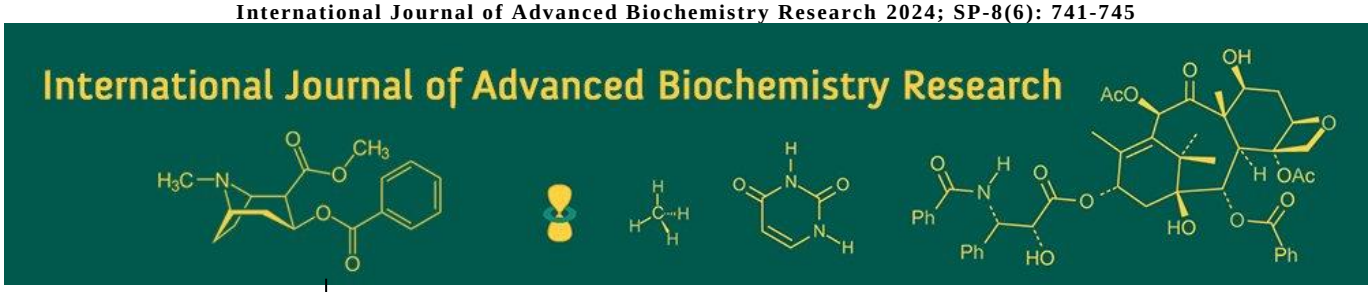

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Age-related involutory changes in bursa of Fabricius of Chabro bird

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Abstract
The study aimed to provide the basic findings about the age-related involutory changes, the study 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. The bursa of Fabricius was a sac-like structure connected to the dorsal wall of the proctodeum of the cloaca by a small stalk. Histologically, the wall of the bursa of Fabricius was comprised of three basic layers tunica mucosa, tunica muscularis and tunica serosa. The tunica mucosa consisted of lining epithelium and lamina propria. The epithelium covering the plicae were divided into two types viz, follicle associated epithelium (FAE) and the interfollicular epithelium (IFE). The epithelium was made up of pseudostratified columnar ciliated epithelium. The lamina propria filled with lymphoid follicles of different shape and size. The tunica muscularis consisted of an outer circular and inner longitudinal smooth muscle layer. The outermost tunica serosa was thin. The involutory changes started from the 3rd group. Depletion of the lymphocytic population from the periphery of the cortex, medulla and separation of cortex from adjacent follicles were noticed at the beginning of involution. An increased convolution of the inter-follicular epithelium (IFE) observed during involution from 3rd group onwards. Fatty changes in the sub epithelial and interfollicular connective tissue and formation of the epithelial cyst, area of epithelial attenuation, vacuolations, folding and detachment were found over the plical surface and in the interplical areas. In the 5th group, the plical epithelium was difficult to identify.

Keywords: Bursa of Fabricius, Chabro bird, involution

Introduction
The role of bursa of Fabricus in immunity was first describes by Glick *et al.* (1956) [8]. It is not only a primary lymphoid organ but also a secondary organ due to the presence of a T cell dependent area i.e. the diffusely infiltrated area which is located on the dorsal bursal duct (Ciriaco *et al.*, 2003 in avian) [5]. It functions as half of the bird's immune system and the size of bursa reflects the overall status of the birds Singh *et al.* (2011) [9]. It has been recognized that the bursa also functions as a peripheral gut-associated lymphoid organ. Antigens presented via the cloaca and bursal lumen can stimulate specific antibody production by bursal lymphocytes (Lupetti *et al.*, 1984 in chicken) [17]. Direct contact between lymphoid elements and material actively taken up from the cloacal lumen occurs regularly within bursal follicles. Thus, bursa also plays a role in local gut immunologic defence. A critical component of the local bursal response is the surface epithelium of the bursa overlying the medullary region of the lymphoid follicles the follicle-associated epithelium (Houssain *et al.*, 1986) [11].
Review of literatures shows no information about the involuntary changes in the bursa of Fabricus in Chabro bird. Therefore, the present research was designed to understand the involuntary changes in the bursa of Fabricius of Chabro birds during their post hatch stages in Chabro bird.

Materials and Methods
The present study was conducted on the bursa of Fabricius of thirty healthy Chabro birds irrespective of sex. The birds were procured at the time of hatching and were grown up to 150 days of age these were divided into five groups i.e. 0, 30, 60, 90 and 150 days after hatching each group contained six birds. The abdominal cavity of each bird was opened

through the careful dissection. The micrometrical studies were conducted with the help of computerized Leica DM750 microscope. The micrometrical observation was subjected to statistical analysis by SPSS-20 software to find the test of significance with age by using one-way ANOVA in various parameters (Snedecor and Cochran, 1994) [23].

Results and Discussion

The wall of the bursa of Fabricius was comprised of three basic layers within outwards; innermost tunica mucosa, middle tunica muscularis and outermost tunica serosa. The tunica mucosa consisted of lining epithelium and lamina propria filled with lymphoid follicles (Fig.1). The tunica muscularis consisted of an outer circular and inner longitudinal smooth muscle layer. The outermost tunica serosa was thin and enclosed the whole organ in all the groups. These findings were similar with the reports given by Hodges (1974) [10] in domestic fowl; Singh *et al.* (2006) [22] and Tamilselvan *et al.* (2017) [24] in Guinea fowl, Kanasiya *et al.* (2018) [12] in Kadaknath birds; Ayman *et al.* (2020) [2] in Sonali Chicken and Yaren and Boydak (2023) [15] in Henna Partridge.

The tunica mucosa of 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) [9] in Quail and Kempashi *et al.* (2017) [13] in Nandanam chicken. The lining epithelium was made up of pseudostratified columnar epithelium while it was simple columnar in crypts. 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.2 and 3) and the interfollicular epithelium (IFE) covering the remaining part of the plicae consisting of darkly stained columnar cells i.e. between the follicles (fig.2 and 3). The present results were in agreement with the observations of Ackerman and Knauff (1959) [1] in chicken and Hodges (1974) [10] in domestic fowl; Villanueva and Beranardo (2015) [25] in Game fowl; Kempashi *et al.* (2017) [13] in Nandanam chicken; Kanasiya *et al.* (2018) [12] in Kadaknath birds; Ayman *et al.* (2020) [2] in Sonali Chicken and Yaren and Boydak (2023) [15] in Henna Partridge. Bockman and Cooper (1973) [4] in Chicken speculated that the FAE cells provide a specialized environment which induces the initial migration of stem cells into the bursal epithelium.

The lamina propria was consisted of connective tissue framework filled with lymphoid follicles of different shape and size in all age groups, these observations were in accordance with the findings of Kempashi *et al.* (2017) [13] in Nandanam chicken and Kanasiya *et al.* (2018) [12] in Kadaknath birds. The individual follicles were separated from each other by layer of interfollicular connective tissue (fig.3 and 4) in all age groups as observed by Tamilselvan et al. (2017) [24] in Guinea fowl and Yaren and Boydak (2023) [15] in Henna Partridge. Each lymphoid follicles were comprised of outer darker cortex and inner pale large medulla separated by a cortico-medullary junction (fig.5). Cortico-medullary junction consisted of a layer of epithelial cells with distinct basement membrane and thin layer of capillary network (fig.3 and 5). These findings were supporting the findings of Tamilselvan *et al*. (2017) [24] in Guinea fowl and Kanasiya *et al.* (2018) [12] in Kadaknath birds.

The involutory changes started from the 3rd group. In the 3rd group, eosinophilic, elongated, and flask-shaped follicles of various sizes were evident (fig.9). Depletion of the lymphocytic population from the periphery of the cortex, and medulla and separation of cortex from adjacent follicles were signs of the beginning of involution (fig.10 and 11) similar observations were made by Tamilselvan *et al.* (2017) [24] in Guinea fowl; Ayman *et al.* (2020) [2] in Sonali Chicken and Yaren and Boydak (2023) [15] in Henna Partridge. The depletion of lymphocytes and replaced by fibroblast cells and adipose tissue, an extension of the follicular medulla, and accumulation of eosinophilic aggregates containing degenerating lymphocytes with pyknotic nuclei were observed. The present study was in concordance with Khenenou *et al.* (2012) [14] in Broiler chicken and Ebru *et al.* (2015) [6] in Long Legged Buzzard they reported that in the regression there was a loss of lymphocytes from the cortex and medulla, the bursa was fibrosed, diminished in size and remains as a small dorsal sac on cloaca for some times after sexual maturity. An increased convolution of the inter-follicular epithelium (IFE) in process of involution was observed from 3rd group in accordance with the study of Leena *et al.* (2009) [16] in domestic fowl and Farner *et al.* (2012) [7] in avian species who reported that an increased convolution of the IFE observed during involution. The cyst was noticed in the epithelium and medulla of lymphoid follicles (fig.6) observation was noticed by Leena *et al.* (2009) [16] in domestic fowl and Yaren and Boydak (2023) [15] in Henna Partridge. Goblet cells were also observed in the epithelium during involution. Epithelial proliferation followed by degradation and exfoliation was noticed from the 3rd group onwards (fig.7). These findings concur with those observed by Scala *et al.* (1989) [20] in ducks and Yaren and Boydak (2023) [15] in Henna Partridge. Vacuolation in the medulla and epithelial cells was noticed in 3rd group. This increased with the advancement of age as mentioned by Bickford *et al.* (1985) [3] in White leghorns and Tamilselvan *et al.* (2017) [24] in Guinea fowl. Fatty changes in the sub-epithelial and interfollicular connective tissue and formation of the epithelial cyst were seen from 2nd group as mentioned by Saifuddin *et al.* (1988) [19] in Shaver cockerels and Tamilselvan *et al.* (2017) [24] in Guinea fowl. Large cysts were found in the medulla in 3rd group and the number increased with the advancement of age (fig.7). Initially, cysts were organized with simple squamous epithelium, and with the advancement of age, the cysts were surrounded by a thin rim of lymphoid cells interposed in dense connective tissue. Free lymphocytes or lymphoid aggregate were seen in the lumen in the early stage of involutions. Changes in plical or interplical epithelium apparently allowed spillage of lymphocytes into the lumen from degenerating follicles (fig. 9 and 11) in the 4th and 5th group. Area of epithelial attenuation, vacuolations, folding, and detachment was noted over the plical surface and in the interplical areas, especially in the interplical area (fig.7). In the 5th group, the plical epithelium is difficult to identify. The significant decrease in the height of epithelium in the 4th and 5th groups in comparison to the 3rd group significantly increased the vacuolated area in the epithelium (fig.7). Similar observations were made by Leena, *et al.* (2009) [16] in domestic fowl, and Tamilselvan *et al.* (2017) [24] in Guinea

fowl. With the loss of lymphocytes from the medulla, the FAE dipped into the follicle giving a button-like appearance (fig.6) similar observations were made by Leena *et al.* (2009) [16] in domestic fowl. The loss of lymphocytes from the cortex and medulla gave the acinar structure of the follicles as observed by Leena *et al.* (2009) [16] in domestic fowl and Tamilselvan *et al.* (2017) [24] in Guinea fowl and Yaren and Boydak (2023) [15] in Henna Partridge. With decrease in the size of the follicles the amount of collagen and reticular fibers increased in the subepithelial and interepithelial connective tissue present study was in accordance with the Tamilselvan *et al.* (2017) [24] in Guinea fowl and Yaren and Boydak (2023) [15] in Henna Partridge. The tunica muscularis layer became disorganized (fig.8) as reported by Leena *et al.* (2009) [16] in domestic fowl and Tamilselvan *et al.* (2017) [24] in Guinea fowl. The involutory changes were started from the 3rd group. Present study was resembled with the Tamilselvan *et al.* (2017) [24] in Guinea fowl reported that the involutory changes started at the age of two months, Singh *et al.* (2006) [22] in Guinea fowl reported that the involution started after 8th weeks of age and Khenenou *et al.* (2012) [14] reported that bursa of Fabricius undergoes a slow anatomical involution started from the 10th week of age with atrophy of the follicles and the complete regression of bursa appears clearly to the 27th week and remains in the fibrous state in adult broilers. Whereas Millicevic *et al.* (1986) [18] in Prelux Broiler chicken noted that involution was started at 24th week of age in fowl.

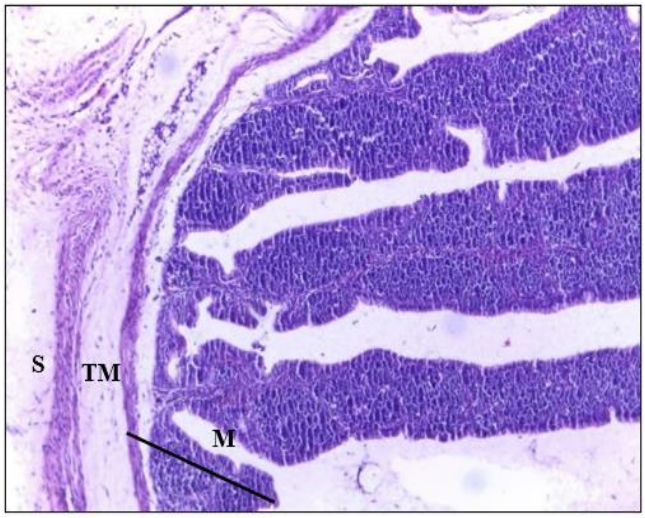

Fig 1: Photomicrograph of bursa of Fabricius of 0 day old chabro bird showing tunica mucosa (M), tunica muscularis (TM) and tunica serosa (S). (H&E x 100)

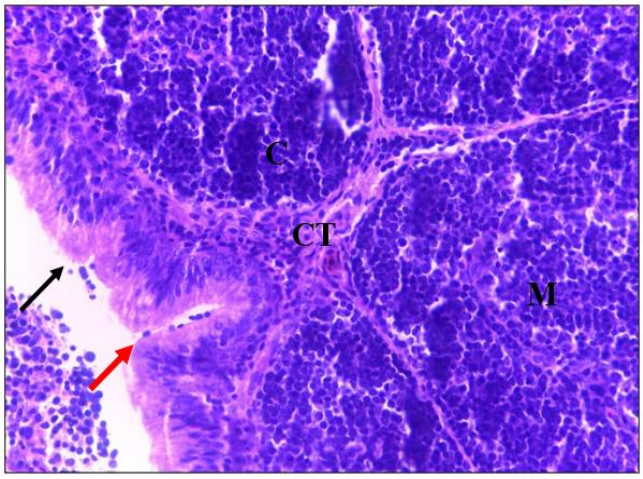

Fig 2: Photomicrograph of bursa of Fabricius of 60 days old chabro bird showing Follicle Associated Epithelium (FAE), Interfollicular Epithelium (IFE), medulla (M), cortex (C) and interfollicular connective tissue (CT). (H&E x 100)

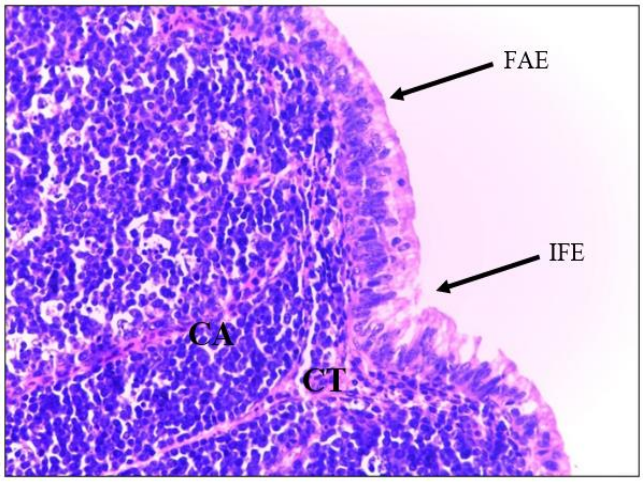

Fig 3: Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing Follicle associated epithelium (FAE), Interfollicular epithelium (IFE), interfollicular connective tissue (CT) and capillaries (CA). (H&E x 400)

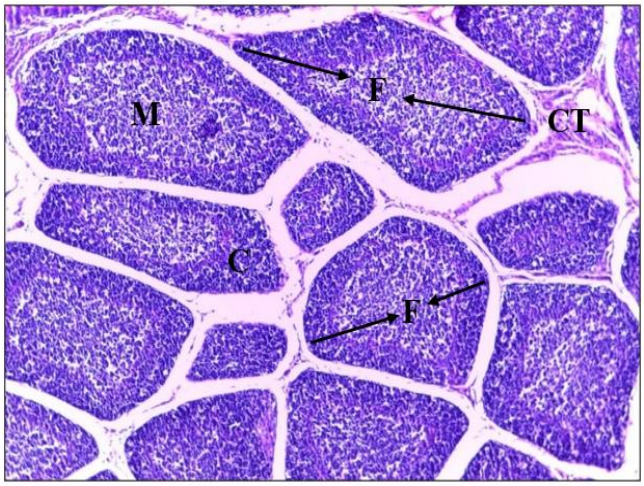

Fig 4: Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing lymphoid follicles (F), interfollicular connective tissue (CT), cortex (C) and Medulla (M). (H&E x 100)

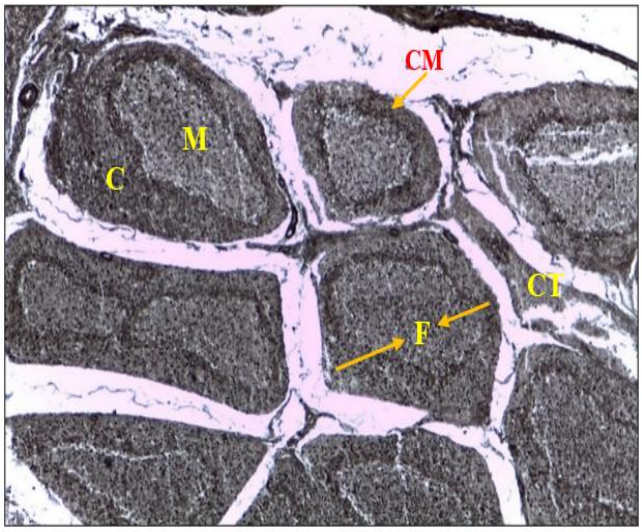

Fig 5: Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing cortico-medullary junction (CM), lymphoid follicles (F), interfollicular connective tissue (CT), cortex (C) and Medulla (M). (Sudan Black-B Stain x 100)

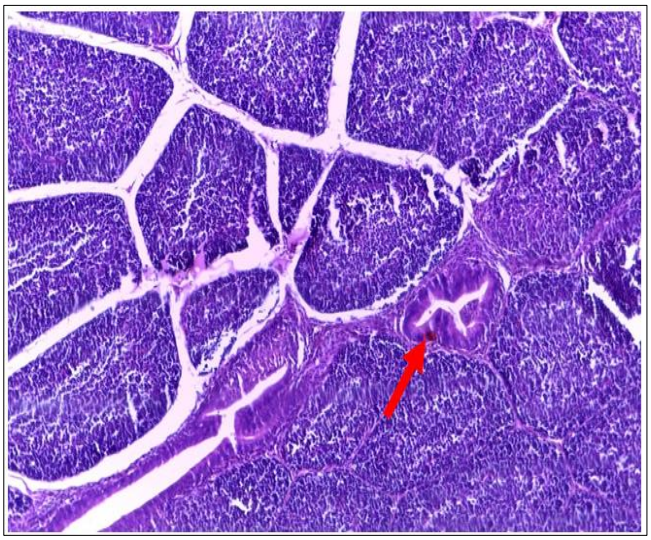

Fig 6: Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing button shaped epithelium in between the follicles (red arrows). (H&E x 100)

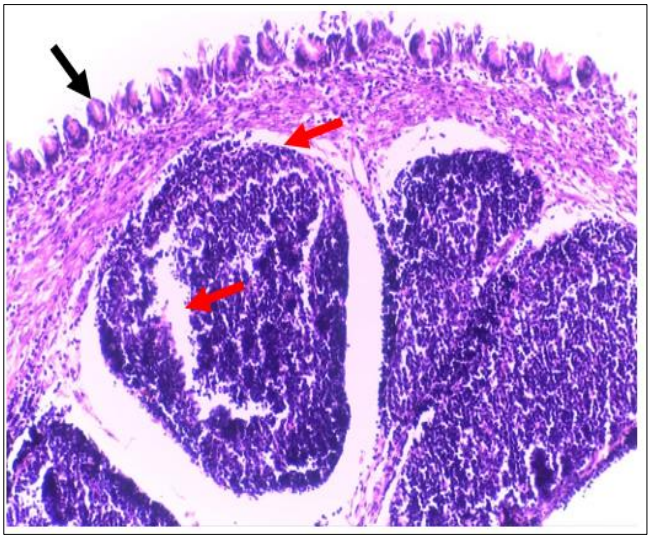

Fig 7: Photomicrograph of bursa of Fabricius of 150 days old chabro bird showing cyst in medulla (red arrows) and epithelial proliferation and vacuolations (black arrow). (H&E x 200)

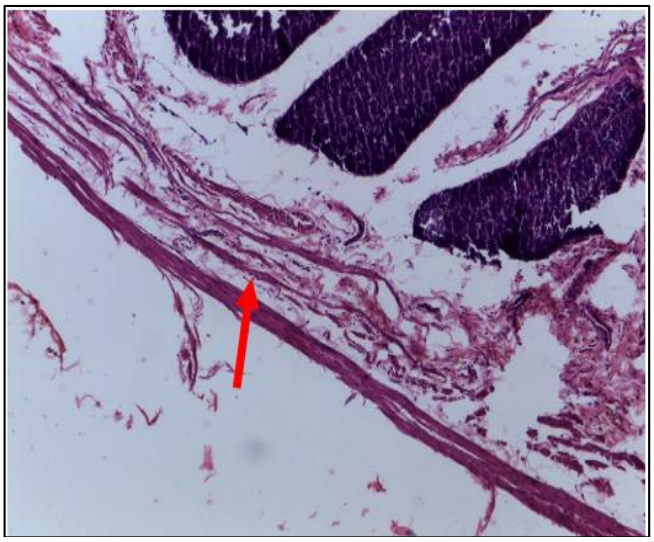

Fig 8: Photomicrograph of bursa of Fabricius of 150 days old chabro bird showing disorganised tunica muscularis (red arrow). (H&E x 200)

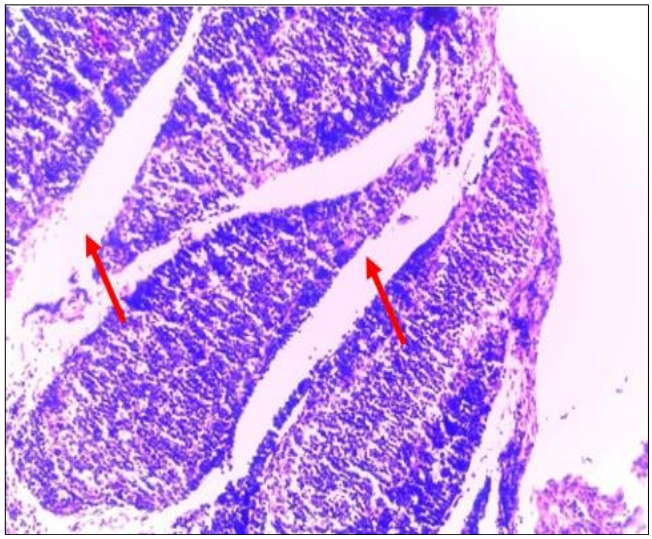

Fig 9: Photomicrograph of bursa of Fabricius of 150 days old chabro bird showing involutory changes flask shaped follicles, epithelial attenuation, vacuolations, folding and detachment (red arrow). **(H&E x 200)**

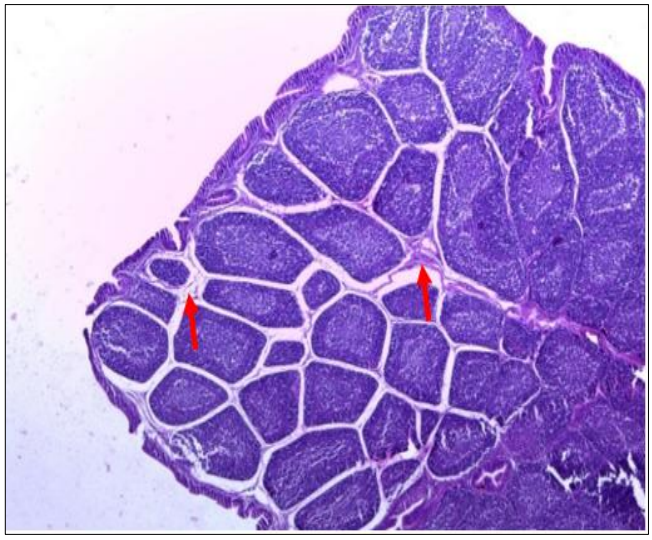

Fig 10: Photomicrograph of bursa of Fabricius of 90 days old chabro bird showing involutory changes (red arrows). (H&E x 200)

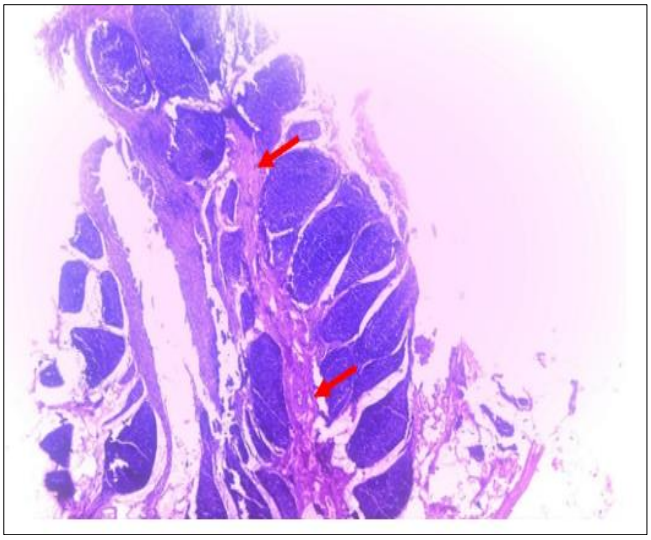

Fig 11: Photomicrograph of bursa of Fabricius of 150 days old chabro bird showing cyst in between the follicles (red arrows). (H&E x 200)

Conclusion

The bursa of Fabricius exhibits a well-defined structural organization, consisting of three distinct layers: the innermost tunica mucosa, the middle tunica muscularis, and the outermost tunica serosa. The tunica mucosa is characterized by its lining epithelium and lymphoid follicles, while the tunica muscularis contains smooth muscle layers. The tunica serosa is a thin, encompassing outer layer. Involution of the bursa begins around the third age group, marked by a reduction in lymphocyte population, fibroblast cell replacement, and structural changes in the follicles. These findings align with previous studies across various avian species, underscoring the consistent anatomical and histological characteristics of the bursa of Fabricius during both its functional and involutory phases.

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