium (IFE) covering the remaining part of the plicae

Table 1: Showing age related changes in tunica mucosa, tunica muscularis and tunica serosa in different age group of Chabro bird.

Parameters	1st Group	2nd Group	3rd Group	4th Group	5th Group
Thickness of Tunica Mucosa (µm)	664.50 ± 26.26^{a} (424.32 – 900.50)	1466.21 ± 3.72^{c} (1421.81 – 1501.18)	1788.95 ± 14.76^{d} (1608.53 – 1968.82)	2004.89 ± 20.82^{e} (1662.98 – 2201.71)	1307.83 ± 8.21^{b} (1273.14 – 1372.35)
Thickness of Tunica Muscularis (µm)	28.62 ± 2.43^{a} (7.50 – 60.79)	76.89 ± 1.39^{b} (61.38 – 89.73)	103.44 ± 2.22^{c} (75.19 – 119.28)	120.05 ± 1.03^{d} (107.56 – 130.13)	113.15 ± 2.48^{d} (98.57 – 123.21)
Thickness of Tunica Serosa (µm)	57.53 ± 2.47^{a} (35.44 – 88.16)	131.04 ± 1.15^{b} (120.33 – 141.90)	170.94 ± 0.78^{c} (161.62 – 178.01)	227.47 ± 3.26^{e} (192.19 – 260.18)	211.61 ± 3.60^{d} (198.24 – 231.19)

Table 2: Showing age related changes in length and width of primary and secondary plicae in different age group of chabro bird.

Parameters		1st Group	2nd Group	3rd Group	4th Group	5th Group
Primary Plicae	Length (µm)	652.28 ± 27.16^{a} (423.11 – 888.27)	1420.10 ± 3.25^{c} (1395.02 – 1451.94)	1665.61 ± 12.12^{d} (1560.33 – 1779.71)	1782.28 ± 15.73^{e} (1650.03 – 1971.61)	1253.11 ± 5.68^{b} (1226.18 – 1300.15)
	Width (µm)	208.23 ± 4.30^{a} (172.13 – 250.57)	585.92 ± 11.70^{b} (480.18 – 685.03)	723.01 ± 1370^{c} (601.34 – 850.28)	847.30 ± 25.66^{d} (673.37 – 1122.34)	999.13 ± 0.90^{e} (994.27 – 1003.37)
Secondary Plicae	Length (µm)	327.84 ± 2.87^{a} (300.19 – 350.72)	882.93 ± 3.74^{c} (844.38 – 917.21)	1047.16 ± 10.13^{d} (981.26 – 1154.78)	1255.71 ± 7.22^{e} (1185.27 – 1327.72)	770.41 ± 17.79^{b} (720.61 – 901.11)
	Width (µm)	134.47 ± 2.79^{a} (109.26 – 160.21)	382.73 ± 7.77^{b} (306.14 – 450.02)	466.87 ± 12.91^{c} (353.38 – 589.73)	586.71 ± 13.68^{e} (480.93 – 712.82)	516.15 ± 12.81^{d} (458.37 – 583.04)

Table 3: Showing age related changes in height of epithelium and height and width of the nucleus in different age group of chabro bird.

Parameters		1st Group	2nd Group	3rd Group	4th Group	5th Group
Height of Epithelium	FAE (µm)	12.26 ± 0.57^{a} (5.44 – 20.44)	32.46 ± 1.25^{b} (19.38 – 47.43)	35.72 ± 3.04^{b} (18.08 – 76.88)	30.69 ± 0.97^{b} (15.89 – 43.40)	13.04 ± 1.43^{a} (0.00 – 32.55)
	IFE (µm)	15.73 ± 0.59^{a} (10.65 – 23.88)	44.06 ± 2.18^{b} (25.05 – 73.74)	42.58 ± 3.02^{b} (19.08 – 84.25)	39.81 ± 0.88^{b} (29.33 – 52.46)	18.81 ± 5.71^{a} (0.00 – 41.61)
Nucleus	Height (µm)	5.28 ± 0.14^{b} (3.41 – 7.02)	6.68 ± 0.19^{c} (4.49 – 9.12)	6.44 ± 0.20^{bc} (3.94 – 8.60)	7.08 ± 0.21^{c} (3.58 – 8.94)	3.55 ± 1.08^{a} (0.00 – 8.07)
	Width (µm)	3.03 ± 0.09^{b} (1.89 – 4.47)	3.68 ± 0.11^{b} (2.14 – 4.81)	3.14 ± 0.10^{b} (2.04 – 4.70)	3.42 ± 0.13^{b} (1.95 – 5.39)	1.96 ± 0.60^{a} (0.00 – 4.79)

consisting of darkly stained columnar cells i.e. between the follicles (Fig. 3). The present results were in agreement with the observations of Ackerman and Knauff (1959) in chicken and Hodges (1974) in domestic fowl; Villanueva and Beranardo (2015) in game fowl; Kempashi *et al*. (2017) in Nandanam chicken and Kanasiya *et al*. (2018) in Kadaknath birds. Bockman and Cooper (1973) in chicken speculated that the FAE cells provided a specialized environment which induced the initial migration of stem cells into the bursal epithelium, Ebru *et al*. (2015) in Long Legged Buzzard reported that FAE cells and mammalian M-cells were similar. Ebru *et al*. (2015) in Long Legged Buzzard reported that IFE and FAE of bursa differed structurally. The author reported that IFE has the features of single layer of columnar epithelium without goblet cells whereas, in the present study it was pseudostratified columnar epithelium. FAE in the bursa of Fabricius was considered to be part of surface epithelium. Bockman and Cooper (1973) and Olah and Glick (1992) in domestic fowl had identified its pinocytotic activity. FAE cells had been reported to be prismatic and similar to absorptive M-cells in mammals. In addition, FAE had been reported to stimulate antibody production by transferring antigen to the medulla following pinocytosis and have a leading role in developing immune response (Whittow, (2000) in avian). Schat and Myers (1991) in birds reported that FAE started to be patchy settled in IFE. However in the present study FAE was observed to continue uninterrupted along the surface of the follicles rather than patchy. The lymphoid follicles in the bursa of Fabricius in the chabro bird was remarkably associated with FAE, it supports the immune response (Ebru *et al*., 2015 in Long Legged Buzzard). They also reported that immune response increased the pinocytosis activity.

Follicular associated epithelium (FAE) was made up of pseudostratified columnar ciliated epithelium, while simple columnar in the crypts. Similar observations were made by Kanasiya *et al*. (2018) in Kadaknath birds. In group 1, the cells of the follicular associated epithelium became distended.

The present observation was in concordance with the findings of Indu *et al*. (2005) in White Pekin duck; Sari and Kurtdede (2007) and Jayachitra *et al*. (2009) in turkeys and Kumar (2014) in Khaki Campbell duck who reported that the communication between mucosal epithelium compartment and FAE occurred through the FAE supporting cell. CT-3 positive cells, which can penetrate the basement membrane since they appear in the mucosal epithelium compartment and in the IFE, were not seen. Olah *et al*. (1978) reported that the interfollicular spaces of the bursal FAE contained vesicles. It is similar to ruminant's ileal payer's patches. The epithelium of Vth group birds was distorted and discontinuous (Fig. 4). Similar observation were made by Leena *et al*. (2009) in domestic fowl and Tamilselvan *et al*. (2017) in Guinea fowl.

The maximum mean height of the follicular associated epithelium (FAE) of bursa of Fabricius was 35.72 ± 3.04 µm in chabro bird at 2nd month (in 3rd group). The height of the follicular associated epithelium (FAE) increased significantly upto 3rd group and then the height started decreasing from the group 3rd to 4th and 4th to 5th. This is in accordance with the observations of Sugimura et al. (1975) and Hashimoto and Sugimura (1976) in ducks.

The maximum mean height of the interfollicular epithelium (IFE) of bursa of Fabricius was 44.06 ± 2.18 µm in chabro bird at 1st month (in 2nd group). The height of the interfollicular epithelium (IFE) increased significantly upto 2nd group and non-significant increase in the height of IFE from 2nd to 3rd and 4th group. There was highly significant decrease in the height of interfollicular epithelium from 4th to 5th group. The result of the present study was in accordance with the observation of the Sugimura *et al*. (1975) and Hashimoto and Sugimura (1976) in duck and Tamilselvan (2017) in Guinea fowl.

There was increase in the height of nucleus from group 1st, 2nd, 3rd and decreased in nuclear height from 4th to 5th group. There was increase in width of epithelium in group 1st and 2nd and decrease in nuclear width in 3rd, 4th and 5th. No specific literature was detected on micrometrical parameters of nucleus of bursa of Fabricius in support of present investigation.

Lamina Propria

The lamina propria consisted of connective tissue framework filled with lymphoid follicles of different shape and size in all age groups.

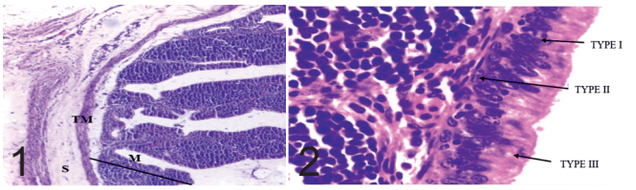

Fig. 1: Photomicrograph of bursa of Fabricius of 0-day old chabro bird showing tunica mucosa (M), tunica muscularis (TM) and tunica serosa (S). (H&E x 100); **Fig. 2:** Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing type I, type II and type III cells. (H&E x 1000)

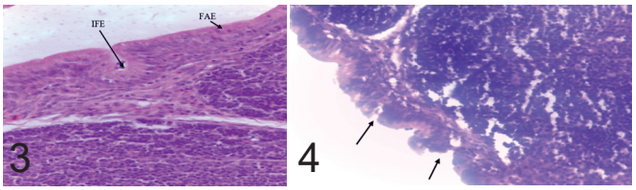

Fig. 3: Photomicrograph of bursa of Fabricius of 60 days old chabro bird showing Follicle Associated Epithelium (FAE) and Interfollicular Epithelium (IFE). (H&E x 1000); **Fig. 4:** Photomicrograph of bursa of Fabricius of 150 days old chabro bird showing discontinuous epithelium. (H&E x 400)

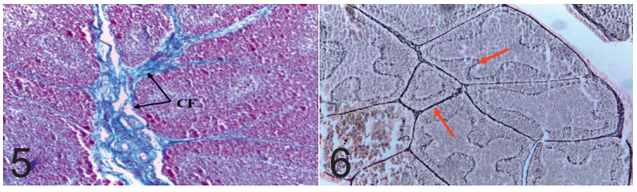

Fig. 5: Photomicrograph of bursa of Fabricius of 60 days old chabro bird showing collagen fibers (CF). (Masson's Trichome x 100); **Fig. 6:** Photomicrograph of bursa of Fabricius of 30 days old chabro bird showing reticular fibres (red arrows) in between the follicles and at the corticomedullary junction. (Gordons & Sweet's stain x 100)

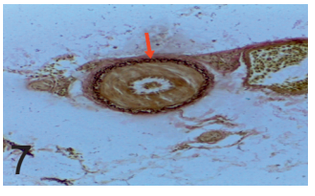

Fig. 7: Photomicrograph of bursa of Fabricius of 30 days old chabro bird showing elastic fibres around the blood vessels (red arrow). (Weigert's method x 200)

These observations were in accordance with the findings of Kempashi *et al*. (2017) in Nandanam chicken and Kanasiya *et al*. (2018) in Kadaknath birds. The connective tissue consisted basically of a network of fine collagen fibres (Fig. 5) and numerous reticular fibres (Fig. 6) surrounding the follicles and scantly elastic fibres especially around blood vessels (Fig. 7). These observations were similar to the findings of Singh *et al*. (2006) and Tamilselvan *et al*. (2017) in Guinea fowl and Ebru *et al*. (2015) in Long Legged Buzzard. The connective tissue was sparse within the corium of the plicae. The follicles took up greater proportion and were

compactly arranged with in each plica. These plicae were mainly consisted of an amount of connective tissue separating the follicles into the follicular septa. The amount of collagen and reticular fibres increased with the advancement of the age. Present study was in accordance with Ackerman and Knouff (1959) and Hodges (1974) in domestic fowl and Tamilselvan *et al*. (2017) in Guinea fowl.

Tunica Muscularis

The middle tunica muscularis layer consisted of an outer circular and inner longitudinal layers of smooth muscle fibres. The longitudinal layer was found to enter the central core of the mucosal plicae (Fig. 1). The present results were similar to the study of Hodges (1974) and Leena *et al*. (2009) in domestic fowl; Jain *et al*. (2010) in CARI Shyama and Vanraja breeds of poultry; Kempashi *et al*. (2017) in Nandanam chicken and Kanasiya *et al*. (2018) in Kadaknath birds.

The main branches of the blood vessels supplying the organ lay between the muscle layers at the base of the plicae and branches of the vessels pass up through the corium of each plicae as reported by Ebru *et al*. (2015) in Long Legged Buzzard.

The maximum mean thickness of the tunica muscularis of bursa of Fabricius was 120.05 ± 1.03 µm at 3rd month of age (in 4th group). There was significant increased in the thickness of the tunica muscularis from group 1st to 2nd, 3rd and 4th and non-significant decrease was noticed from 4th to 5th group (Table 1). No specific literature was detected on micrometrical parameters of tunica muscularis of bursa of Fabricius in support of present investigation.

Tunica Serosa

A thin serosal layer was composed of connective tissue fibres. It was mostly predominant in collagen fibres with few elastic fibres. The observations of present study were in accordance with the findings of Hodges (1974); Dellman and Brown (1993) and Leena *et al*. (2009) in domestic fowl, Jain *et al*. (2010) in CARI shyama and Vanraja; Ebru *et al*. (2015) in Long Legged Buzzard and Kempashi *et al*. (2017) in Nandanam chicken. The maximum mean thickness of the tunica serosa of bursa of Fabricius was 227.47 ± 3.26 µm at 3rd month of age (in 4th group). There was significant increase in the thickness of the tunica serosa from group 1st to 2nd, 3rd and 4th group and significant decrease from 4th to 5th group (Table 1). No specific literature was detected on the micrometrical parameters of tunica serosa of bursa of Fabricius in support of present investigation.

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