Egypt. J. Vet. Sci. **Vol. 56,** No. 1, pp. 127-138 (2025)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Histomorphometrical and Immunohistochemical Study of the Maturation of Bursa of Fabricius in Rose Male Broiler Chicken at Post-Hatching Period

Shayma Z. Ameen[1], Yahya A. Mohammed[1] and Mahdi A. Abdullah[2*]
[1]Department of Theriogenology, Anatomy and Physiology, College of Veterinary Medicine, University of Duhok, Iraq.
[2]Department of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Iraq.

THE PRESENT STUDY was conducted on 180 male chicks (RMBCs) that were categorized into nine groups based on the post hatching age: D1, D4, D7, D14, D21, D28, D35, D42 and D58. To accomplish this research, routine and special stains as well as mouse anti-chicken; monoclonal anti-Bu-1 antibody markers were employed. The epithelium of the bursa of Fabricius possessed simple columnar epithelium, M-cells and intraepithelial lymphocytes. The M-cells were characterized by vesicular nuclei and pale cytoplasm that did not stain with H&E, PAS and Diastase-PAS. The lymphoid follicles of the bursa consisted mainly of lymphocytes as well as lymphoblasts, reticular cells, plasma cells, macrophages and dendritic cells. The bursal lymphoid follicles obviously appeared at D1 old and then continued during bursal age. At D7 old, a layer of cuboidal cells associated with blood capillaries clearly appeared in the corticomedullary border. The number of the lymphoid follicles gradually decreased significantly ($P < 0.05$) with the advance age, whereas the diameter of the same follicles increased significantly ($P < 0.05$) with progress of age also. The Bu-1 positive B-lymphocytes were chiefly located in cortex and medulla of the lymphoid follicles from D1-D58 old. The study concluded that the maturation of the lymphoid follicles of the bursa in this breed was depending on age.

Keywords: Rose Broiler Chicken, Bursa of Fabricius, Histomormphmetric, Immunohistochemistry.

Introduction

The Ross 308 chicken is the world's number one broiler breeder brand. It is considered as a hybrid breed: (the Ross chicken from Scotland and the Cobb chicken from the United Kingdom). To meet the growing demand for animal protein, world poultry meat production soared from 9 to 122 million tons between 1961 and 2017 [1]. Chickens are playing a significant role in the national economy and reducing poverty by supplying meat and eggs. The immune system of the chicken is very helpful in preventing disease and helping to ensure maximum productive potential is realized [2]. The acquired or adaptive immune system of all vertebrates (including birds) is mediated by immunocompetent cells which can be categorized into two arms: humoral immunity (humoral response B-cells) and cellular immunity (cell mediated response T-cells) and some other cells like phagocytic and adherent cells [3,4]. The bursa of Fabricius (BF) is responsible for the development and differentiation of B-lymphocytes and immunoglobulin isotype

*Corresponding author: Mahdi Ali Abdullah, E-mail: mahdi.ali@uod.ac., Tel.: 07507344094
(Received 15/02/2024, accepted 01/04/2024)
DOI: 10.21608/EJVS.2024.268880.1837
©2025 National Information and Documentation Center (NIDOC)

switch causes [5]. It has been recognized that the bursa is also considered as a secondary gut-associated lymphoid organ. It has been clearly demonstrated that developmental changes in lymphoid organs of birds can be associated with a drastic change in their body functions [3]. The structural development of the BF can affect the susceptibility of chickens to infectious bursal disease viruses [6]. The Microfolds (M-cells) are present in the epithelium of the BF [7]. The antigen sampling and processing by M-cells is critical to the initiation of immune responses in the avian mucosa-associated lymphoid tissues (MALT) [8]. Though some work on BF of broiler chicken [9], in sonali chicken [10] has been done. According to review of literature, there is no overall study on post hatching maturation of this organ in RMBCs in Kurdistan-Region of Iraq. Keeping this fact in mind and viewing the increased popularity of the RMBCs, the present study is undertaken to develop a baseline data in this breed. Therefore, the present research is designed to understand the morphological, morphometrical and immunohistochemical characteristics of the BF of RMBCs during their postnatal growth.

Material and Methods

Ethical approval

The study protocol and animal experimentation were approved in line with the guidelines set by the Animal Welfare and Experimentation Ethics Committee at Duhok University, college of veterinary medicine, (DR1996919CV approved on June 11, 2019).

Animals and study design

A total of 180 newly hatched (one day age) Rose male broiler chicks(RMBCs)-genetic line 308 (Avex and Garanti Company) were apparently healthy and unvaccinated. These birds were obtained from Jeen hatchery in Marina Village-Duhok Province, Kurdistan-Region of Iraq. The birds were transported in sterile cages and kept in the sterile room under strict hygienic conditions that had dimensions (length 3m × width 2.5m × height 2.40m) in a poultry Farm, College of Veterinary Medicine, University of Duhok. Then, the food and water were provided ad libitum. As well as the uniformity of feeding history, management practices were taken into consideration as much as possible during the whole of the study [11]. The 180 chicks were categorized into nine groups based on the age starting from day one (D1) of hatching: D1, D4, D7, D14, D21, D28, D35, D42 to D58. Each age group was composed of 20 chicks (10 chicks were used for anatomical study, while the other 10 chicks were employed for histomorphometrical and immunohistochemical study. The first group deals with it immediately, while the other group's deals with it later.

Collection of samples

Chickens from each group were authanized by inhalation anaesthesia with chloroform. After authanization the feathers were removed manually. Each bird put on a back and make a surgical incision in the midline of the abdomen, then reflect the peritoneum and take the organ after removing the adjacent tissue. The BF was collected after careful separating it from the adjacent organs. Thereafter, the BF washed thoroughly with 1% phosphate buffer saline or 0.9% normal saline [12]. The samples of BF were taken after making a longitudinal section from pole to pole **(Fig. 1)**. Some samples of the organ were fixed independently in 10% neutral buffered formalin for 24- 48 hours and another sample was immersed in Bouins solution for 4-18 hours [12].

Preparation tissue specimens

All samples were processed using a routine paraffin technique at the laboratory of Duhok Research Center, College of Veterinary Medicine, University of Duhok, Iraq. Tissue specimens were washed with running tap water after fixation. The specimen was dehydrated with ascending grade of ethyl alcohol followed by clearing in xylene, then infiltrated and embedded in paraffin wax (58-60 ^{o} C) [12]. Serial and step serial tissue sections with 4- 5 µm thick were prepared by using a rotatory microtome (Leica RM 2245, Germany) and stained by the following dyes [12]:

1. Harris hematoxylin and eosin stain (H&E) was used to demonstrate the general tissue architecture.
2. Masson's trichrome stain (MT) was employed to identify the presence of collagen and muscle fibers.
3. Silver impregnation (SI) was utilized for the demonstration of the reticular fibers.
4. Periodic acid-Schiff's (PAS) was performed to demonstrate neutral polysaccharides and different types of glycoproteins.
5. Diastase- Periodic acid-Schiff's (Diastase-PAS) was used to detect glycogen and establish the glycoproteins.

Morphometrical and Statistical analysis

The sections treated with H&E and MT stains were used for the morphometric analysis. Image measurements were captured and examined using a light microscope (Olympus *CX22*, Japan) equipped with a digital microscopic using the Omax 18.0 MP USB 3.0 camera (code, A35180U3; USA).The measurements include the following parameters: number of lymphoid follicle {in 10x microscopic field}, mean lymphoid follicle diameter, thickness of cortex & medulla of BF per specific area. Approximately ten cross-sections were measured from the BF using a medium power lens (10 x magnifications) for each of the mentioned parameters. All data were presented as means ± SE (standard error) and were analyzed by using one -way analysis of variance (ANOVA) with significant level set on $P < 0.05$. Specific differences among the different post hatching age groups were examined using Tukey test by using JMP Pro 14.3.0 (JMP Pro Statistical Discovery LLC; Marlow, Buckinghamshire, England

Immunohistochemical approaches

The Anti-Bu-1 antibody served as the primary marker for the B-lymphocytes (BCs+) in the BF indicating their localization, distribution, expression and density during various post-hatching maturation. Three-µm-thick paraffin sections were utilized and processed according to the specified procedure provided by the primary antibody's (mouse anti-chicken; monoclonal anti-Bu-1 antibody [Catalogue no. MA5- 28700], Thermo fisher scientific; Invitrogen USA Company).

Results

The BF of RMBCs were thoroughly investigated at post-hatching D42 old, because this day was considered a slaughter day [13]. Subsequently, the findings from this day were compared with those obtained from other post-hatching days.

Anatomically, in the present study the BF of RMBCs was a smooth globular to ovoid shaped organ situated dorsal to the caudal third of the cloaca **(Fig 2)**.

Histological and Histochemical findings of BF

The bursal wall of RMBCs at D42 old comprehend of three layers from the inside to outside: Tunica mucosa (TMU), Tunica muscularis (TM) and Tunica serosa (TS) **(Fig. 3)**. The bursal TMU thrown into number of longitudinal folds known as the bursal plicae which directed to the bursal lumen. The TMU was comprised of epithelium and lamina propria (LP). Core of highly vascularized connective tissue of the collagen and reticular fibers **(Fig. 4)** as well as individual smooth muscle fibers from the TM formed the center of the plicae.

Thin septa of reticular and collagen fibers originated from the core of the plicae surrounding each lymphoid follicle (LF) **(Fig. 4 and 5).** Each plica was covered by two types of surface epithelium; Inter follicular epithelium (IFE) (pseudostratified columnar epithelium) alternating with follicle associated epithelium (FAE) (simple columnar epithelium) **(Fig.6)**. The IFE was formed of columnar and basal cells that rest on the basement membrane. The nuclei of columnar cells were oval in shape which present at different levels in the epithelium, while the basal cells possessed rounded to oval nuclei. The cytoplasm of columnar cells stained pink with eosin **(Fig. 7a and b)**.

The most cytoplasm of the former cells is intensely stained with PAS **(Fig. 8)** and Diastase-PAS. The cytoplasm of basal cells stained pink with eosin and negatively with PAS and Diastase-PAS**.** The FAE comprehends mostly one layer of columnar cells. These cells possessed oval to rounded light staining nuclei which situated mostly at the upper third of the epithelium. The cytoplasm of the FAE cells appeared slightly pink with eosin **(Fig. 6)** and did not stain with PAS and Diastase-PAS.

Among columnar cells of both the IFE and FAE, the M-cells were noticed mostly scattered singly in the upper third of the epithelium. The shape of these cells varied from rounded to oval. The nuclei of M-cells were vesicularly rounded to oval. The cytoplasm of M-cells did not stain with eosin **(Fig. 7a, b)**, PAS and Diastase-PAS **(Fig. 8)**. The intra epithelial lymphocytes (IELs) and intra luminal lymphocytes (ILLs) were present in the IFE, FAE and luminal bursa. These cells contained rounded dark stained nuclei and were surrounded by a narrow rim of unstained cytoplasm **(Fig. 6 and 8)**. The BF possessed mostly oval to irregular shape LFs which filled the LP within the plicae **(Fig. 3).** Each LF was composed of a peripheral dark staining cortex and a central light staining medulla **(Fig. 9)**. Only the medulla of LFs was contacted directly with FAE **(Fig. 6)**. The network of reticular fibers was present in the cortex of the LFs, while the medulla devoid from these fibers **(Fig. 4)**. The corticomedullary border (CMB) that

separates the cortex from the medulla is composed of a single layer of cuboidal cells associated with blood capillaries **(Fig. 9)**.

Both cortex and medulla are a population of cells that are distributed randomly and include; numerous lymphocytes, lymphoblasts, plasma cells, reticular cells, macrophages and dendritic cells. The boundaries of these cells could not be demonstrated under the light microscope, so these cells were identified by their nuclei. The nucleus of lymphoblast was a large open face with one or two prominent nucleoli. The lymphocytes were characterized by dark heterochromatic nuclei. The nuclei of plasma cells had cartwheel appearance. The reticular cells possessed large light oval nuclei. The nucleus of macrophages was bean shaped. The dendritic cells possessed pear to elongated vesicular nucleus mostly contained peripheral heterochromatin with condensed nucleolus **(Fig. 10)**.

The TM of the BF was composed of inner (circular) and outer (longitudinal) layers of smooth muscle fibers.

The TS was formed of layers of loose connective tissue containing blood vessels, nerves, trunk, and adipose tissue that was invested by the mesothelial layer of peritoneum.

Post-hatching maturation of BF

The layers of the bursal wall from D1-D58 old were similar to those of the D42 old, but the thickness of these layers increased with the progress age. At D1 old the plicae were small in size leaving wide spaces between them and became more matured with the advanced age. The epithelial cells in both IFE and FAE were similar to those of the epithelium at D42 old, except that the M-cells were decreased in number. The LFs appeared small and the CMB was not well differentiated, because of the absence of the layer of cuboidal cells. The cell population of the LFs was similar to those of D42 old, and increased in number during aging of bursa **(Fig. 11)**. At D4 old, the LFs appeared more organized compared with D1 old and the CMB was poorly clear.

At D7 old, the LFs were fully organized and the layers of cuboidal cells in the CMB were clearly visible**.** The epithelium was more maturated and the number of M-cells were obviously increased **(Fig. 12)**. All the elements that had been mentioned in the BF from D14 to D58 old were similar to those of the D42 old, except at D58 old the LFs appeared very large in diameter **(Fig. 13).**

Histomorphometrical findings of BF

The number of the LFs of BF was gradually decreased significantly ($P < 0.05$) with the progress of age from D1-D35 old, while from D35-D58 old was decreased non-significantly. Furthermore, the diameter of the LFs from D1-D58 old was increased significantly ($P < 0.05$). The thickness of the cortex of the LFs was increased significantly ($P < 0.05$) from D21-D58 old, whereas the thickness of medulla was increased significantly ($P < 0.05$) from D7-D58 old. As shown in **(Fig. 14 and 15)**.

Immunohistochemical findings of BF

Generally, the (BCs+) were mainly located in both cortex and medulla of the LFs. Post-hatching immunhistochemical result showed that at D1 and D4 old, the LFs possessed moderate expression for (BCs+) whereas, from D7 to D58 old, both cortex and medulla of the LFs showed strong expression for (BCs+). Furthermore, the medulla of the LFs was referred to as a stronger expression than the cortex. As shown in (**Fig. 16)**

Discussion

Histological observation of BF

The current study showed that the surface epithelium of BF of RMBCs included both the IFE (pseudostratified columnar epithelium) alternating with FAE (simple columnar epithelium) confirming the findings of [14,9] in broiler chicken. In contrast, [5] in Guinea Fowl stated that the IFE was stratified cuboidal type and FAE was pseudostratified columnar type. These changes in the epithelium of BF were largely due to variability among breeds. In RMBCs the cytoplasm of columnar cells of the IFE intensely stained with PAS and Diastase-PAS positive reaction, while the cytoplasm of columnar cells of the FAE was not stained with the same stain. Similar findings were noted by [15] in Nandanam chicken and [16] in Chabro Bird. The results exhibited the PAS and Diastase- PAS positive reactions of these cells indicating the presence of neutral glycoprotein material in their cytoplasm. The post hatching of our results revealed that there were no cytological and histochemical differences of the columnar cells in both IFE and FAE. Our date showed the cytological features of the M-cell were similar to that of [17] in chicken. M-cells were highly specialized for the phagocytosis and transcytosis of macromolecules from the gut lumen particulate antigens and pathogenic or commensal microorganisms across epithelium. After transcytosis across the FAE, antigens exit

into the intraepithelial pocket beneath the M-cell basolateral membrane which contained various populations of lymphocytes, dendritic cells and mononuclear phagocytes, such as macrophages [18,19,20]. The present investigation revealed that the M-cells were observed in both types of epithelium; IFE and FAE of the BF. Contrarily to present study, [21] in turkey and chicken exhibited that the M-cells were present in the FAE of the BF. The occurrence of the M-cells in the IFE of our study might be immature cells. In this aspect, the presence of M-cells in the FAE of Peyer's patches were regarded as a mature cell, while the M- cells that were located in the IFE were considered as immature or undifferentiated cells [22]. The post-hatching maturation of M-cells in the BF did not show any changes in their characteristic and position from D1-D58 old, except that increased in their number with the advanced age. The study found that the BF of RMBCs was composed of LFs. Each LF possessed a dark cortex and light medulla. The junction between the cortex and medulla was formed by a layer of cuboidal cells associated with blood capillaries. This was similar to the results of [23,24] in broiler chicken and [25] in duck. Olah *et al*. [26] in chicken was named this junction between the cortex and medulla as cortecomedullary arch that played a role in migration of B-lymphocytes in the bloodstream. The post-hatching of the current result revealed that the cortex and medulla of LFs were less organized at D1 old, because of the absence of the CMB, but at D7-58 old, this border became obvious. Similar findings were reported by [9] in Marshall broiler chicken. Both cortex and medulla comprised a population of cells; lymphocytes, lymphoblasts, reticular cells, macrophages, plasma cells and dendritic cells. This population of cells was similar to those of the cells in broiler chicken [9]. There were no differences in the population of cells from D1-D58 old, except that these cells increased in number with advanced age.

Our investigation result and other researchers; [10] in Sonali chicken and [27] in turkey were in agreement about the directly contacting medulla of the LFs with FAE. This contact was very important to activation of B-lymphocytes to produce an immune response. In this area of contact, the antigenic particles were recognized by dendritic cells and the B-lymphocytes activated and differentiated [28].

The number of LFs of the BF was decreased significantly ($P < 0.05$) from D1-D58 old, whereas the diameter of LFs of the same organ was increased significantly at the same value. This correlation between the number and diameter of LFs was conversely and this might be due to increasing the diameter of the LFs that affected their number.

The results showed that the thickness of both cortex and medulla of the bursal LFs were increased significantly with advanced age confirming with the findings of [10] in Sonali chicken. After hatch the important parameter for the development of BF and distribution of B-lymphocyte was the thickness of both cortex and medulla of the LFs [29]. In this manner, [30] in chicken mentioned that the thinner bursal cortex of the LFs mean delay of B-lymphocytes migration from the bursal medulla to the cortex and this delay could be the reason for the lower stimulation of B-lymphocytes by particles of antigens. Also the same author explained that the widened bursal cortex area could be an indicator of higher B-lymphocytes stimulation. Furthermore, significant increase of the total diameter and thickness of both cortex and medulla of bursal LFs was a natural phenomenon resulting from proliferation of B- lymphocytes within the BF. Davison, [4] documented this fact, since mentioned the proliferation and activation of B-lymphocytes of the BF and then their migration to the secondary lymphatic organs.

Immunohistochemical observation of BF

The present immunohistochemical results indicated that the (BCs+) were mainly located in both cortex and medulla of the LFs confirming with the findings of [15] in Nandanam chicken and [31]. However, Al-Ogaili and Hameed, [32] reported that around 98% of the lymphocyte population in the BF expressed the Bu-1 marker in chicken, while Sayegh *et al.* [33] and Kozlu *et al.* [34] also reported results for CD79a, CD79b and CD79αcy when examining B-receptor complexes in the BF in chicken and turkey. Our results showed BF provides a microenvironment essential for proper B-lymphocyte maturation. It became clear that interactions between immature B-lymphocytes and epithelium were required for B-lymphocytes differentiation. As previously mentioned in RMBCs both cortex and medulla of LFs possessed plasma cells. It was well known that the plasma cells responsible for production of antibodies. In this manner, Ratcliffe *et al*. [35] suggested that the majority of B-lymphocytes in the BF produced IgM (+), IgG (+) and IgA (+). The current post-hatching immunohistochemical

results revealed that there were increases in the expression of the (BCs+) in both cortex and medulla with the progress of age confirming to the report of Jeyachandra *et al.* [15] in Nandanam chicken.

Conclusion

Our results conclude that the maturation of the histological and immunohistochemical structures of the lymphoid tissue of Bursa of Fabricius in Rose Male Broiler chickens was mainly age dependent.

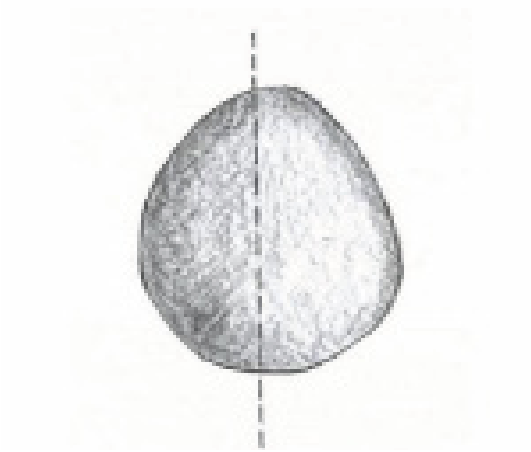

Fig.1. Schematic showed: BF in RMBCs at D42 old chick.

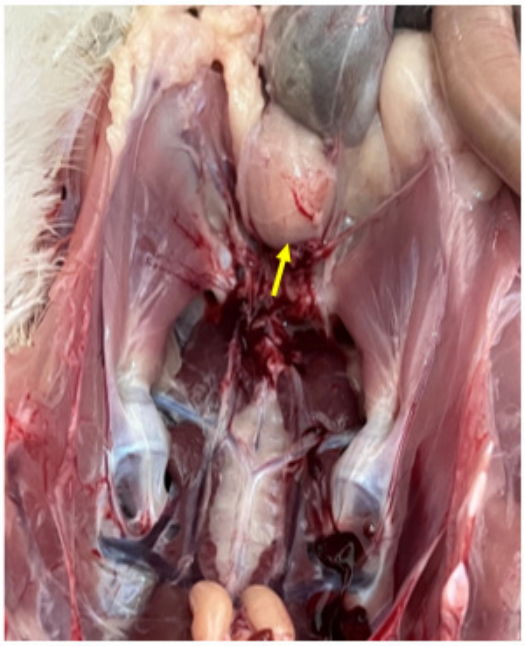

Fig. 2. Photomacrograph of the RMBCs at D42 old showing BF (yellow arrow).

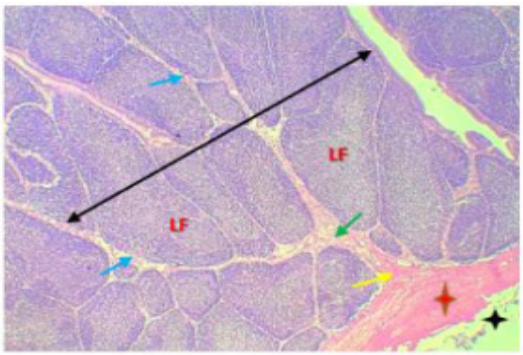

Fig. 3. Photomicrograph of BF of RMBCs at D42 showing: Plicae (black arrow), Core of connective tissue in the center of plica (green arrow), Thin septa (blue arrow), smooth muscle fibers (yellow arrow) lymphoid follicle (LF), TM (red star), TS (black star). H & E 4x.

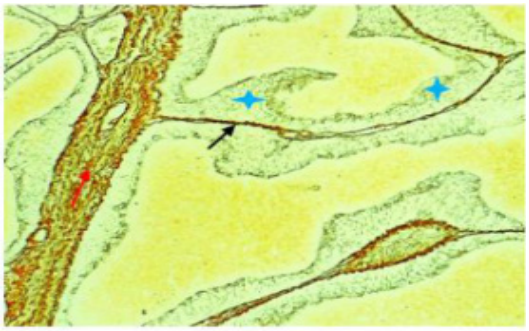

Fig. 4. Photomicrograph of BF of RMBCs at D42 showing: The reticular fibers in the center of plicae (red arrow), in thin septa (black arrow) and in the cortex of lymphoid follicles (blue star). SI 10x.

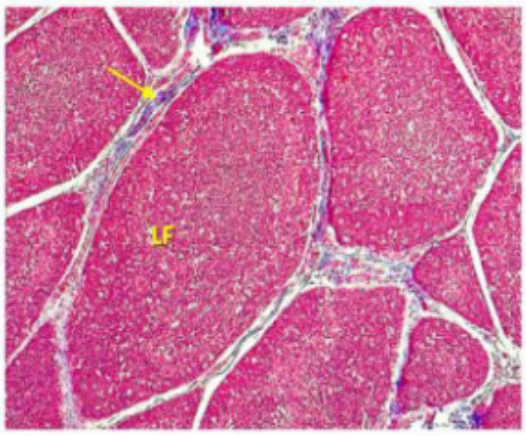

Fig. 5. Photomicrograph of BF of RMBCs at D42 showing: The collagen fibers (yellow arrow), lymphoid follicles (LFs). MT 10x.

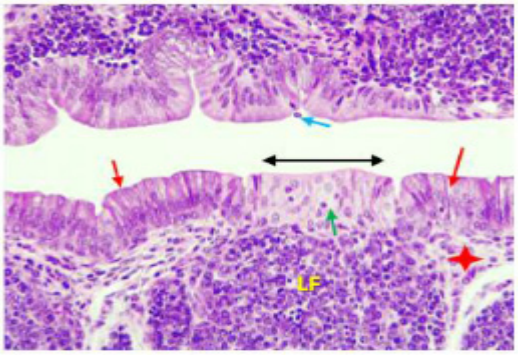

Fig. 6. Photomicrograph of BF of RMBCs at D42 showing: Inter follicular epithelium (red arrow), Follicular associated epithelium (black arrow), Intra luminal lymphocyte (blue arrow), intra epithelial lymphocyte (green arrow), lymphoid follicles (LF), Lamina propria (red star). H & E 40x.

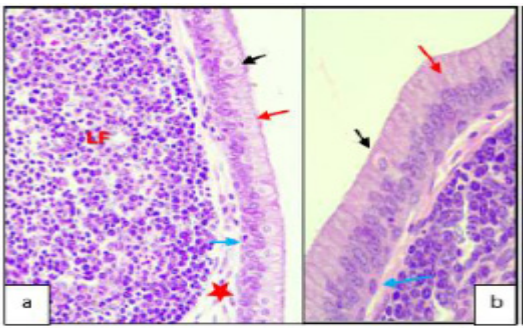

Fig.7. Photomicrograph of BF (inter follicular epithelium) of RMBCs at D42 showing: Columnar cells (red arrow), Basal cells (blue arrow) and M-cells (black arrow), lymphoid follicles (LF), Lamina propria (red star). H & E 40x (a) and 100x (b)

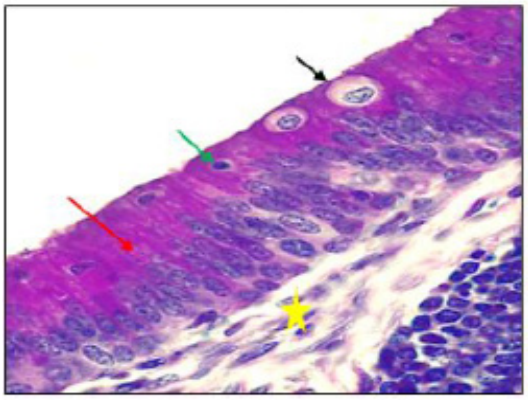

Fig.8. Photomicrograph of BF (inter follicular epithelium) of RMBCs at D42 showing: M-cells (black arrow), columnar cells (red arrow), Intra epithelial lymphocyte (green arrow), Lamina propria (yellow star). Diastase- PAS 100X.

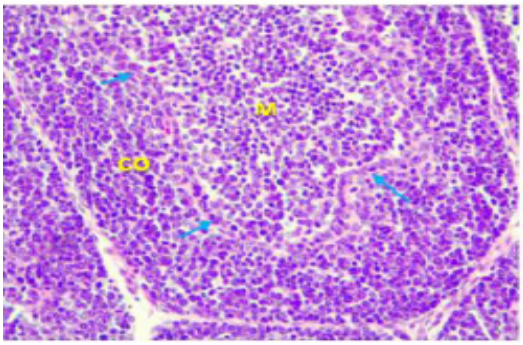

Fig. 9. Photomicrograph of LF (lymphoid follicle) of RMBCs at D42 showing: layer of cuboidal cells (blue arrow), Cortex (CO) and Medulla (M). H & E 40x.

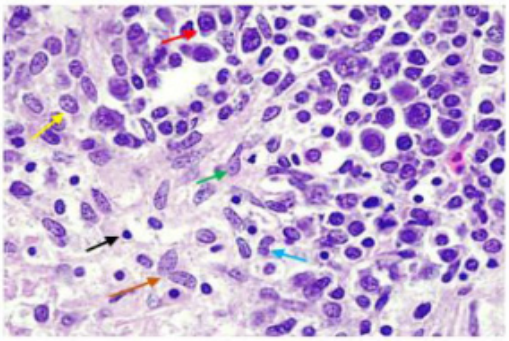

Fig. 10. Photomicrograph of BF (medulla of lymphoid follicle) in RMBCs at D42 showing: Lymphoblast (yellow arrow), Lymphocyte (black arrow), reticular cell (brown arrow), Plasma cell (red arrow) and Macrophage (blue arrow), Dendritic cell (green arrow). H & E 100x.

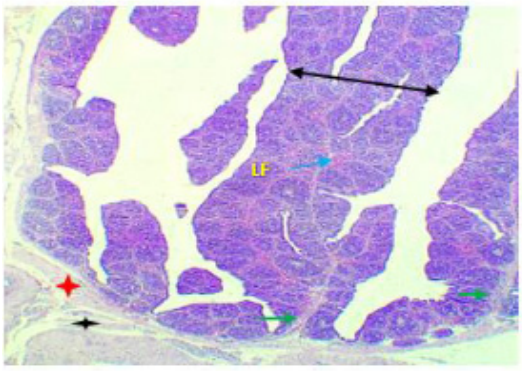

Fig. 11. Photomicrograph of BF of RMBCs at D1 showing: Plicae (black arrow), Core of connective tissue in the center of plica (green arrow), Thin septa (blue arrow), lymphoid follicle(LF), TM (red star), TS (black star) .H & E 4x.

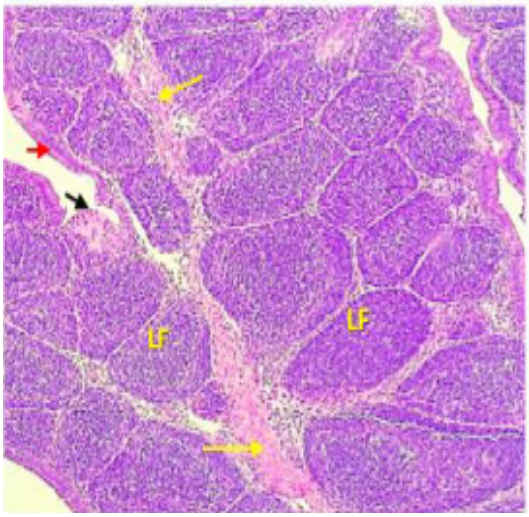

Fig. 12. Photomicrograph of BF of RMBCs at D7 showing: lymphoid follicles (LF), inter follicular epithelium (red arrow), follicular associated epithelium (black arrow), Core of connective tissue in the center of plicae (yellow arrow). H & E 10x.

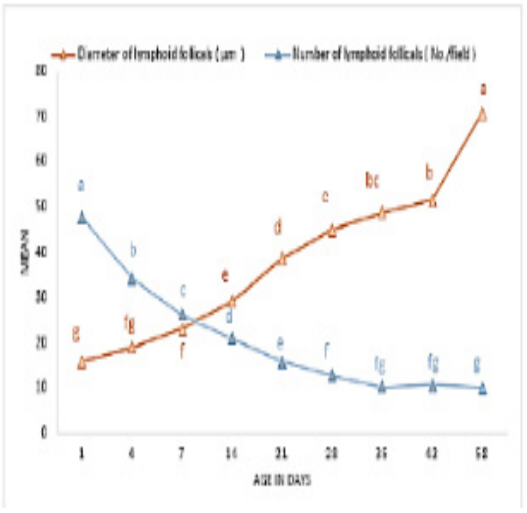

Fig. 14. Analysis of diameter and number of lymphoid follicle of BF; Mean ± Standard Error. Data with different letters with in the same line (a–g) differ significantly (P < 0 .05)

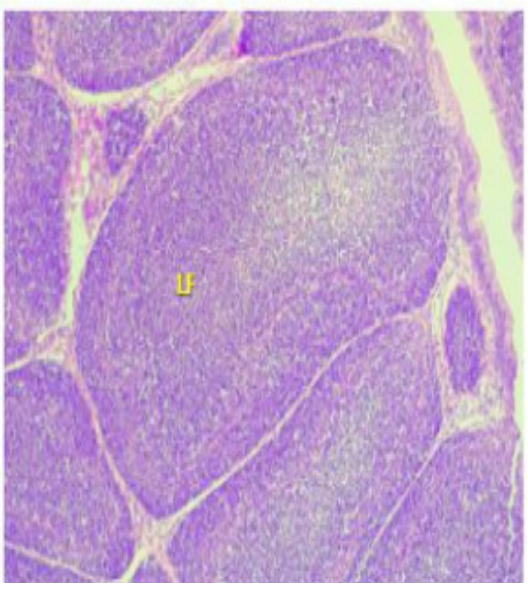

Fig. 13. Photomicrograph of BF of RMBCs at D58 showing: lymphoid follicle (LF). H & E 10x.

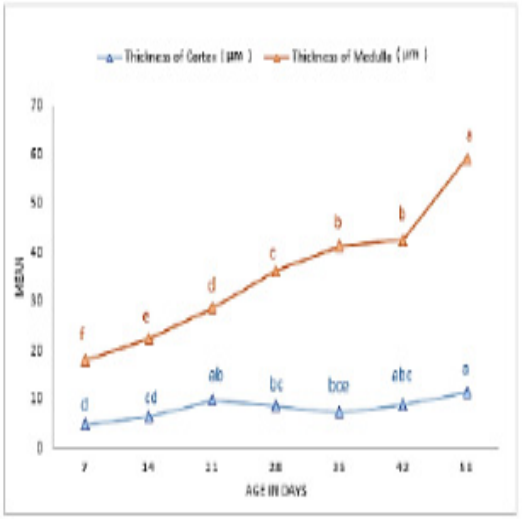

Fig.15. Analysis of thickness of cortex and medulla of lymphoid follicle of BF; Mean ± Standard Error. Data with different letters with in the same line (a–f) differ significantly (P < 0 .05)

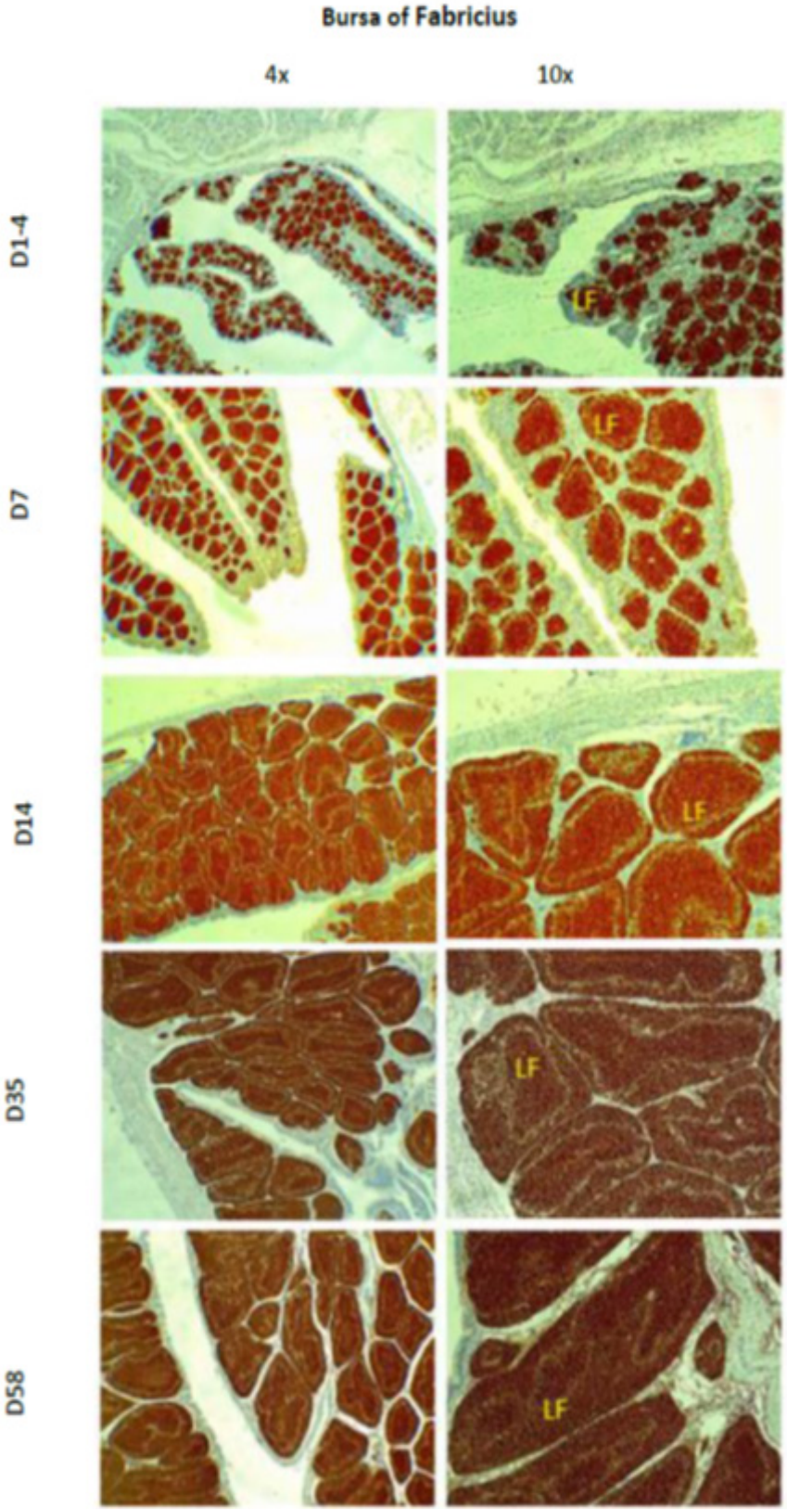

Fig. 16. Immunohistochemical expression pattern of Bu-1 positive B-cells in the BF of the RMBCs at different post hatching ages (D1-4, D7, D14, D35 and D58) showing distribution of (BCs+) in LF of BF.

References

1. FAO, F. Gateway to poultry production and products. *Food and agriculture organisation of the united nations* from http://www.fao.org/poultry-production-products/produ ction/en/(2020).
2. Tizard, I. R. Veterinary Immunology-E-Book. *Elsevier Health Sciences* (2017).
3. Ciriaco, E., Píñera, P. P., Díaz-Esnal, B. and Laurà, R. Age-related changes in the avian primary lymphoid organs (thymus and bursa of Fabricius). *Microscopy Research and Technique*, **62**(6), 482-487(2003).
4. Davison, F. The importance of the avian immune system and its unique features. In *Avian Immunology* (pp. 19-). Academic Press (2022).
5. Tamilselvan, S., Jayachitra, S. and Balasundaram, K. Age related histological changes of bursa of Fabricius in guinea fowl (Numida meleagris). *International Journal of Livestock Research*, **7**(7), 172-176 (2017).
6. Zhao, S., Jia, Y., Han, D., Ma, H., Shah, S. Z. A., Ma, Y. and Teng, K. Influence of the structural development of bursa on the susceptibility of chickens to infectious bursal disease virus. *Poultry Science*, **95**(12), 2786-2794 (2016).
7. Balic, A., Chintoan-Uta, C., Vohra, P., Sutton, K. M., Cassady-Cain, R. L., Hu, T. and Vervelde, L. Antigen sampling CSF1R-expressing epithelial cells are the functional equivalents of mammalian M cells in the avian follicle-associated epithelium. *Frontiers in Immunology*, **10**,2495(2019).
8. Casteleyn, C., Doom, M., Lambrechts, E., Van Den Broeck, W., Simoens, P. and Cornillie, P. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. *Avian Pathology*, **39**(3), 143-150 (2010).
9. Udoumoh, A. F., Nwaogu, I. C., Igwebuike, U. M. and Obidike, I. R. Pre-hatch and post-hatch development of the bursa of Fabricius in broiler chicken: a morphological study. In *Veterinary Research Forum* (Vol. 13, No. **3**, p. 301). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran (2022, September).
10. Ayman, U., Alam, M. R. and Das, S. K. Age-related development and histomorphological observations of bursa of Fabricius in sonali chicken. *J. Adv. Biotechnol. Exp. Ther.,* **3**(1), 20-28 (2020).
11. Sun, Q. F. and Pan, H. H. RFID-Based Intelligent Management System of Poultry House. *Applied Mechanics and Materials*, **433**, 1511-1514 (2013).
12. Suvarna, K. S., Layton, C., &. and Bancroft, J. D. *Bancroft's theory and practice of histological techniques*. Elsevier health sciences (2018).
13. Coban, O., Lacin, E., Aksu, M. I., Kara, A. and Sabuncuoglu, N. The impact of slaughter age on performance, carcass traits, properties of cutup pieces of carcasses, and muscle development in broiler chickens. *European Poultry Science/ Archiv* für *Geflügelkunde*, **78**, 2014.60 (2014).
14. Khan, M. Z. I., Masum, M., Khan, M. Z. I., Aziz, A. R., Nasrin, M. and Siddique, M. N. H. Histomorphology of the lymphoid tissues of broiler chickens in Kelantan, Malaysia (Histomorfologi Tisu Limfa Ayam Pedaging di Kelantan, Malaysia). *Sains Malaysiana*, **43**(8), 1175-1179 (2014).
15. Jeyachandra, K., Kannan, T. A., Basha, S. H., Raja, A. and Geetha, R. Histochemical and immunohistochemical observations of bursa of Fabricius in Nandanam chicken. *International Journal of Veterinary Science*, **6**(2), 121-126 (2017).
16. Singh, A., Singh, S. P., Farooqui, M. M., Prakash, A., Pathak, A., Sharma, A. and Vishen, A. Histochemical Observations on Bursa of Fabricius of Chabro Bird. *Science*, **23,** 163-171 (2019).
17. KITAGAWA, H., HIRATSUKA, Y., IMAGAWA, T. and UEHARA, M. Distribution of lymphoid tissue in the caecal mucosa of chickens. *The Journal of Anatomy*, **192**(2), 293-298 (1998).
18. Mabbott, N. A., Baillie, J. K., Hume, D. A. and Freeman, T. C. Meta-analysis of lineage-specific gene expression signatures in mouse leukocyte populations. *Immunobiology*, **215(**9-10), 724-736 (2010).
19. Hume, D. A., Mabbott, N., Raza, S. and Freeman, T. C. Can DCs be distinguished from macrophages by molecular signatures?. *Nature Immunology*, **14**(3), 187-189 (2013).
20. Kimura, S. Molecular insights into the mechanisms of M-cell differentiation and transcytosis in the mucosa-associated lymphoid tissues. *Anatomical Science International,* **93**(1), 23-34 (2018).

21. Olah, I. and Glick, B. Follicle-associated epithelium and medullary epithelial tissue of the bursa of Fabricius are two different compartments. *The anatomical Record*, **233**(4), 577-587(1992).

22. Mabbott, N. A., Donaldson, D. S., Ohno, H., Williams, I. R. and Mahajan, A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunology*, **6**(4), 666-677 (2013).

23. Oliyar, A. V., Skliarov, P. M., Masiuk, D. M., Bilyi, D. D., Logvinova, V. V. and Lieshchova, M. A. Effect of β-mannanase enzyme supplementation on the morphofunctional state of broiler chickens' immunocompetent organs. *Regulatory Mechanisms in Biosystems,* **11**(4), 579-587(2020).

24. Dahariya, N., Sathapathy, S., Patra, R., Mishra, U. K., Sahu, S. K., Samal, L. and Joshi, S. K. Age wise histological studies on the major lymphoid organs in various chicken genotypes. *Journal of Animal Research*, **10**(5), 811-819 (2020).

25. Deka, A., Mahanta, J. D. and Perumal, P. Anatomy of Bursa of Fabricius of Pati duck (Anas platyrhynchos domesticus) of Assam at Different Stages of Development. *International Journal of Bio-resource and Stress Management*, **11**(1), 57-63 (2020).

26. Olah, I., Nagy, N. and Vervelde, L. Structure of the avian lymphoid system. In *Avian Immunology,* Academic press, pp. 11-44, (2014).

27. Penchev, G. Age-related histology of the bursa of Fabricius in bronze turkeys (Meleagris meleagris gallopavo). *Bulgarian Journal of Veterinary Medicine*, **25**(3), 372-378 (2022).

28. Zmrhal, V. and Slama, P. Immunomodulation of avian dendritic cells under the induction of prebiotics. *Animals*, **10**(4), 698 (2020).

29. Chrzastek, K., Madej, J. P. , Mytnik, E . and Wieliczko, A. The influence of antibiotics on B-cell number, percentage, and distribution in the bursa of Fabricius of newly hatched chicks. *Poultry Science*, **90**(12), 2723–2729 (2011).

30. Madej, J. P., Stefaniak, T. and Bednarczyk, M. Effect of in ovo-delivered prebiotics and synbiotics on lymphoid-organs' morphology in chickens. *Poultry Science*, **94**(6), 1209-1219 (2015).

31. Igyártó, B. Z., Nagy, N., Magyar, A. and Oláh, I. Identification of the avian B-cell-specific Bu-1 alloantigen by a novel monoclonal antibody. *Poultry Science*, **87**(2), 351-355 (2008).

32. Al-Ogaili, A. S. and Hameed, S. S. Development of lymphocyte subpopulations in local breed chickens. *Veterinary World*, **14**(7), 1846 (2021).

33. Sayegh, C. E., Demaries, S. L., Pike, K. A., Friedman, J. E. and Ratcliffe, M. J. The chicken B-cell receptor complex and its role in avian B-cell development. *Immunological Reviews*, **175**(1), 187-200 (2000).

34. Kozlu, T., Sari, E. K., Bozkurt, Y. A. and Kurtdede, N. Immunohıstochemıcal staınıng of cd3, cd79acy and s-100 on bursa fabrıcius, thymus and spleen of turkeys (Meleagrıs gallapavo). *Indian Journal of Animal Research*, **53**(11), 1450-1454 (2019).

35. Ratcliffe, M. J. and Härtle, S. B cells, the bursa of Fabricius, and the generation of antibody repertoires. In *Avian immunology* (pp. 71-99). Academic Press (2022).

دراسة نسجية قياسية ونسجية مناعية للنظوج جراب فابريشيا لذكور دجاج لحم نوع روز في الفترة ما بعد الفقس

شيماء زهير امين[1] ، يحيى احمد محمد [1] و مهدي علي عبد الله[2]
[1] قسم الولادة والتشريح و الفسلجة - كلية الطب البيطري - جامعة دهوك - العراق.
[2]قسم الامراض و الاحياء المجهرية كلية الطب البيطري جامعة دهوك - العراق.

تم اجراء البحث الحالي على١٨٠ دجاج ذكر لحم نوع روز، حيث تم تصنيفها الى تسع مجاميع على اساس العمر ابتداءا من الايام؛١،٤،٧،١٤،٢١،٢٨،٣٥،٤٢،٥٨. تم استخدام الصبغات الروتينية والخاصة وكذلك الاجسام المضادة وحيدة النسيلة ضد بوا (الفار ضد الدجاج) لانجاز هذا البحث. تمتلك ظهارة جراب فابريشيا على الخلايا الظهارية العمودية خلايا ميكروفولد و الخلاايا اللمفاوية ما بين الظهارة. تمتاز خلايا ميكروفولد بانوية حويصلية وهيولي شاحب اللون لا يصطبغ بالهيماتوكسيلين والايوسين،الباس،الديستاز المقترن مع الباس. تتالف الجريبات اللمفاوية لجراب فابريشيا من الخلايا اللمفيه بصورة رئيسة فضلا عن الارومات اللمفية، الخلايا الشبكيه، الخلايا البلازمية، البلعميات الكبيرة والخلايا الجذعيه. تظهر الجريبات اللمفاوية في جراب فابريشيا بصورة واضحة في اليوم الاول من عمر الطير و بعد ذلك تستمر مع تقدم العمر. تظهر طبقة من الخلايا المكعبة المصاحبة للاوعية الدموية الشعرية في الحاجز القشري النخاعي في اليوم السابع. انخفض عديد الجريبات اللمفاوية لجراب فابريشيا تدريجيا وبشكل ملحوظ عند مستوى معنوي ($P < 0.05$) مع تقدم العمر بينما يزداد قطر الجريبات نفسها وبشكل ملحوظ عند مستوى معنوي ($P< 0.05$) مع تقدم العمرايضا. تتواجد الخلايا اللمفية نوع بي الموجبة لمستضد بوا ١ بشكل رئيسي في قشرة ونخاع الجريبات اللمفاوية من اليوم الاول ولغاية اليوم الثامن والخمسون من عمر الطير. استنتجت الدراسة الحالية بان نضوج الجريبات اللمفاوية لجراب فابريشيا في هذه السلالة تعتمد على عمر الطير.