Research Note: Effect of age on the distribution of lymphocytes in the oviduct in Turkey breeder hens

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ABSTRACT Considering the lack of research on the local immunity of the reproductive tract in other poultry species than chickens, the aim of this study was to determine the contribution of T and B cell subpopulations in different parts of breeder turkey oviduct mucous membrane with the use of flow cytometry. In addition, the study aimed to establish an impact of bird age and different stages of the egg production cycle on the systemic and local oviduct-related immune system structure. Our study results demonstrated a lower percentage of T lymphocytes in 32-wk turkey hens followed by a successively increasing population of these cells up to the 38th week of bird's life. The results of our study have also shown a similar dependency between birds' age and number of B lymphocytes. In addition, we demonstrated a decrease in the number of immune system cells in the oviduct, blood, and spleen of turkey hens in the late and end laying period. The differences reported in the number of lymphocyte subpopulations in the reproductive system of laying turkey hens at various stages of the production cycle may, to some extent, explain the frequency and periods of increased predilection to the incidence of infectious diseases in birds under field conditions.

Key words: turkey hen, oviduct, flow cytometry, lymphocyte

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INTRODUCTION

Apart from central lymphatic organs of the avian immune system, secondary immunological structures, including the reproductive tract associated lymphoid tissue, play an important role in both local and systemic immunity.

A study performed by Withanage et al. (1997) on healthy egg-laying hens led to the identification of various subpopulations of T lymphocytes both in the ovary and oviduct. Cells are concentrated mainly in the ovary, infundibulum, and vaginal part of the oviduct. The $CD4^+$ T lymphocytes are localized mainly in the parietal lamina propria, while smaller counts are found in the submucous membrane and muscular layer, especially in the vicinity of capillary vessels. The $CD8^+$ T cells are distributed uniformly in the lamina propria, muscular layer, and submucous membrane of the oviduct. Some of the $CD8^+$ T lymphocytes can be found intercellularly in the epithelium lining the oviduct.

Kimijima et al. (1990) demonstrated that IgY^+ B cells occurred mostly in the superficial layers of the mucus, among epithelial cells, and were strongly connected with the glandular tissue along the whole length of the oviduct. The IgA^+ and IgM^+ B cells are present in all parts of the oviduct but are most numerous in the glandular tissue of the magnum. Also, the study by Withanage et al. (1997) demonstrated the presence of B lymphocytes in the oviduct of egg-laying hens. The IgA^+ B cells are mostly found under the epithelium, while being far less numerous in the submucous and muscular layers of the oviduct. The highest number of these cells is found in the magnum, isthmus, and vaginal parts of the oviduct. In turn, the IgY^+ B cells occur mostly under the epithelium and near the glands of the magnum and isthmus. Finally, the IgM^+ B cells are present mainly in the isthmus and the magnum of the oviduct under the epithelium. The strategic localization of B cells in the avian reproductive system most probably implicates their local stimulation toward the production and secretion of specific antibodies against invading antigens.

In addition, Zheng et al. (1998) concluded that the functioning of the oviduct-related immune system in egg-laying hens was influenced by sex hormones, the

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level of which is age-dependent. Based on the cited research, it was proven that local immunity within the reproductive system becomes more effective during the time of attaining sexual maturity, whereas percentages of T and B lymphocytes in the mucous membrane of the oviduct decrease steadily with age.

Considering the lack of research reports on local immunity of reproductive tract in other poultry species than chickens, the aim of the present study was to determine the contribution of T and B cell subpopulations in different parts of breeder turkeys' oviduct mucous membrane and the impact of age and different stages of egg production cycle on local oviduct-related immune system structure.

MATERIAL AND METHODS

Experimental and animal handling procedures were conducted in accordance to the approval of Local Ethical Commission for Animal Experiments in Olsztyn (resolution no. 23/2016).

Experimental Design and Sampling

The experiment was carried out on a commercial farm of Hybrid XL turkey breeder hens (Grelavi S.A., Poland), localized in the Warmia and Mazury Province in Poland. Samples were collected in 5 stages of birds' life which represented different physiological periods of their reproductive system functioning, that is, in the 32nd week of birds' life (laying onset–sampling 1), 38th wk (laying peak–sampling 2), 44th wk (late laying–sampling 3), 50th and 56th wk, that is, at the end of the laying (sampling 4 and 5, respectively). During each sampling, blood (2 mL/per bird) was collected from the wing vein of turkey breeder hens (n = 5) to test tubes with anticoagulant (BD Vacutainer K2E; BD, Holdrege, NE). Furthermore, after euthanasia of 5 birds and separation of oviducts, mucous membrane samples from the relevant sections (3 parts of magnum [the initial, middle, and final section], isthmus, and 3 parts of uterus [the initial, middle, and final section]) were obtained. Spleen samples (n = 5) were also collected, and eventually all samples were subjected for flow cytometry analysis.

Isolation of Lymphocytes

Mononuclear cell isolation from blood and spleen samples was performed with the use of Histopaque (Sigma-Aldrich, Taufkirchen, Germany) density gradient. In the case of oviduct mucous membrane, Percoll (Sigma-Aldrich, Taufkirchen, Germany) density gradient was used to isolate the mononuclear cells.

Blood Samples and Spleen

Blood samples were diluted (1:1) with phosphate buffered saline (PBS; Sigma-Aldrich, Taufkirchen, Germany), and 2.5 mL was gently layered on 2.5 mL of Histopaque.

Spleen samples (0.3 g) were homogenized using a manual Dounce tissue grinder (Kimble, Rockwood, TN) in 3 ml of complete cell culture medium. Homogenized samples were filtrated through 70-μm mesh, nylon, sterile cell strainers (Falcon; Thermo Fischer Scientific, Bartlesville, OK), filtrates were washed twice in culture medium. Finally, cell pellets were resuspended in 2.5 mL of RPMI-1640 and gently layered on 2.5 mL of Histopaque.

After density gradient centrifugation at 400 × g at room temperature for 30 min, with the break off, isolated mononuclear cells populations were collected from the interphase and washed twice in PBS with 5% fetal bovine serum (FBS; Sigma-Aldrich, Taufkirchen, Germany).

Oviductal Samples

Oviduct mucous membrane samples (0.3 g each) were cut into small (2 mm) pieces and then digested at 38°C for 35 min with the use of collagenase type IV solution (RPMI-1640, 1% HEPES, 1% Penicillin-Streptomycin, 200 Collagen Digestion Units/ml; Sigma-Aldrich, Taufkirchen, Germany). After filtration through 70-μm mesh, nylon, sterile cell strainers (Falcon), samples were washed twice in culture medium (RPMI–1640, 1% MEM nonessential amino acids solution, 10% FBS, 1% Penicillin-Streptomycin, 1% sodium pyruvate, 1% HEPES; Sigma-Aldrich, Taufkirchen, Germany) to remove the residues of collagenase solution. After centrifugation (450 g, 10 min, 20°C) supernatants were discarded, cell pellets were resuspended in 2.5 ml of 40% Percoll density gradient and gently layered on 2.5 mL of 60% Percoll. After density gradient centrifugation (1,900 g, 20 min, 20°C, breaks off), isolated mononuclear cells were collected from the interphase and washed twice in PBS with 5% FBS.

A Vi-cell XR automatic cell counter viability analyzer (Beckman Coulter, IN) was used to determine the viability and the absolute lymphocyte counts (ALC)/ml in each sample.

Flow Cytometry

The percentages of the subpopulations of turkey hens' blood, spleen, and oviductal mucous membrane T and B lymphocytes were determined by flow cytometry.

Two hundred and fifty thousand of viable lymphocytes from samples were stained with monoclonal Mouse anti-Chicken CD4—FITC (clone 2-35; Bio-Rad, Hercules, CA) and CD8—Alpha:RPE (clone 11-39; Bio-Rad, Hercules, CA) for T lymphocytes, or polyclonal Goat anti-Chicken IgM—FITC for B cells (Bio-Rad, Hercules, CA), incubated for 30 min on ice and washed twice in PBS. Relative cell counts (RCCs) of T and B lymphocytes in samples were established with FACS Canto II (BD, Holdrege, NE) and FlowJo 7.5.5 (Tree Star, Ashland, OR). The antibodies used in the study were tested

for cross-reactivity with turkeys' immune cells (Li et al.1999).

Absolute cell counts (**ACCs**) of T and B cells were calculated with the use of the following formula: ACC = (ALC*RCC)/100%. Data were expressed as the mean ACC of $CD4^+$ and $CD8^+$ and IgM^+ lymphocytes for oviduct samples or as mean RCC for blood and spleen samples.

Statistical Analysis

The ANOVA with repeated measurements was used to determine differences in the contribution of T and B cell subpopulations in blood and spleen samples and in the ACC in different parts of breeder turkeys' oviduct mucous membrane at various stages of the egg production cycle.

All calculations were made using Statistica 13.1 software (StatSoft, Krakow, Poland) and Graphpad Prism 6 software (San Diego, CA). Differences were found statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Owing to its morphology and topography, the avian reproductive system is a predilection site for infections induced by various pathogens and may lead to functional impairments of the ovary and the oviduct; hence, the appropriate and efficient functioning of the local immune mechanisms is of outmost significance in this respect. The morphology of immunological structures associated with the reproductive system of birds has been relatively well described in a domestic hen, while little is known on this system's morphology in other species of commercially produced birds (Kimijima et al., 1990; Withanage et al., 1997; Zheng et al., 1998). Most of the works addressing the avian reproductive system and its immune system are based on histological examinations. Research is missing that would make use of precise analytical techniques to determine the percentage contribution of subpopulations of immune system cells in the reproductive system of turkey breeder hens.

Table 1 presents the contribution of T and B cell subpopulations in blood and spleen samples of breeder turkey hens in each sampling. No significant differences were found in percentage of $CD4^+$ and $CD8^+$ cells in blood of turkey breeder hens between the successive sampling periods. The percentage of $CD4^+/CD8^+$ cells was significantly higher in blood at sampling 2 than at sampling 1 ($P = 0.029$) and in case of IgM^+ cells at sampling 5 compared with sampling 1, 2, and 3 ($P = 0.041$; $P = 0.049$; and $P = 0.020$, respectively).

In spleen samples, the percentage of $CD4^+$ cells was significantly lower at sampling 5 than at sampling 2, 3, and 4 ($P = 0.002$; $P = 0.02$; and $P = 0.01$, respectively). The contribution of $CD8^+$ cells subpopulations was significantly higher at sampling 1 than at sampling 4 and 5 ($P = 0.006$ and $P = 0.001$, respectively) and at sampling 3 than at sampling 5 ($P = 0.037$). No significant differences were detected in $CD4^+/CD8^+$ cells subpopulation. At sampling 3 and 4, the percentage of IgM^+ cells was higher than that at sampling 1 ($P = 0.038$ and $P = 0.035$, respectively) and sampling 5 ($P = 0.029$ and $P = 0.027$).

The results of flow cytometry analysis of spleen and blood mononuclear cells provided in our study are similar to the findings reported by Kubińska et al. (2014). On the other hand, Suresh et al. (1993) demonstrated that the mean percentage of $CD4^+$ cells in the peripheral blood and spleens of 10-week-old turkeys was 29.8 and 26.3, respectively. In our study, the mean contribution of $CD4^+$ cells in blood and spleens was 11.38% and 23.24%, respectively. Furthermore, results of our study indicated the variability of the mean percentage of $CD8^+$ cells in blood (3.47%) and spleen (36.87%) in comparison to 13.6 and 15.5%, respectively, demonstrated earlier by Suresh et al. (1993). These differences may result from, i.a., age, sex, physiological status, hormonal metabolism, environmental conditions,

Table 1. Percentages of T and B cell subpopulations[1] in blood and spleen of breeder turkey hens at 5 different time points (sampling 1–32nd wk of birds' life, 2–38th wk, 3—44th wk, 4—50th wk, and 5–56th wk).

	Sampling time				
Blood samples	1	2	3	4	5
T cell subpopulations					
$CD4^+$	10.52^{a}	11.11^{a}	11.26^{a}	15.89^{a}	8.01^{a}
$CD8^+$	2.48^{a}	3.27^{a}	4.81^{a}	4.30^{a}	2.49^{a}
$CD4^+$ $CD8^+$	0.3^{b}	0.94^{a}	$0.56^{a,b}$	$0.72^{a,b}$	$0.64^{a,b}$
B cell population					
IgM^+	4.51^{b}	4.69^{b}	4.39^{b}	$5.95^{a,b}$	7.10^{a}
Spleen samples					
T cell subpopulations					
$CD4^+$	$22.24^{a,b}$	28.54^{a}	24.92^{a}	25.84^{a}	14.65^{b}
$CD8^+$	46.44^{a}	$38.16^{a,b,c}$	$39.88^{a,b}$	$32.48^{b,c}$	27.4^{c}
$CD4^+$ $CD8^+$	2.57^{a}	4.06^{a}	2.47^{a}	2.21^{a}	2.16^{a}
B cell population					
IgM^+	8.66^{b}	$11.88^{a,b}$	12.62^{a}	12.30^{a}	$9,08^{b}$

[a-c]Mean values in a line for equal samples with different superscript letters are significantly different (Tukey's test, $P < 0,05$).

[1]Measured by flow cytometry (see the Materials and Methods section).

Table 2. Mean ACC (±SD) of T and B cell subpopulations[1] in oviductal mucosa of breeder turkey hens at 5 different time points (sampling 1–32nd wk of birds' life, 2–38th wk, 3–44th wk, 4–50th wk, and 5–56th wk).

			Sampling time				
Part of oviduct	Section	Cells subpopulation	1	2	3	4	5
Magnum	MI	$CD4^+$	40,462.2^{b} ± 37,868.1	19,0232.5^{b} ± 91,988.2	329,290^{a} ± 192,847.2	166,450^{b} ± 75,401.91	111,542^{b} ± 56,987.52
		$CD8^+$	72,354.2^{b} ± 68,312.7	18,3622.5^{a,b} ± 78,645.1	327,586^{a} ± 244,828.1	157,848^{a,b} ± 52,518.4	98,350^{b} ± 65,831.1
		$CD4^+$ $CD8^+$	5,499.6^{a} ± 6,765.2	11,249.5^{a} ± 3,019.8	7,913.6^{a} ± 4,020.5	13,789.8^{a} ± 8,360.5	6,039.4^{a} ± 2,886.3
		IgM^+	13,708.6^{b} ± 12,198.9	53,084.5^{a,b} ± 29,946.8	95,956.8^{a} ± 60,272.2	36,902.6^{b} ± 13,890.3	28,867^{b} ± 20,930.5
	MM	$CD4^+$	48,825.6^{a} ± 38,944.7	125,315^{a} ± 27,898.3	213,602^{a} ± 15,930.6	190,326^{a} ± 174,357.9	103,290^{a} ± 102,670.5
		$CD8^+$	164489,2^{a} ± 130,997	161,697^{a} ± 37,772.7	267,946^{a} ± 53,074.3	219,784^{a} ± 143,939	107,188^{a} ± 115,634
		$CD4^+$ $CD8^+$	6,910.3^{a} ± 6,315.4	7,492.7^{a} ± 2,644.7	11,454.6^{a} ± 4,343.9	17,581.4^{a} ± 16,097.2	8,452.6^{a} ± 9,394
		IgM^+	20,665.9^{b} ± 15,271.8	51,656^{a,b} ± 15,621.9	79,943.2^{a} ± 14,542.7	50,108^{a,b} ± 41,162.3	30,343.6^{a,b} ± 31,439.7
	MF	$CD4^+$	28,638^{b} ± 22,643.3	82,595^{b} ± 20,918.8	242,074^{a} ± 90,638.2	120,672^{a,b} ± 70,329.7	125,938^{a,b} ± 43,877.6
		$CD8^+$	69,260.6^{b} ± 65,312.5	180,360^{a,b} ± 35,483.5	321,892^{a} ± 189,379.4	126,538^{a,b} ± 74,396.1	109,516^{a,b} ± 20,045.4
		$CD4^+$ $CD8^+$	4,699.8^{a} ± 6,718.4	7,712.5^{a} ± 2,203.2	12,783.8^{a} ± 4,784.6	12,363.4^{a} ± 10,569	10,179^{a} ± 4,120.1
		IgM^+	12,868.7^{b} ± 10,044	38,586.2^{b} ± 7,575.9	110,552.6^{a} ± 63,671.7	30,555.2^{b} ± 14,083.1	35,425^{a,b} ± 6,896.6
Isthmus		$CD4^+$	43,770.4^{a} ± 25,272	137,085^{a} ± 74,994.7	166,284^{a} ± 98,005.5	154,712^{a} ± 108,884.7	97,230^{a} ± 11,462
		$CD8^+$	211,000^{a} ± 190,065.7	501,260^{a} ± 394,200.7	633,970^{a} ± 342,546.2	19,588^{a} ± 97,624.1	159,972^{a} ± 5,890
		$CD4^+$ $CD8^+$	12,130.6^{a} ± 11,637.6	32,593^{a} ± 10,231.1	26,448.4^{a} ± 9,359.9	24,488.4^{a} ± 27,295.7	15,555.4^{a} ± 2,079.2
		IgM^+	25,370^{a} ± 24,647.7	92,650.2^{a} ± 61,052.9	92,185.8^{a} ± 42,577.7	47,790^{a} ± 36,996.8	45,634.8^{a} ± 3,440.1
Uterus	UI	$CD4^+$	79,048^{b} ± 73,037.9	268,400^{a,b} ± 204,968.7	523,776^{a} ± 251,701.4	216,354^{a,b} ± 81,228.2	190,850^{a,b} ± 172,853.8
		$CD8^+$	231,564.2^{a} ± 237,443.5	292,050^{a} ± 153,701.2	564,616^{a} ± 390,492.5	212,928^{a} ± 45,403.7	281,162^{a} ± 137,909.9
		$CD4^+$ $CD8^+$	10,178.3^{a} ± 13,259.7	15,546.2^{a} ± 13,826.4	26,716.6^{a} ± 11,235.4	23,596.2^{a} ± 14,143.9	21,291.8^{a} ± 15,070
		IgM^+	23,123.7^{b} ± 18,844.6	87,712^{a,b} ± 48,426.6	145,226.8^{a} ± 67,345.1	49,525^{b} ± 15,673.9	61,439.6^{a,b} ± 42,021.8
	UM	$CD4^+$	42,957.2^{b} ± 55,787.7	206,560^{a,b} ± 65,534.5	315,206^{a} ± 39,340.2	192,846^{a,b} ± 104,027.7	136,828.8^{a,b} ± 122,138.8
		$CD8^+$	141,307.4^{a} ± 220,472.1	229,665^{a} ± 159,756.8	344,350^{a} ± 177,754.1	160,334^{a} ± 62,560.9	232,456^{a} ± 120,043.9
		$CD4^+$ $CD8^+$	7,823.4^{a} ± 12,469.6	10,523.2^{a} ± 7,741.2	19,760.6^{a} ± 10,413.1	19,360.4^{a} ± 14,674.6	14,523.6^{a} ± 10,414.3
		IgM^+	17,476.8^{b} ± 24,264	65,421.2^{a,b} ± 20,779.8	89,947^{a} ± 20,844.5	41,012.4^{a,b} ± 21,088.3	47,990.4^{a,b} ± 27,572.2
	UF	$CD4^+$	39,676.2^{c} ± 37,257.2	175,677.5^{a,b} ± 60,152.12	291,364^{a} ± 103,108.9	165,554^{a,b} ± 61,122.6	83,589^{b,c} ± 40,212.8
		$CD8^+$	147,949^{a} ± 209,412.5	267,170^{a} ± 177,607	305,388^{a} ± 179,660.3	152,196^{a} ± 47,694.2	196,852^{a} ± 182,473.2
		$CD4^+$ $CD8^+$	6,926.8^{a} ± 10,845	10,143^{a} ± 4,783.5	16,340^{a} ± 4,349.1	15,424.2^{a} ± 8,812.9	10,909.6^{a} ± 7,596.8
		IgM^+	15,632.5^{b} ± 14,036.7	77,430.2^{a} ± 38,894.4	80,674.2^{a} ± 36,101.6	37,564.8^{a,b} ± 11,266.6	34,396^{a,b} ± 24,023.2

[a-c]Mean values in a line for equal samples with different superscript letters are significantly different (Tukey's test, $P < 0.05$).
Abbreviations: MF, final section of magnum; MI, initial section of magnum; MM, middle section of magnum; UF, final section of uterus; UI, initial section of uterus; UM, middle section of uterus.
[1]Measured by flow cytometry (see the Materials and Methods section).

differences in methods of lymphocyte isolation and staining, and antibody isotypes used in the study, as well as the birds breed (Li et al. 2000; Kushima et al. 2004; Schmidt et al. 2009).

No statistical differences in absolute lymphocyte counts between particular section of magnum and uterus were observed.

Table 2 presents that in the initial part of magnum, the ACC of $CD4^+$ cells was significant higher at sampling 3 than at sampling 1, 2, 4, and 5 ($P = 0.0006$, $P = 0.045$, $P = 0.019$, and $P = 0.0009$, respectively). The number of $CD8^+$ cells was higher at sampling 3 related to sampling 1 and 5 ($P = 0.011$ and $P = 0.030$). No significant differences were found in ACC of $CD4^+$ and $CD8^+$cells between the successive sampling periods. The mean ACC of IgM^+ cells was higher at sampling 3 than that at sampling 1, 4, and 5 ($P = 0.003$, $P = 0.016$, and $P = 0.021$, respectively).

No significant differences were found in percentage of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ cells in the mucous membrane of the middle section of magnum between the successive sampling periods. At sampling 3, the ACC of IgM^+ cells was higher related to sampling 1 ($P = 0.007$).

In the final section of magnum, the mean ACC of $CD4^+$ was significantly higher at sampling 3 than at sampling 1 and 2 ($P = 0.002$ and $P = 0.023$, respectively), and ACC of $CD8^+$ was higher at sampling 3 than at sampling 1 ($P = 0.014$). No significant differences were detected in $CD4^+/CD8^+$ cells subpopulation. At sampling 3, absolute IgM^+ cells count was higher related to sampling 1, 2, and 4 ($P = 0.006$, $P = 0.043$, and $P = 0.026$, respectively).

No significant differences were found in mean ACC of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ cells, and IgM^+ cells in the mucous membrane of isthmus between the successive sampling periods.

The mean ACC of $CD4^+$ cells in the initial section of uterus was higher at sampling 3 than that at sampling 1 ($P = 0.012$) and in case IgM^+ ACC at sampling 3 compared to sampling 1 and 4 ($P = 0.008$ and $P = 0.036$). No significant differences were detected in $CD8^+$ and $CD4^+/CD8^+$ cells subpopulation.

In the middle part of the uterus, the mean ACC of $CD4^+$ cells and IgM^+ cells was higher at sampling 3 than at sampling 1 ($P = 0.0012$, $P = 0.003$, respectively). In the ACC of $CD8^+$ and $CD4^+/CD8^+$ cells subpopulation, no significant differences were detected.

Table 2 presents that in the final section of the uterus at sampling 1 and 5, the mean ACC of $CD4^+$ cells was significantly lower related to sampling 3 ($P = 0.0004$ and $P = 0.002$, respectively) and in sampling 1 compared to sampling 2 and 4 ($P = 0.042$ and $P = 0.038$, respectively). Moreover, the ACC of IgM^+ cells was higher at sampling 2 and 3 than at sampling 1 ($P = 0.031$ and $P = 0.0009$). No significant differences were found in $CD8^+$ and $CD4^+/CD8^+$ cells subpopulation.

As demonstrated by earlier research, changes observed throughout the production cycle in the structure and functioning of the local immune system of the reproductive system of hens result in the immunosuppression and, consequently, in the increased susceptibility of birds to viral and bacterial infections. Johnston et al. (2012) reported the lowest percentage of T lymphocytes in the oviduct of hens at the beginning of the laying period, and a significant increase in the population of these cells on day 165 of bird life. A similar correlation between the age of birds and the population number of $CD3^+$ cells in different sections of the reproductive system was described by Zheng et al. (1998). These authors observed also vast differences in the population number of B lymphocytes, each time demonstrating their lower number in the sexually immature birds compared to the birds in the laying period; however, the percentage of these cells increased along with birds age depending on oviduct section. The aforementioned findings correspond with results of our study which demonstrated a lower percentage of T and B lymphocytes in 32-week turkey hens followed by a successive increase in the population of these cells till the peak of the laying period. In addition, we demonstrated a decrease in the number of immune cells in blood, spleen, and oviduct samples in the late and the end of laying period, when the number of produced eggs was successively depleting. Zheng et al. (1998) explain these phenomena with a change in progesterone and estrogen production profile in birds depending on the degree of their sexual maturity, as the enhanced production of these hormones at the peak of the laying period affects an increase in the number of immune system cells in the reproductive system.

An immunohistochemical study conducted by Withanage et al. (1997) described the distribution of T and B lymphocytes in the reproductive tract of laying hens. Results obtained in our study enable concluding that the mean ACC of $CD4^+$ lymphocytes in turkeys' oviduct was higher in the magnum and uterus than in the isthmus (Table 2). In addition, authors demonstrated that the number of $CD8^+$ cells was higher in the isthmus than in the magnum and uterus. The same tendency was demonstrated in our study carried out with turkey breeder hens where the mean $CD8^+$ ACC in the isthmus was higher than that in the magnum and the uterus. Moreover, the analysis of data collated in Table 2 demonstrated that no significant differences were detected between the relevant sections of the magnum and the uterus, reflecting the fact that immune system cells are evenly distributed throughout the oviductal mucous membrane of every part.

Considering low variable among percentage of T and B lymphocytes in both the spleens and the blood samples, the results of our study should be considered as representing the physiological status of birds and provide grounds for further research concerning the immune system of the reproductive tract structure in turkey hens.

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