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Research article

Respiratory and urogenital tract associated lymphoid tissues in native chicken *(Gallus gallus domesticus)* of Bangladesh

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A B S T R A C T

This study specifies the morphological features and distribution of lymphoid tissues in the respiratory and urogenital tract to establish the impact of chicken age significance on tract-associated lymphoid tissues. The different organs of the respiratory and urogenital tract of 1-day, 30-day, 90-day, and 180-day-old male and female native chickens were freshly taken and stained with hematoxylin and eosin (H & E). The mean of intraepithelial lymphocytes (IELs) in the trachea of male and female native chickens has a significant variation in 180 days of age ($P \leq 0.05$). The mean of IELs in the lungs of male native chickens has a significant variation with their age ($P \leq 0.05$). The mean of aggregated lymphoid tissues in the lungs of male native chickens has a significant variation between 90 days and 180 days of age ($P \leq 0.05$). The mean of IELs in the kidneys of both male and female native chickens and ureters only in female native chickens has a significant variation with their age ($P \leq 0.05$). The mean of IELs in the infundibulum, isthmus, and uterus of female native chickens has a significant variation between 90 days and 180 days ($P \leq 0.05$). A higher number of IELs in the uterus compared to other segments of the oviduct was observed at 180 days of chicken. There was a significant variation in the mean of IELs in the urogenital tract of female chickens from male chickens. There was no significant variation ($P > 0.05$) of aggregated lymphoid tissues based on age in the native chicken. The IELs were in various sizes, from small lymphocytes with little cytoplasm to larger cells with obvious cytoplasm. The aggregated lymphocytes were a distinct, distinguishable structure under bright-field light microscopy. It was in the lamina propria, typically immediately below the basement membrane. The aggregates did not allow for the definite identification of germinal centers. These results suggest that the distribution of lymphoid tissue in the respiratory and urogenital tract of native chickens increases with aging and is relatively high in specific parts of the tract.

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1. INTRODUCTION

The development of urogenital tract-associated lymphoid tissues is central, e.g., the Thymus and Bursa. The thymus-dependent component is represented by the smaller lymphocytes and is responsible for cell-mediated immunity (CMI), including immune surveillance (Janeway Jr et al., 1988), whereas the bursa-dependent component is represented by the larger lymphocytes which transformed into plasma cells in the tissue and plays an important role in humoral immunity (HI). The peripheral or secondary lymphoid tissues apparently depend

on the central lymphoid tissue for their origin, development, and function. In chicken, peripheral lymphoid tissues include the spleen and all the mucosa-associated lymphoid tissues (MALT) including the respiratory tract, urogenital tract, and alimentary tract with Peyer's patches (Kajiwara et al., 2003; Khatri and Sharma, 2009; Lee et al., 2010).

The features, number, and organization of lymphoid tissues differ within the various segments of the tract with age. The histological observation revealed that the tract-associated lymphoid tissues in the form of isolatory lymphocytes, diffuse lymphocytes, and lymphoid follicles (isolatory or aggregated) are often present in the lamina propria submucosa in the digestive tract, respiratory tract, and urogenital tract, where the muscularis mucosa is usually incomplete. In addition, lymphoid tissue was much more abundant in the lamina propria submucosa of some specific parts of the tract with changes in age. Most of the farmers in our country rear native chickens (*Gallus gallus domesticus*) that are scavengers in nature and attain a weight of around 1000 gm at 6 months of age (Islam et al., 2008). It is popular among the people in Bangladesh for its unique taste of meat (and may be due to more nutrient meat) compared with the commercial broiler. These scavenging native chickens are fed by kitchen waste, seeds and grains, garden left-over, insects, green grasses, and all other human refusals that would otherwise go to waste (Rahman et al., 2003). Due to their scavenging nature, the postnatal lymphoid tissues especially mucosa-associated lymphoid tissues (MALT), lamina propria-associated lymphoid tissues (LALT), submucosa-associated lymphoid tissues (SALT), and Peyer's patches (PPs) of native chicken contain more immune-competent cells than that of high yielding birds (Khan et al., 2007; Islam et al., 2008). During their scavenging habits, microorganisms enter through their feeds in the digestive tract in a descending way and similarly, air-transmitted microorganisms enter the urogenital tract and respiratory tract in an ascending and descending way, respectively. Hence, as a usual mechanism, there is lodgment of migratory lymphocytes as the primary protection against the organism by producing immunity after converting it into plasma cells.

However, information about the tract-associated lymphoid tissues of native chickens is scarce, since most investigations were performed in high-yielding birds (Moral et al., 1998; Nagy and Oláh, 2010). Concerning the above important point of view, the proposed research undertaken on respiratory and urogenital tract-associated lymphoid tissues in native Chicken *(Gallus gallus domesticus)* of Bangladesh. Although several studies have been done about native chicken, little attention has been made to the systemic study of tract-associated lymphoid tissues. Therefore, The specific objective of the study was to elucidate the distribution of lymphoid tissues in the respiratory and urogenital tract in native chicken of Bangladesh.

2. MATERIALS AND METHODS

Ethical statement

The birds were sacrificed, and samples were collected from the carcasses, avoiding and minimizing the conditions affecting animal welfare according to standard guidelines and procedures of the FIU (FIU, 2003).

Rearing of chicken in scavenging method

The day-old chicks (native chickens) were collected from the five household farmers and reared up to sacrifice in one farmer's house at Hathazari, Chattogram. About 75 chicks were reared using the scavenging method around the home range for up to six months at Hathazari, Chattogram, where sufficient feed and water were supplied. Fifteen (15) birds from each group were sacrificed a total of 60 birds.

Sacrificing of chicken for collection of samples

The chickens were sacrificed by excess chloroform inhalation. The samples from the respiratory and urogenital tract were carefully collected from 15 chickens of each category, i.e., day-old chicks (D1), followed by D30, D90, and D180. The samples were washed with normal saline for histomorphological study.

Fixation of samples

Samples were immersed in Bouin's solution for a period of 2 hours for prefixation and then immersed in neutral buffered formalin for a period of 72 hours for complete fixation.

Tissue processing and staining for microscopic examination

After washing of fixed tissue, dehydration was performed through the passing of tissue samples into successive ascending concentrations of alcohol. After the completion of dehydration, the tissue specimens were passed through successive changes of xylene for cleaning until the alcohol from the tissue was replaced. When the tissue samples were clearly transparent, i.e., cleaning was completed by xylene. The specimen was to be placed into melted paraffin in the oven at 58-60°C to evaporate the xylene and the tissue space was infiltrated with melted paraffin. After completion of infiltration, the tissue samples were placed in between two L-shaped angles and then filled with melted paraffin to make a paraffin block. Then microscopic section was cut at 6µm thickness using a sliding microtome machine (Leica SM2010R V1.2 English-09/2008 Sliding Microtome Machine, Germany) with the help of a disposable Carbon blade. The water bath was set at 60°C temperature. The slide was then labeled by a diamond pencil as marked with a tag. Gelatin was used as an adhesive to attach the section to a glass slide. Then the slide was dried in air for 12 hours before staining. The tissues were stained with hematoxylin and eosin according to standard protocol (Cardiff et al., 2014) for visualization under the light microscope.

Histomorphometry

To obtain histomorphometric data, a microscopic study of lymphoid tissues of the tract at different aged chickens was performed and photomicrographs were taken using a photomicroscope (AmScope Trinocular Compound Microscope with 1.3 MP Camera, Model T490 B-MT) and AmScope image measuring software (x86, 3.7.3036 version).

Data analysis

All data obtained from this study were entered and stored in Microsoft Excel Professional 2020 (Microsoft Corporation, USA) followed by transferred to statistical software, STATA-16 (STATA Corp., Texas, USA) to perform statistical analysis. An unpaired sample t-test was done to compare the means of different variables between the two groups. A p-value equal to or less than 0.05 ($P \leq 0.05$) was considered significant for this test. Results were expressed as arithmetic mean ± standard deviation (Mean ± SD).

3. RESULTS

Histological features indicated that the native chicken trachea was lined by a ciliated pseudostratified columnar epithelium consisting of goblet, and basal cells. The completely encircled tracheal cartilage rings overlap and interlock with adjacent rings. This study revealed that the frequency of intraepithelial lymphocytes (IELs) and aggregated lymphoid tissue in the trachea of the male native chicken was recorded at 2 and nil in 1 day, 3 and nil in 30 days, 5 and nil in 90 days, and 8 and 3 in 180 days, respectively (Table 1; Figure 1 and 2). The frequency of IELs and aggregated lymphoid tissue in the trachea of the female native chicken was measured at 3 and nil in 1 day, 5 and nil in 30 days, 6 and nil in 90 days, and 9 and 3 in 180 days, respectively (Table 1; Figure 1 and 2). The mean of IELs in the trachea of male and female native chickens has a significant variation at 180 days of age ($P \leq 0.05$) (Table 4). The study observed that the lungs in native chickens formed parabronchi which are located in the center of the pulmonary lobule, and which originate from secondary bronchi. The parabronchi were lined by simple squamous epithelium. The study also explored that the frequency of IELs and aggregated lymphoid tissue in the lungs of the male native chicken was at 3 and 1 in 1 day, 9 and 3 in 30 days, 11 and 8 in 90 days, and 13 and 5 in 180 days, respectively (Table 1; Figure 1 and 2). The frequency of IELs and aggregated lymphoid tissue in the lungs of the female native chicken was at 4 and 2 in 1 day, 9 and 3 in 30 days, 11 and 5 in 90 days, and 12 and 8 in 180 days, respectively (Table 1; Figure 1 and 2). The mean of IELs in the lungs of male native chickens has a significant variation with their age ($P \leq 0.05$) (Table 4). The mean of aggregated lymphoid tissues in the lungs of male native chickens has a significant variation between 90 days and 180 days of age ($P \leq 0.05$) (Table 5).

The present study histologically featured that the kidneys of all studied birds were divided into cortical and medullary parts. The cortex is

composed of large and small renal corpuscles, each renal corpuscle consists of Bowman's capsule and glomerulus. The parietal layer of Bowman's capsule of renal corpuscles is usually lined by a layer of simple squamous epithelium. The proximal and distal convoluted tubules and collecting tubules were lined by simple cuboidal epithelium. The Medulla of the kidney was composed of thin and thick segments of the Henle's loop and collecting ducts, these structures were lined by simple cuboidal epithelium. The collecting ducts continued forming the papillary ducts, lined by simple columnar epithelium. The macula densa was shown to be surrounded by juxtaglomerular cells and peripolar cells that contributed to unique juxtaglomerular apparatus. The current study registered that the frequency of IELs in the kidney of male native chicken was 1, 5, 10, and 11 for 1 day, 30 days, 90 days, and 180 days, respectively (Table 2; Figure 3). The frequency of aggregated lymphoid tissue in the kidney of male native chicken was 1 (1 day), 2 (30 days), 2 (90 days), and 4 (180 days) (Table 2; Figure 3). The frequency of IELs and aggregated lymphoid tissue in the kidney of the female native chicken was recorded at nil & 1 in 1 day, 4 & nil in 30 days, 30 & 5 in 90 days, and 5 & 2 in 180 days, respectively (Table 3; Figure 3). The mean of IELs in the kidneys of both male and female native chickens has a significant variation with their age ($P \leq 0.05$) (Table 6, 7). The ureter's mucosal folds were mainly isometric, giving the lumen a stellate appearance in cross-section. The wall of the ureter was composed of tunica mucosa, submucosa, muscularis, and serosa. Mainly simple columnar epithelium lined the tunica mucosa of the proximal area of the ureter. Although, in the middle and distal areas, the epithelium varied from simple columnar to pseudostratified columnar. The acinar glands were found in the lamina propria of tunica mucosa. The frequency of IELs and aggregated lymphoid tissue in the ureter of the male native chicken was found at 3 & 3 in 1 day, 13 & 3 in 30 days, 3 & 1 in 90 days, and 8 & 1 in 180 days, respectively (Table 2; Figure 4). The frequency of IELs and aggregated lymphoid tissue in the ureter of the female native chicken was found at nil & 1 in 1 day, 8 & 2 in 30 days, 9 & 2 in 90 days, and 2 & 1 in 180 days, respectively (Table 3; Figure 4). The mean of IELs in the ureters only female native chickens has a significant variation with their age ($P \leq 0.05$) (Table 6).

The reproductive system of the female chicken is of two parts: the ovary and the oviduct. Overlying the tunica albuginea is a serous membrane covered with a single layer of modified mesothelial cells called the ovarian surface epithelium. The microscopic examination of the ovary displayed that the ovarian surface epithelium appeared as simple squamous, simple cuboidal, or low columnar. Ovarian surface epithelium often has a cuboidal appearance where it overlies small follicles. Large follicles that protrude from the surface of the ovary are typically covered by simple squamous surface epithelium. The follicular stoma, an avascular line along which a follicle will ovulate, is characterized by a more proliferative, low-columnar surface epithelium. The follicular wall of an oocyte was a delicate structure composed of multilayers and encased by connective tissue.

Table 1. Frequency of IELs and Aggregate lymphoid tissue per 5 microscopic fields (40X) in the respiratory system of the male and female native chickens.

Name of the organs	Age (days)	Intraepithelial lymphocytes (N)		Aggregated Lymphoid tissue (N)	
		Male chicken	Female chicken	Male chicken	Female chicken
Trachea	1	2	3	0	0
	30	3	5	0	0
	90	5	6	0	0
	180	8	9	3	3
Lungs	1	3	4	1	2
	30	9	9	3	3
	90	11	11	8	5
	180	13	12	5	8

Table 2. Frequency of IELs and Aggregate lymphoid tissue per 5 microscopic fields (40X) in the urogenital system of the male native chickens.

Name of the organs	Age (days)	Intraepithelial lymphocytes (N)	Aggregated Lymphoid tissue (N)
Testes	1	1	1
	30	1	1
	90	2	1
	180	2	2
Vas deference	1	1	1
	30	2	1
	90	3	3
	180	5	2
Epididymis	1	1	1
	30	1	1
	90	1	1
	180	2	2
Kidney	1	1	1
	30	5	2
	90	10	2
	180	11	4
Ureter	1	3	3
	30	13	3
	90	3	1
	180	8	1

Table 3. Frequency of IELs and Aggregate lymphoid tissue per 5 microscopic fields (40X) in the urogenital system of the female native chickens.

Name of the organs	Age (days)	Intraepithelial lymphocytes (N)	Aggregated Lymphoid tissue (N)
Ovary	1	1	3
	30	2	2
	90	0	1
	180	3	1
Oviduct	1	3	1
	30	3	1
Infundibulum	90	6	3
	180	23	10
Magnum	90	6	2
	180	13	3
Isthmus	90	2	1
	180	7	3
Uterus	90	5	5
	180	27	2
Kidney	1	0	1
	30	4	0
	90	30	5
	180	5	2
Ureter	1	0	1
	30	8	2
	90	9	2
	180	2	1

Table 4. IELs per 5 microscopic fields (40X) in the respiratory system of the male and female Native Chicken.

Name of the organs	Age (Days)	Male chicken		Female chicken	
		Mean± SD	P-value	Mean± SD	P-value
Trachea	1	0.4±0.55		0.6±0.55	
	30	0.6±0.55	*	1.0±0.00	*
	90	1.0±0.00	*	1.2±0.45	*
	180	1.6±0.55	**	1.8±0.45	**
Lungs	1	0.6±0.89		0.8±0.45	
	30	1.8±0.45	**	1.8±0.45	*
	90	2.2±0.84	**	2.2±1.64	*
	180	2.6±0.89	**	2.4±1.14	*

* Non-significance (P > 0.05); ** Significance (P ≤ 0.05)

Table 5. Aggregated Lymphoid tissues per 5 microscopic fields (40X) in the respiratory system of the male and female Native Chicken.

Name of the organs	Age (Days)	Male chicken		Female chicken	
		Mean± SD	P-value	Mean± SD	P-value
Trachea	1	0.0±0.00		0.0±0.00	
	30	0.0±0.00	-	0.0±0.00	-
	90	0.0±0.00	-	0.0±0.00	-
	180	0.6±0.55	*	0.6±0.55	*
Lungs	1	0.2±0.45		0.4±0.55	
	30	0.6±0.55	*	0.6±0.89	*
	90	1.6±0.89	**	1.0±0.71	*
	180	1.0±0.00	**	1.6±0.55	*

* Non-significance (P > 0.05); ** Significance (P ≤ 0.05)

Each follicle consisted of a developing yolk-filled oocyte with a spherical nucleus. The oocyte was surrounded by multiple layers: the theca externa, theca interna, membrane granulosa, and perivitelline membrane. The frequency of IELs and aggregated lymphoid tissue in the ovary of the female native chicken was recorded at 1 and 3 in 1 day, 2 and 2 in 30 days, nil and 1 in 90 days, and 3 and 1 in 180 days, respectively (Table 3; Figure 5).

Different segments of the reproductive tract of chickens (infundibulum, magnum, isthmus, uterus, and vagina) were not distinguishable up to 30 days-old chickens. Growth of the oviduct was noticed at around 90 days of age and at 180 days of development, the different segments appeared significantly. The frequency of IELs and aggregated lymphoid tissue in the oviduct of the female native chicken was found at 3 and 1 in 1 day, and 3 and 1 in 30 days, respectively (Table 3; Figure 6). The histological structure of the infundibulum showed that the tunica mucosa was quite rugged with an extensive fold. The mucosal folds of the infundibulum were lined with columnar secretory ciliated epithelium. The lamina propria submucosa consisted of loose connective tissue, blood vessels, and contained a tubular gland. The tunica muscularis constituted of two layers of smooth muscle fibers; inner circular and outer longitudinal. The frequency of IELs and aggregated lymphoid tissue in the infundibulum of the female native chicken was found at 6 and 3 in 90 days, and 23 and 10 in 180 days, respectively (Table 3; Figure 7).

The tunica mucosa of the magnum was composed of simple columnar ciliated epithelium. The lamina propria submucosa contained loose fibro-cellular connective tissue, several capillaries, and branched tubular glands. The tunica muscularis constituted of two layers of smooth muscle fibers; inner circular and outer longitudinal. The frequency of IELs and aggregated lymphoid tissue in the magnum of the female native chicken was found at 6 & 2 in 90 days, and 13 & 3 in 180 days, respectively (Table 3; Figure 8).

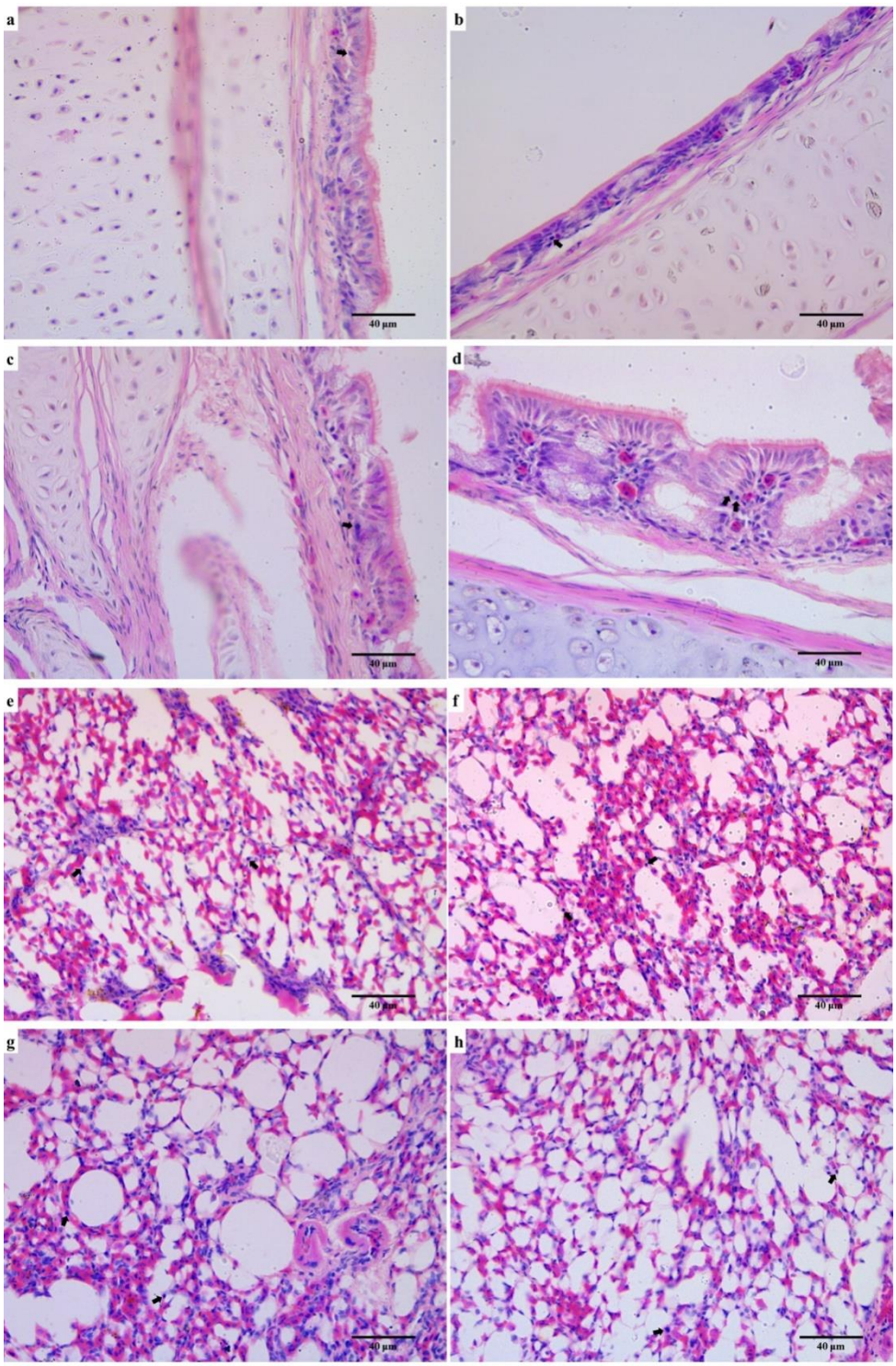

Figure 1. Hematoxylin and eosin staining of the trachea (a; female of 1 day, b; male of 30 days, c; male of 90 days, and d; female of 180 days) and lungs (e; female of 1 day, f; female of 30 days, g; male of 90 days, and h; male of 180 days) from the respiratory system of the native chicken. The arrow in the images indicated IELs in the lining epithelium. Scale for low magnification: 100 µm, for high magnification: 40 µm.

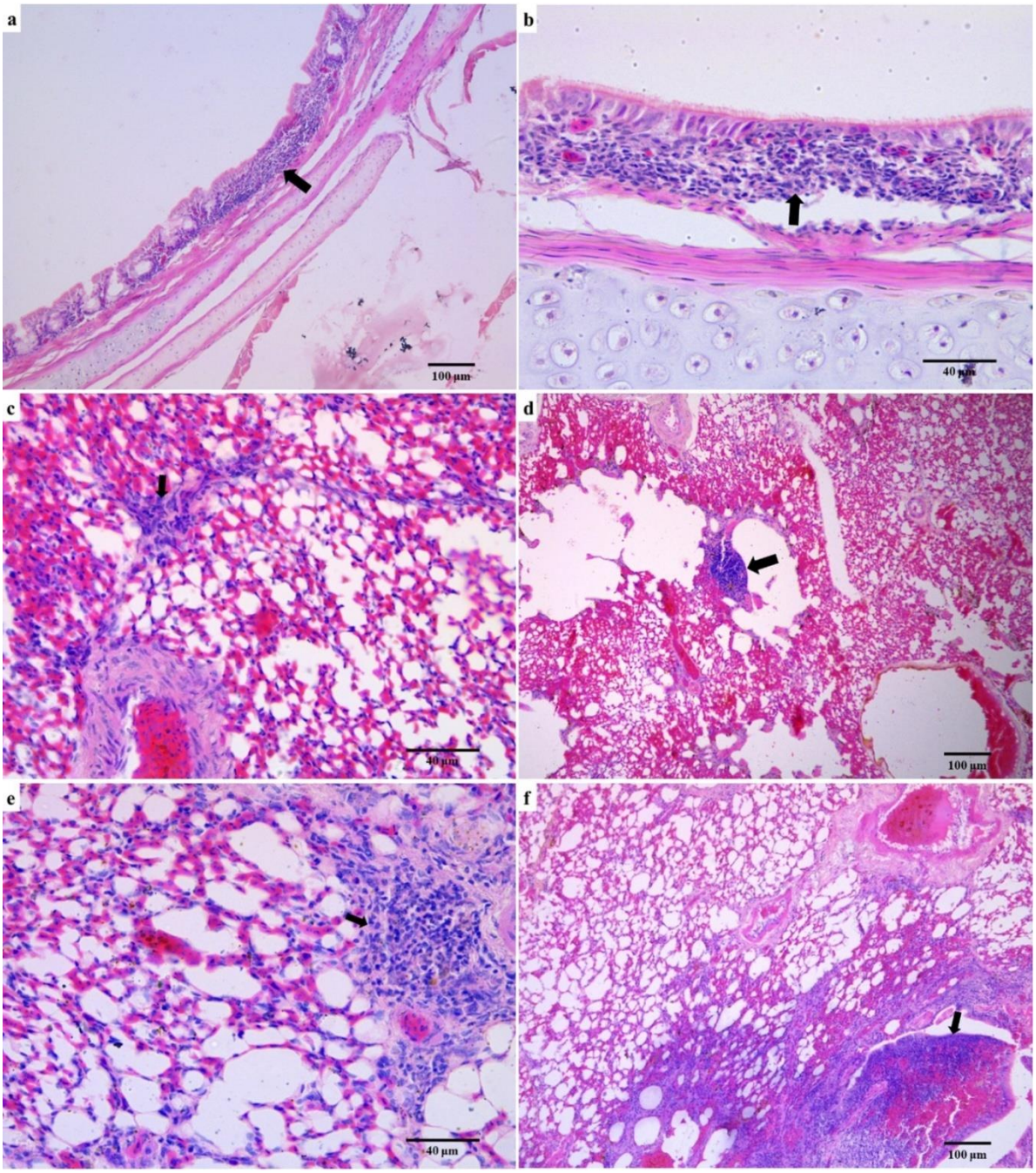

Figure 2. Hematoxylin and eosin staining of the trachea (a; female of 180 days, b; male of 180 days) and lungs (c; female of 1 day, d; male of 30 days, e; male of 90 days, and f; female of 180 days) from the respiratory system of the native chicken. The arrow in the images indicated aggregated lymphoid tissues in the lamina propria and lungs parenchyma. Scale for low magnification: 100 µm, for high magnification: 40 µm.

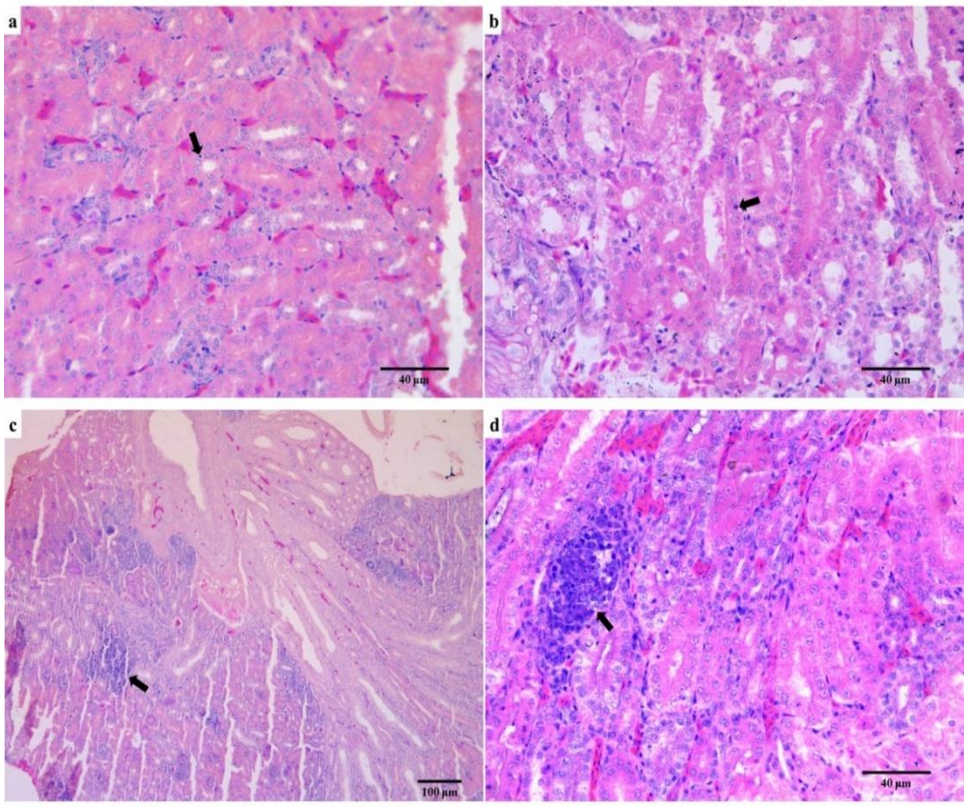

Figure 3. Hematoxylin and eosin staining of the kidney of the native chicken. The arrow in the images indicated IELs (a; male of 30 days, and b; female of 90 days,) and aggregated lymphoid tissues (c; female of 1 day, and d; male of 180 days).

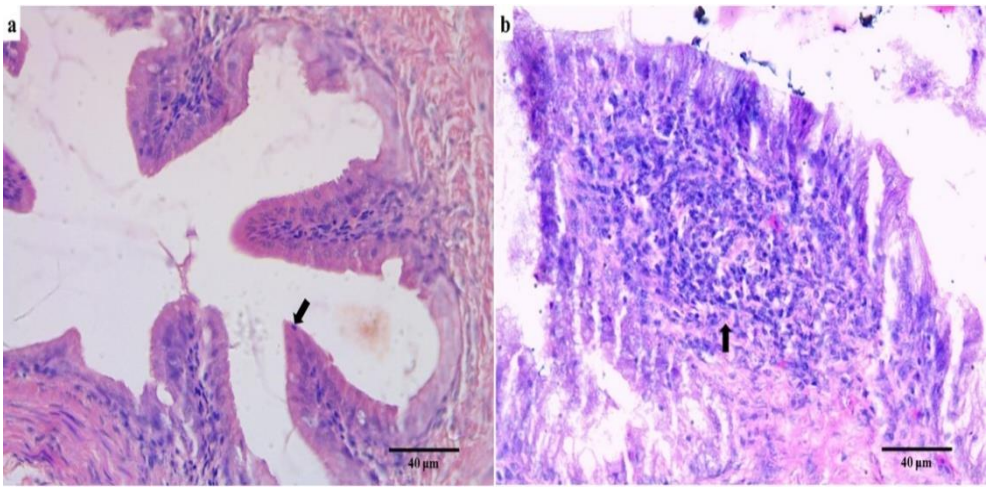

Figure 4. Hematoxylin and eosin staining of the ureter of the native chicken. The arrow in the images indicated IELs (a; male of 30 days,) and aggregated lymphoid tissues (b; female of 180 days). Scale for high magnification: 40 µm.

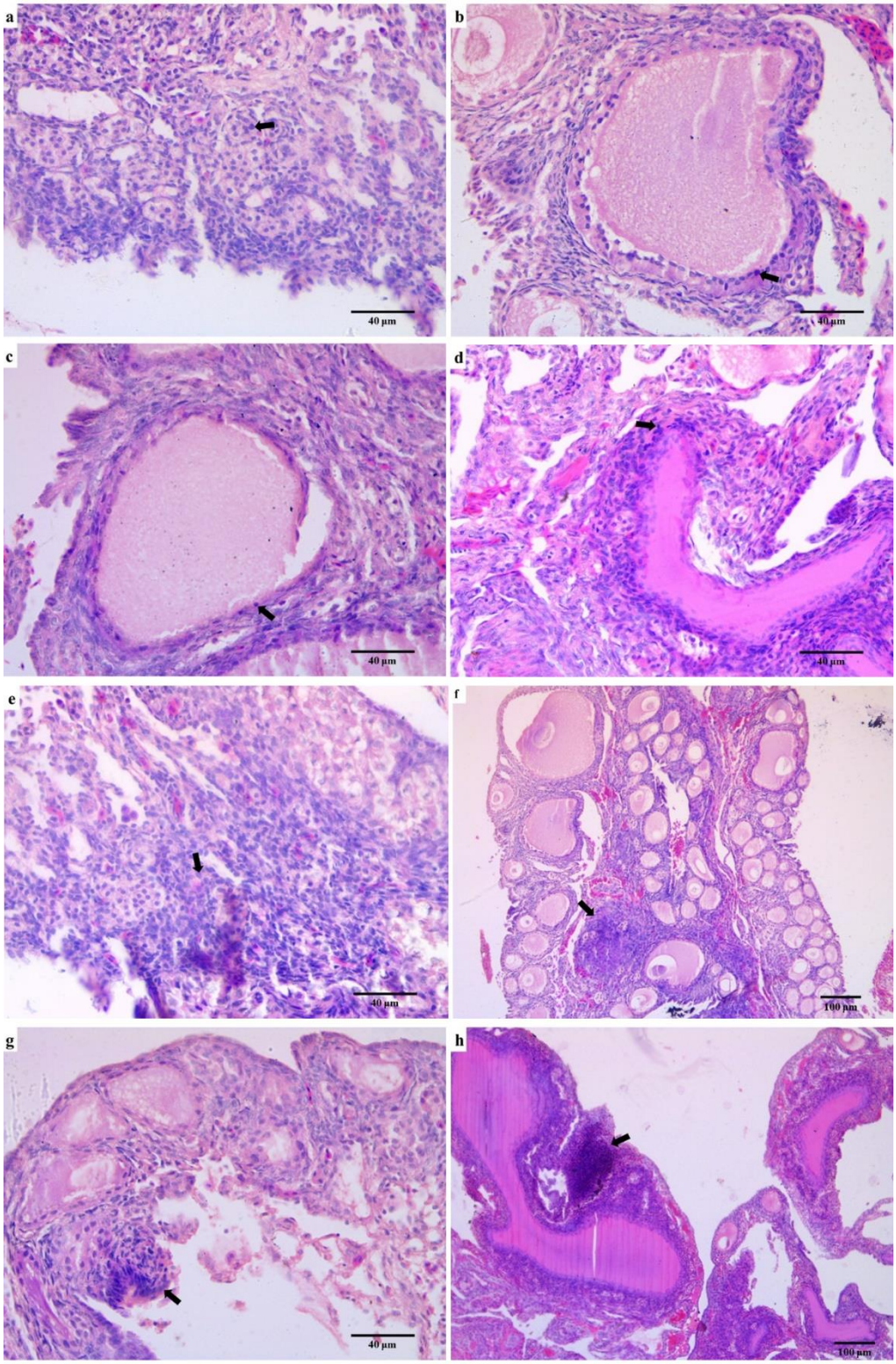

Figure 5. Hematoxylin and eosin staining of the ovary from the genital system of female native chicken. The arrow in the images indicated IELs (a; 1 day, b; 30 days, c; 90 days, and d; 180 days) and aggregated lymphoid tissues (e; 1 day, f; 30 days, g; 90 days, and h; 180 days). Scale for low magnification: 100 µm, for high magnification: 40 µm.

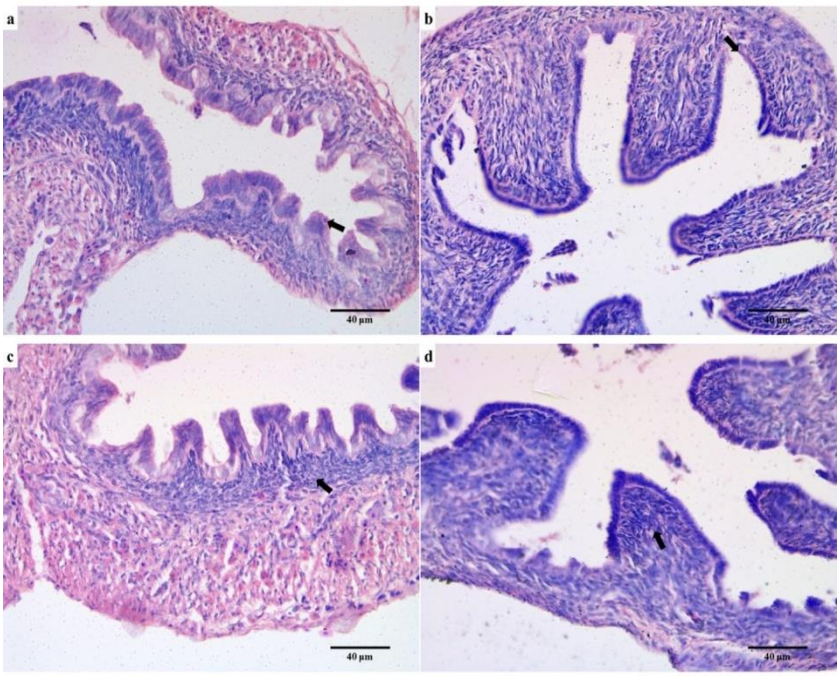

Figure 6. Hematoxylin and eosin staining of the oviduct from the genital system of female native chicken. The arrow in the images indicated IELs (a; 1 day, and b; 30 days) and aggregated lymphoid tissues (c; 1 day, and d; 30 days). Scale for high magnification: 40 µm.

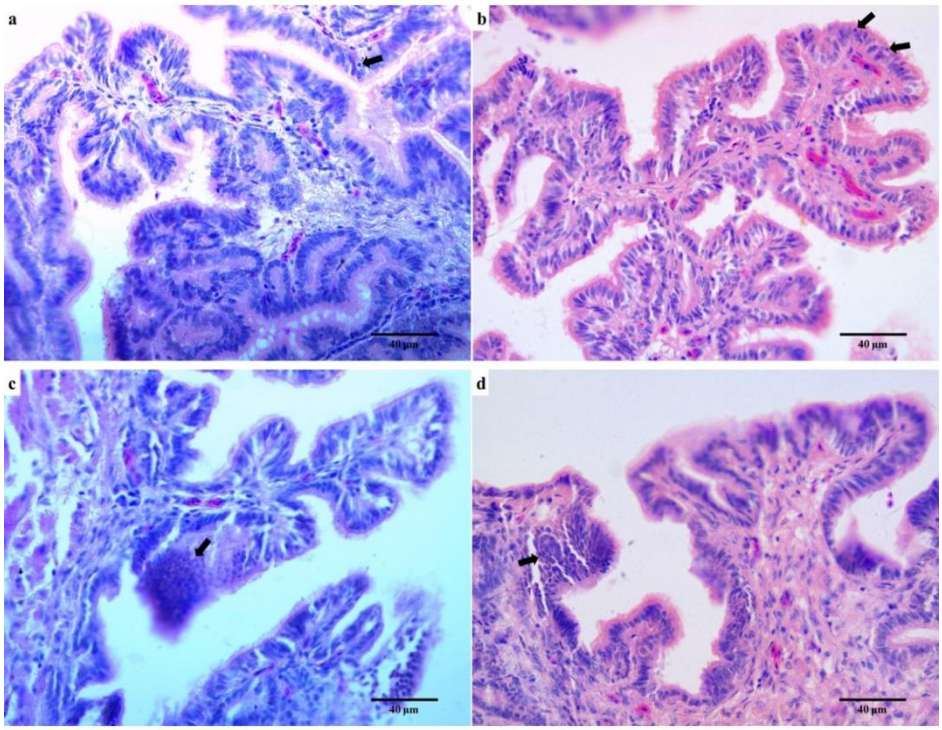

Figure 7. Hematoxylin and eosin staining of the infundibulum from the genital system of female native chicken. The arrow in the images indicated IELs (a; 90 days, and b; 180 days) and aggregated lymphoid tissues (c; 90 days, and d; 180 days). Scale for high magnification: 40 µm.

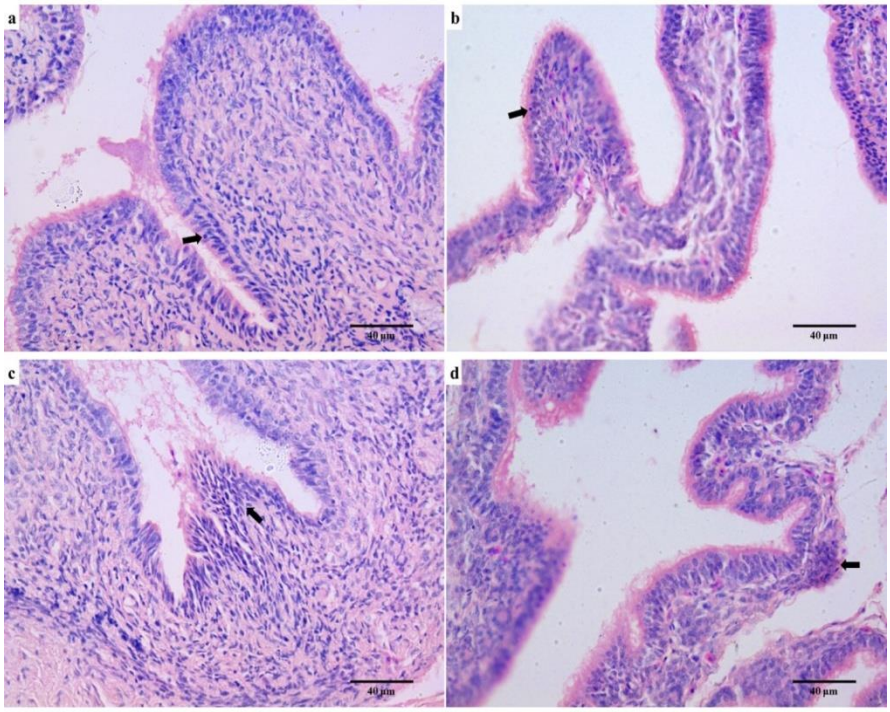

Figure 8. Hematoxylin and eosin staining of the magnum from the genital system of female native chicken. The arrow in the images indicated IELs (a; 90 days, and b; 180 days) and aggregated lymphoid tissues (c; 90 days, and d; 180 days). Scale for high magnification: 40 µm.

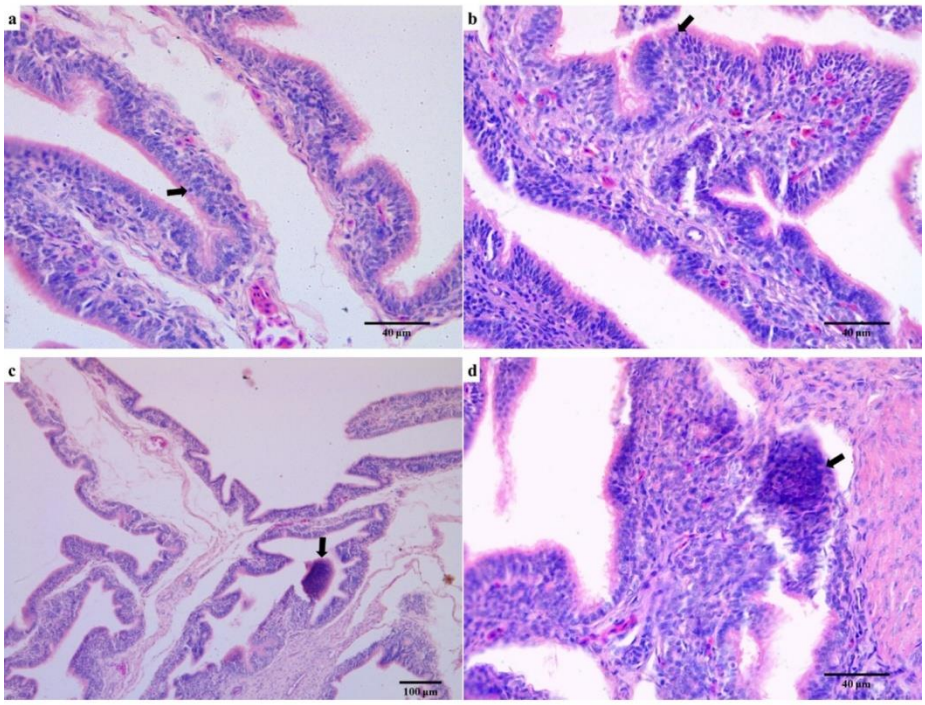

Figure 9. Hematoxylin and eosin staining of the isthmus from the genital system of female native chicken. The arrow in the images indicated IELs (a; 90 days, and b; 180 days) and aggregated lymphoid tissues (c; 90 days, and d; 180 days). Scale for low magnification: 100 µm, for high magnification: 40 µm.

The mucosal surface epithelium of the isthmus was lined by a ciliated pseudo-stratified columnar. The lamina propria submucosa of the isthmus showed the presence of loose connective tissue, blood vessels, and branched tubular glands. The tunica muscularis mucosa was divided into two layers, an inner longitudinal layer, and an outer circular layer, and was thicker than that of the magnum. The frequency of IELs and aggregated lymphoid tissue in the isthmus of the female native chicken was found at 2 & 1 in 90 days, and 7 & 3 in 180 days, respectively (Table 3; Figure 9).

The surface epithelium of the uterus was lined by pseudostratified columnar ciliated and non-ciliated secretory cells. The highly vascularized loose connective tissue that made up the lamina propria submucosa includes branched tubular glands. The tunica muscularis mucosa was thicker than the preceding segments and arranged in two layers, inner longitudinal and outer circular. The frequency of IELs and aggregated lymphoid tissue in the uterus of the female native chicken was found at 5 & 5 in 90 days, and 27 & 2 in 180 days, respectively (Table 3; Figure 10).

The mucosal folds of the vagina were lined by pseudostratified columnar ciliated and non-ciliated secretory cells. The lamina propria submucosa consisted of without tubular glands. The muscular coat was composed of thick inner longitudinal and thin outer circular smooth muscle layers. The tunica serosa of the oviduct was formed by loose connective tissue covered by mesothelium. The mean of IELs in the infundibulum, isthmus, and uterus of female native chickens has a significant variation between 90 days and 180 days ($P \leq 0.05$) (Table 6). A higher number of IELs in the uterus than in other segments of the oviduct was observed at 180 days of chicken (Table 3).

The male reproductive system of chicken consists of the testes, epididymis, ductus deferens, ejaculatory region, and mating organ. Histologically, we revealed that the seminiferous tubules were different in shape and size and filled the parenchyma of the testis. The seminiferous tubule was lined by multiple layers of epithelial cells, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa from the basement to the lumen of the tubule, respectively. Sertoli cells were located between primary spermatocytes, they have eccentric round nuclei. The interstitial tissue of the testis was formed of connective tissue that contained Leydig' cells. The frequency of IELs and aggregated lymphoid tissue in the testes of the male native chicken was found at 1 & 1 in 1 day, 30 days, 2 & 1 in 90 days, and 2 & 2 in 180 days, respectively (Table 2; Figure 11). Whereas the lining epithelium of the epididymis and vas deferens is the pseudostratified columnar and columnar epithelium, respectively. The frequency of IELs and aggregated lymphoid tissue in the epididymis of the male native chicken was found at 1 & 1 in 1 day, 30 days, 90 days, and 2 & 2 in 180 days, respectively (Table 2; Figure 12). The frequency of IELs and aggregated lymphoid tissue in the vas deference of the male native chicken was found at 1 & 1 in 1 day, 2 & 1 in 30 days, 3 & 3 in 90 days, and 5 & 2 in 180 days, respectively (Table 2; Figure 13).

4. DISCUSSION

The histological structures of the trachea at different ages of native chicken were similar to the findings of AL-Taai, (2021), briefly the lamina propria has loose connective tissue and submucosa contains tracheal cartilaginous rings, numerous seromucous glands, and large blood vessels. The IELs were observed in the lining epithelium of different ages of chicken but aggregated lymphatic tissues in lamina propria were observed only 180 days chicken. This result was accepted by Mokhtar & Hussien, 2020 who reported that IELs could be observed in the trachea sections as small darkly stained cells located mainly in the basal borders of the epithelium. Fagerland and Arp (1993) further reported that there were age-related differences in the number of lymphocyte infiltration in the epithelial lining and aggregated lymphoid tissues in the lamina propria and submucosa of the respiratory tract. Almost all the IELs in the upper respiratory and bronchi are T lymphocytes. The IELs often have more CD8+ cytotoxic/suppressive T cells than CD4+ T-helper cells. The primary function is to maintain the integrity of the mucosal barrier and defend the epithelial against pathogenic agents (Goto et

al., 2000). The study demonstrated that the lungs in native chicken formed a large number of parabronchi which is located in the center of the pulmonary lobule. This finding was supported by Reese et al. (2006) who reported that the parabronchial tissue is the fundamental functional part of the chicken lung, where the gaseous exchange takes place. The IELs and aggregated lymphoid tissues were found in the lung of male chicken whereas statistically significant variations were observed attributed to their ages. The results were supported by Fagerland and Arp (1993) who reported that the IELs were progressively more numerous with the increasing age of broiler chicken. Similar results were reported by Po et al. (2009) who found that the number of IELs and the number and size of aggregated lymphoid tissues were significantly related to the ages of individuals.

The result revealed the histological features of the kidneys of different aged native chickens. The results were supported by Deepa et al., 2020, who reported that the majority of the kidney's surface was made up of the cortex. The cortex of chickens included three different types of nephrons: small reptilian-type nephrons at the cortex's surface, large mammalian-type nephrons close to the medulla, and intermediate-type nephrons in the cortex's deeper region. The intraepithelial lymphocytes and aggregated lymphoid tissues were found in the kidney whereas statistically significant variations were observed with their ages for IELs. In another study, Hadipour (2010) reported that the number of lymphocytes and aggregated tissues depends on aging. Furthermore, it was also reported that the increased number of aggregated and intraepithelial lymphocytes in birds were observed while they were affected by infectious diseases. Similar types of findings were observed in the cortex and medulla of kidney after being affected by the infectious bronchitis virus (Chousalkar et al., 2007; Toffan et al., 2013).

Table 6. IELs and Aggregated Lymphoid tissues per 5 microscopic fields (40X) in the urogenital tract of the female Native Chicken.

Name of the organs	Age (Days)	Intraepithelial lymphocytes		Aggregated Lymphoid tissues	
		Mean± SD	P-value	Mean± SD	P-value
Ovary	1	0.2±0.45		0.6±0.89	
	30	0.4±0.89	*	0.4±0.55	*
	90	0.0±0.00	*	0.2±0.45	*
	180	0.6±0.55	*	0.2±0.45	*
Oviduct	1	0.6±0.89		0.2±0.45	
	30	0.6±0.89	*	0.2±0.45	*
Infundibulum	90	1.2±1.30		0.6±0.55	
	180	4.6±1.82	**	2.0±1.58	*
Magnum	90	1.2±0.84		0.4±0.55	
	180	2.6±1.67	*	0.6±0.89	*
Isthmus	90	0.4±0.55		0.2±0.45	
	180	1.4±1.14	**	0.6±0.55	*
Uterus	90	1.0±1.00		1.0±1.00	
	180	5.4±3.65	**	0.4±0.55	*
Kidney	1	0.0±0.00		0.2±0.45	
	30	0.8±1.30	**	0.0±0.00	*
	90	6.0±4.06	**	1.0±1.23	*
	180	1.0±1.23	**	0.4±0.55	*
Ureter	1	0.0±0.00		0.2±0.45	
	30	1.6±1.52	**	0.4±0.55	*
	90	1.8±1.48	**	0.4±0.55	*
	180	0.4±0.89	**	0.2±0.45	*

* Non-significance (P > 0.05); ** Significance (P ≤ 0.05)

Table 7. IELs and aggregated Lymphoid tissues per 5 microscopic fields (40X) in the urogenital tract of the male Native Chicken.

Name of the organs	Age (Days)	Intraepithelial lymphocytes		Aggregated Lymphoid tissues	
		Mean± SD	P-value	Mean± SD	P-value
Testes	1	0.2±0.45		0.2±0.45	
	30	0.2±0.45	*	0.2±0.45	*
	90	0.4±0.89	*	0.2±0.45	*
	180	0.4±0.89	*	0.4±0.89	*
Vas deference	1	0.2±0.45		0.2±0.45	
	30	0.4±0.89	*	0.2±0.45	*
	90	0.6±0.89	*	0.6±0.89	*
	180	1.0±1.23	*	0.4±0.55	*
Epididymis	1	0.2±0.45		0.2±0.45	
	30	0.2±0.45	*	0.2±0.45	*
	90	0.2±0.45	*	0.2±0.45	*
	180	0.2±0.45	*	0.4±0.89	*
Kidney	1	0.2±0.45		0.2±0.45	
	30	1.0±0.71	**	0.4±0.55	*
	90	2.0±2.00	**	0.4±0.55	*
	180	2.2±2.28	**	0.8±0.84	*
Ureter	1	0.6±0.89		0.6±0.89	
	30	2.6±1.82	*	0.6±0.55	*
	90	0.6±1.34	*	0.2±0.45	*
	180	1.6±1.52	*	0.2±0.45	*

* Non-significance (P > 0.05); ** Significance (P ≤ 0.05)

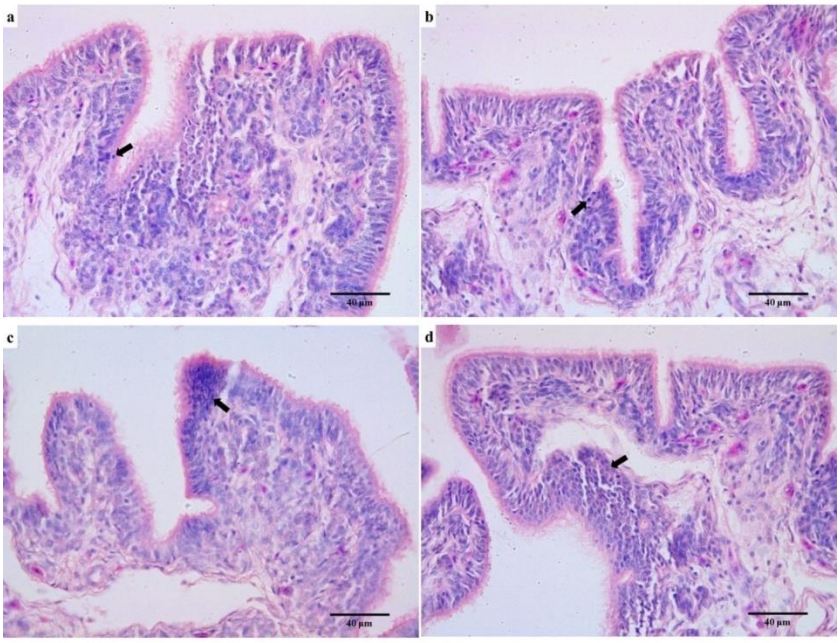

Figure 10. Hematoxylin and eosin staining of the uterus from the genital system of female native chicken. The arrow in the images indicated IELs (a; 90 days, and b; 180 days) and aggregated lymphoid tissues (c; 90 days, and d; 180 days). Scale for high magnification: 40 µm.

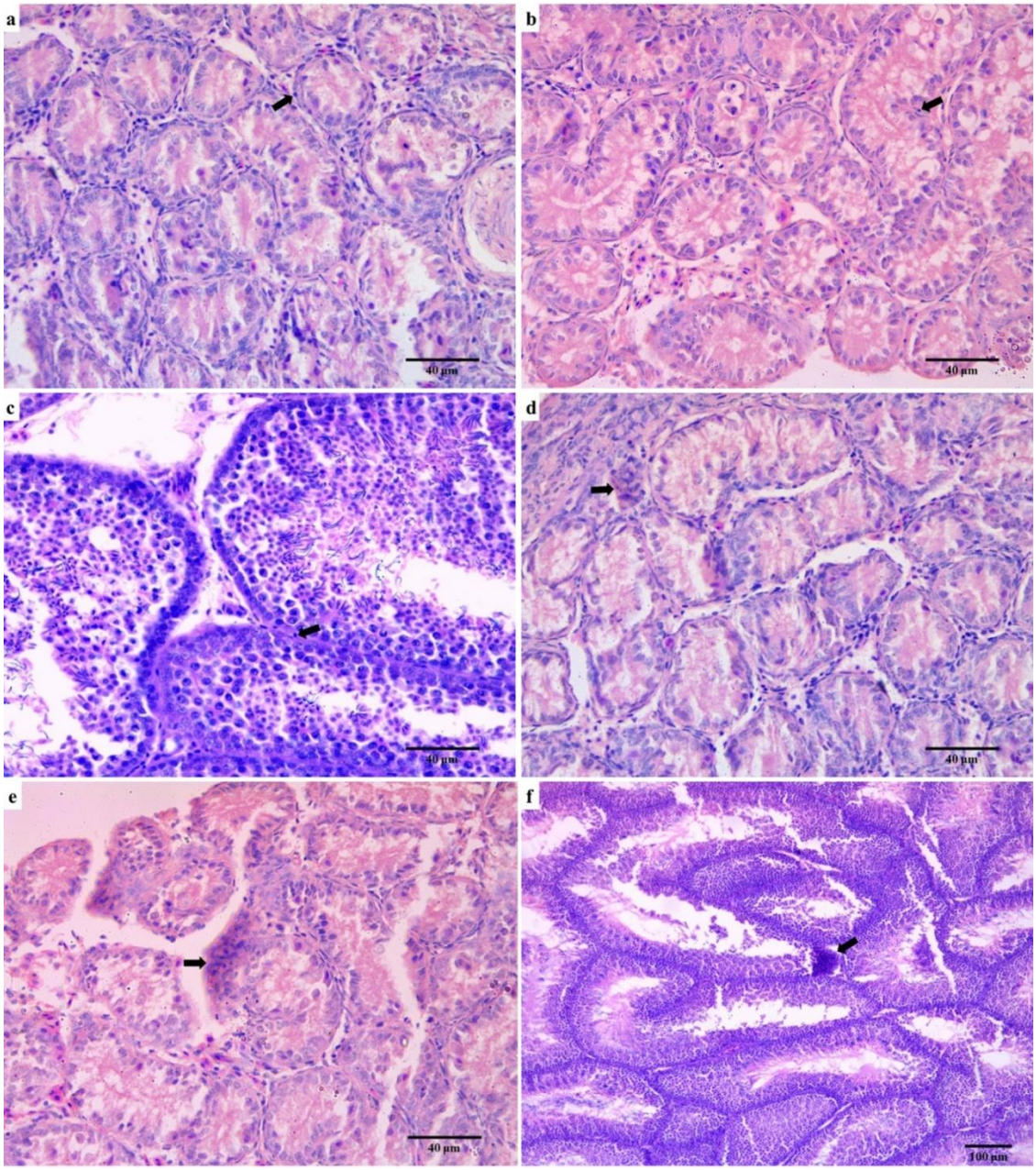

Figure 11. Hematoxylin and eosin staining of the testes from the genital system of male native chicken. The arrow in the images indicated IELs (a; 30 days, b; 90 days, and c; 180 days) and aggregated lymphoid tissues (d; 30 days, e; 90 days, and f; 180 days). Scale for low magnification: 100 µm, for high magnification: 40 µm.

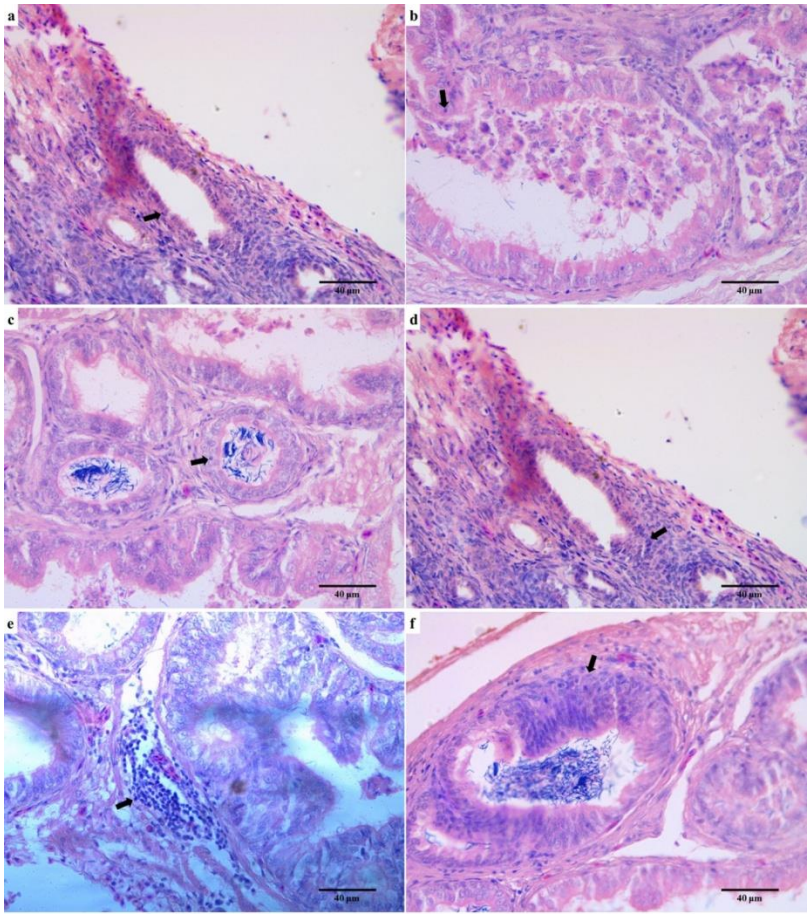

Figure 12. Hematoxylin and eosin staining of the epididymis from the genital system of the male native chicken. The arrow in the images indicated IELs (a; 30 days, b; 90 days, and c; 180 days) and aggregated lymphoid tissues (d; 30 days, e; 90 days, and f; 180 days). Scale for high magnification: 40 µm.

In the present study it was observed that in the proximal part of the ureter the simple columnar epithelium changed into the pseudostratified columnar epithelium. The intraepithelial lymphocytes and aggregated lymphatic tissues in lamina propria were observed in the ureter. Similar results were reported by Islam et al. (2001), who found that Rhode Island red (RIR) chickens had more lymphatic tissues in their lamina propria than White leg horn (WLH) chickens. Besides, Josifidis et al. (1989) reported that in the infectious individuals the number of lymphocytes and plasma cells increased in the submucosa and adjacent muscularis externa. Contrastingly, Oliaii and Mobini (2017), reported that the lamina propria did not contain any lymphatic tissues in any parts of the ureter of Japanese quail.

The study identified that the ovarian surface epithelium appeared as simple squamous, simple cuboidal, or low columnar. The results were supported by Apperson et al. (2017), who reported a single layer of granulosa cells around each ovum serves as the boundary for the follicle. Granulosa cells can be columnar, pseudostratified columnar, or polyhedral in form in highly developed follicles. It's possible that certain tiny white follicles are lack of distinct granulosa and thecal layers. The study also observed intraepithelial lymphocytes and aggregated lymphoid tissues in the ovary of the native chicken. The results were also supported by Bradaric et al. (2013) who reported that the "resident" lymphocytes were exist in the normal ovary and are found close to the follicles' outer cell layer, as well as in the stroma and surface epithelium of the ovary. In the stroma and medullary areas, B cells were observed rather often. Besides, Tregaskes et al. (1996) reported that comparatively large concentration of T lymphocytes in the follicles play an important

role in healthy ovarian function. Bradaric et al. (2013) added that the CD8+ cells were the dominant T cell sub-type in both ovarian stroma and in ovarian follicles compared to CD4+ cells. In another study by Barua and Yoshimura (1999) it was reported that the follicle-associated T lymphocytes, macrophages, and B cells are increased in the ovary during ovarian maturity and then decreased with aging and this report was similar with the finding of this study.

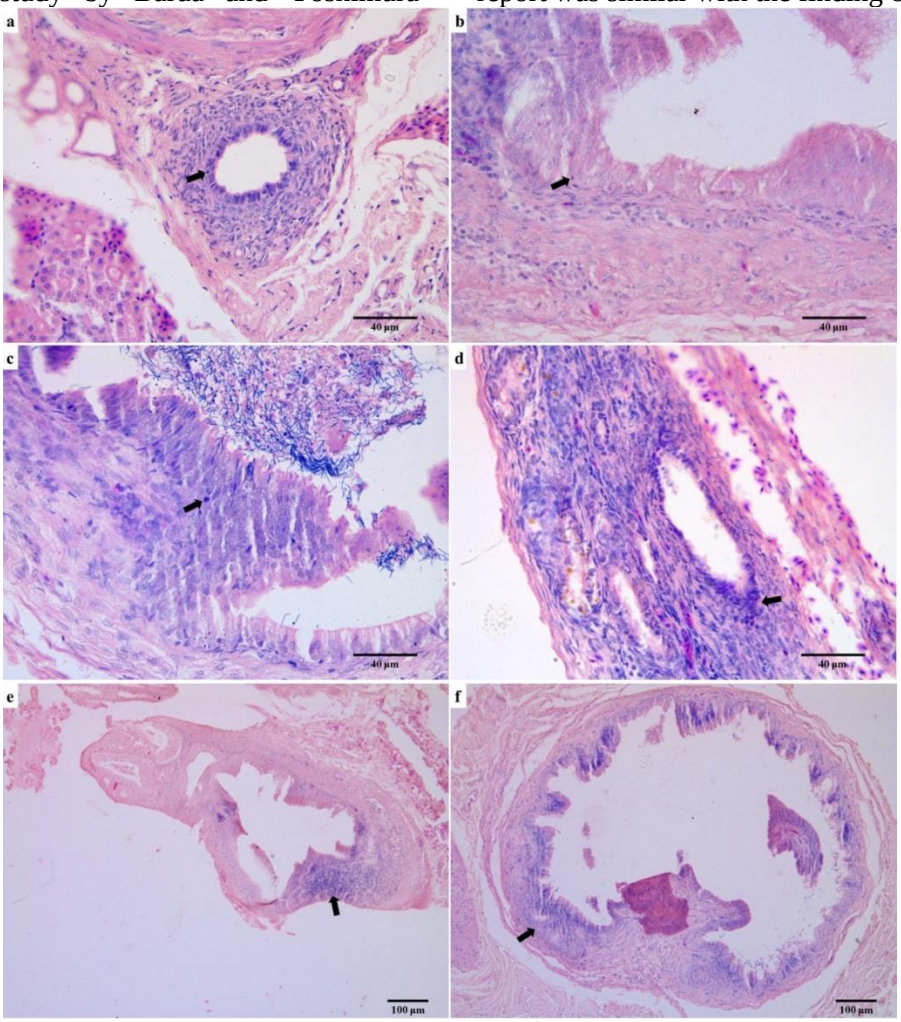

Figure 13. Hematoxylin and eosin staining of the vas deference from the genital system of the male native chicken. The arrow in the images indicated IELs (a; 30 days, b; 90 days, and c; 180 days) and aggregated lymphoid tissues (d; 30 days, e; 90 days, and f; 180 days). Scale for low magnification: 100 µm, for high magnification: 40 µm.

In the study, we observed that the mucosal folds of the infundibulum were lined with columnar secretory ciliated epithelium. The surface epithelium of the isthmus and uterus was lined by pseudostratified columnar ciliated and non-ciliated secretory cells. Similar results were reported by Wani et al. (2017) who found that the magnum's tubular glands had distinguishing characteristics. According to their various functions and roles in egg formation, each section of the tract differed from the others in terms of the shape, size, and number of folds as well as the amount of muscle present. The study also identified that the total of IELs in the infundibulum, isthmus, and uterus of female native chickens has a significant variation between 90 days and 180 days ($P \leq 0.05$). A closely consistent result was reported by Kowalczyk et al. (2020) where T lymphocyte percentage was lower in 32-week-old turkey hens, and then the population of these cells gradually increased until the 38th week of the bird's life. Almost similar result was reported by Johnston et al. (2012) who found that T lymphocyte percentages were lowest in the

oviduct of chicken at the start of the laying period and significantly increased on day 165 of the bird's life. This study found a higher number of IELs in the uterus than the other segments of the oviduct, observed at 180 days of chicken. A similar result was reported by Kowalczyk et al. (2020) who found that the mean IELs in the section of the uterus were higher in adults than in immature birds. There was a significant correlation between avian age and the population of lymphocyte cells in different parts of the reproductive system. From the infundibulum to the uterus, the frequency of the lymphocyte peaked at 15 weeks; in the vagina, it peaked at 21 weeks (Khan et al., 1997). According to the lymphocyte population in the blood, estrogen promotes B lymphocytes to migrate from lymphoid organs into the oviduct through peripheral blood circulation. On the other hand, Chousalkar et al. (2007) suggested that in any viral infection the number of infiltrated lumphocytes increase in the oviduct of chicken whereas in this study we didn't consider any infected birds.

In the present study, the histological structure of testes revealed that Leydig's cells were present in the interstitial tissue as well as the germinal epithelium that lined the seminiferous tubules. These findings were similar with the findings of Razi et al. (2010) and Sun et al. (2019). In present study the IELs and aggregated lymphoid tissues were found in the testes and it was supported by Osman (1980), who reported that the intraepithelial lymphocytes in the tubuli recti, rete testis, and the lumina of the terminal segment and the lymphocytes were also present among the cells of the boundary tissue. Besides, Monleon et al. (2008) added that the affected testes were infiltrated by heterophils, lymphocytes, plasma cells, and macrophages in the interstitial tissue.

In the present study, it was found that the lining epithelium of the epididymis and vas deferens is the pseudostratified columnar and columnar epithelium, respectively and this result was supported by Bull et al. (2007) and Althnaian, (2022). The study also found that the epididymis and vas deference had IELs and aggregated lymphoid tissues in the lining epithelium and lamina propria, respectively. Similar result was reported by Aire and Malmquist (1979), who found that in the epithelial layer of the domestic chicken's excurrent ducts, lymphocytes were found to be more concentrated towards the basal lamina than everywhere else. Moreover, Qu et al. (2020) added that the increased infiltration of lymphocytes in the testes and epididymis depended on age and infection by the pathogen.

The present study revealed the morphological appearance of IELs and aggregated lymphoid tissues in the urogenital tract of the native chicken. The IELs displayed a variety of sizes, from small lymphocytes with little cytoplasm to larger cells with obvious cytoplasm. The result was supported by Wilson et al. (1986) who reported that the cytotoxic T-cell markers are predominant in intraepithelial lymphocytes (IELs), whereas T-helper characteristics are less frequently observed. The IELs have been considered to play a key role in the defense system of the urogenital tract. Their primary function is to maintain the integrity of epithelial cells, regulate tract homeostasis, maintain epithelial barrier function, and rapidly respond to infection (Sheridan and Lefrançois, 2010). The distribution of IELs and the lymphocytes located in other histological layers of the urogenital tract have established the baseline for the parameters of quantitative analysis of lymphocytes in the native chicken of Bangladesh, and this finding could be utilized for the diagnosis of various diseases.

5. CONCLUSION

In conclusion, the distribution of lymphoid cells and tissues significantly depends on the ascending age. There was a significant variation in the mean of IELs in the urogenital tract of female chickens from male chickens whereas, there was no significant variation of aggregated lymphoid tissues with the native chicken ages.

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