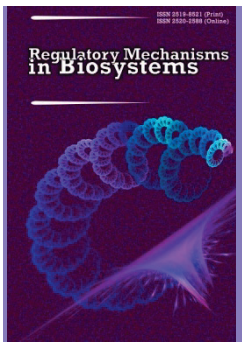


Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(3), 453–462
doi: 10.15421/022464

Morphological and functional spleen development in crossbreed rabbits

I. I. Myroshnychenko, M. A. Lieshchova

Dnipro State Agrarian and Economic University, Dnipro, Ukraine

Article info

Received 25.05.2024
Received in revised form 21.06.2024
Accepted 17.07.2024

Dnipro State Agrarian and Economic University, Serhii Efremov st., 25, Dnipro, 49600, Ukraine.
Tel. +38-067-256-24-86.
E-mail: lieshchova.m.o@dsau.dp.ua

Myroshnychenko, I. I., & Lieshchova, M. A. (2024). Morphological and functional spleen development in crossbreed rabbits. Regulatory Mechanisms in Biosystems, 15(3), 453–462. doi:10.15421/022464

The accelerated growth of muscle mass in productive broiler breeds is often associated with delayed organ development of integral body systems, particularly the immune structures. The spleen is the largest secondary immune organ in the mammalian body and is responsible for initiating immune responses to blood-borne antigens. It can only perform this function successfully if all of its tissue components are fully morphologically and functionally mature. The spleen was studied in meat production rabbits (early maturing crossbreed Hyplus) at 1, 10, 20, 30, 60 and 90 days of age. Morphological studies included anatomical dissection, clarification of topography, determination of mass parameters, preparation of smears and histological sections. Spleen histological sections were stained with haematoxylin and eosin, according to Van Gieson, and impregnated with silver nitrate, followed by microscopy. Qualitative and quantitative indicators of spleen cellular and tissue component development were determined using ImageJ software. It was found that in crossbred rabbits the topography of the spleen corresponds to general anatomical principles of localisation, has a fixed place and is an anatomically formed organ. In day-old animals, the histological differentiation of the spleen is limited to the connective tissue stroma and the parenchyma. The organ's parenchyma is formed by reticular tissue, with hematopoietic and lymphoid cells among the cells, without differentiation into white and red pulp. In 10-day-old rabbits, the white pulp is represented by the periarterial lymphatic sheath (PALS) and marginal zones. Single primary lymphoid nodules without germinal centres are seen in the spleen from 20 days of age. By 30 days of age, the white splenic pulp has all the major structural and functional zones, including formed lymphoid nodules with germinal centres and mantle zones. During the second and third months of life, the spleen gradually increases the relative area of all white pulp functional zones, reaching a maximum at 90 days of age. In productive rabbits, the cellular composition of the splenic white pulp is represented by lymphocytes (small, medium, large), reticular cells, macrophages and plasma cells. During postnatal ontogenesis, the number of small lymphocytes increases in all white pulp functional zones, reaching maximum values in 90-day-old animals. On the contrary, the relative number of medium and large lymphocytes decreases. The number of reticulocytes in the periarterial lymphatic sheath and lymphoid nodules zones does not change significantly, and in the marginal zone it decreases substantially by the end of the study. The results of determining the timing of morphological and functional maturation of immunocompetent structures in the spleen of meat rabbits are of great biomedical and economic importance. They will serve as a control for comparing changes in rabbit spleen lymphoid tissues during the development of pathological processes, as well as under the influence of external factors.

Keywords: rabbit breeding; white splenic pulp; cellular composition; morphometric parameters; organ topography; lymphoid tissue; organs of haemo- and lymphopoiesis.

Introduction

The spleen is an unpaired multifunctional organ that belongs to the peripheral organs of mammalian haematopoiesis and lymphopoiesis. It is characterised by a high sensitivity to the effects of various environmental factors and living conditions. The spleen is responsible for the formation of an immune response when antigens enter the bloodstream, and also participates in the screening of blood cells that have completed their cell cycle, performs blood and iron storage functions, and is actively involved in the production of biologically active substances (Abdel-Fattah et al., 1999; Kashchenko, 2004; Di Ianni et al., 2005; Cesta, 2006; Dunaievska et al., 2024). Monocytes are deposited in the spleen from where they subsequently migrate to damaged organs (Lewis et al., 2019). This organ serves as a universal haematopoietic organ in the early stages of life (Cenariu et al., 2021). Anatomically, the spleen is the only organ of the immune system located in the direction of blood flow from the aorta to the portal vein system, through which a significant amount of blood constantly passes, so it is also associated with the filtration system (Avilova et al., 2017).

In mammals, the spleen consists of a dense pulp surrounded by a capsule and separated by wide trabecula. The organ stroma (capsule and trabecula) is a dense fibrous tissue containing significant amounts of collagen, elastic fibres and fibroblasts. Smooth muscle cells are found within the fibrous structure. The capsule itself is made up of three layers: outer (superficial), middle (intermediate) and inner (deep). These layers differ in thickness and histoarchitecture, i.e. the degree to which the fibrous structures develop. Together with the trabecula, the capsule forms a musculoskeletal system in the spleen which helps it to adapt quickly to changes in organ volume under the influence of various factors (Ikegami et al., 2016; Avilova et al., 2017). Splenic trabecula are divided into vascular, connective and radial ones. Vascular trabecula originate from the splenic hilum and branch into the parenchyma, where arteries, veins and nerves pass. Connective trabecula have no vessels and branch laterally from the vascular trabecula. Radial trabecula originate from the inner surface of the capsule and are directed radially in the parenchyma (Tarantino et al., 2011; Avilova et al., 2017).

The red splenic pulp is composed of reticular tissue and combines heterogeneous populations of haematopoietic, migrating and circulating blood cells. Its main part is represented by venous sinusoids – spaces filled with blood – and pulp tracts composed of reticular fibres and macrophages (Steiniger, 2015). In the interspaces between the cords, there are also various cellular elements, in particular: erythrocytes, granulocytes, lymphocytes, plasmocytes and plasmoblasts, which arrive there as a result of

migration from the nodules and periarterial lymphatic sheath (PALS) of the white pulp as a result of antigenic species differentiation (Tekhver, 1970; Hruzdeva, 2000).

The white pulp has a more complex structural organisation. It consists of periarterial lymphatic sheaths (PALS), which are located around the pulp arteries, and lymphoid nodules (LNs), which are formed from the PALS directly in the areas where the pulp arteries branch off. Each nodule is a spherical dense accumulation of lymphocytes, immunoblasts and macrophages. Each LN contains a central artery, which is mostly eccentrically located, and four zones: periarterial zone, light centre, mantle and marginal zones (Tekhver, 1970; Cesta, 2006; Dunajevska, 2016).

The development of the mammalian spleen begins with the mesenchyme of the mesenteric region of the gastrointestinal tract, namely near the posterior wall of the omentum, together with the great curvature of the stomach due to the protrusion of duodenal or pancreatic epithelial tissue (Tekhver, 1970; Komakhydze, 1971; Dunajevska et al., 2021). The structure of the spleen, especially tissue/cell ratios, depends on the species and age characteristics of animals. Based on studies by Losco (1992) and Seymour et al. (2006), fetal spleen represents a cluster of primitive reticular cells. In rodents (mice and rats), haematopoietic cells are the first cells to appear after two weeks of embryonic development.

In carnivores (dogs), lymphocytes first appear at seven weeks of embryonic development, while rodent spleens contain little or no white pulp at birth (Cesta, 2006). In rats, the first lymphocytes accumulate in the PALS region from 2 days of age, and dendritic cell precursors appear from 5 days, after which active nodule development begins. At the same time, immunological function is activated at the age of 2 weeks, during the formation of intercellular contacts of antigen-presenting dendritic cells. In rodents, the spleen reaches its peak development during puberty, after which it undergoes gradual involution (Losco, 1992).

Today, there are at least three classifications of the spleen according to its morphological and functional organisation. Based on the histological and morphometric evaluation of its functional zones, four main categories can be distinguished. The first category includes animals in which the spleen has a well-defined depot function (ungulates and carnivores). The second category includes animals with spleens whose main function is immune and bactericidal (mice and rats). The third category includes mammals (humans and cattle) whose spleens combine both protective and depot functions to an equal extent – the 'mixed type'. And the fourth category includes those mammals whose spleens are underdeveloped and functionally inactive (rabbits and guinea pigs) (Tekhver, 1970; Komakhydze, 1971). There is also a somewhat simplified classification in which the spleen is classified as either a depot type (carnivores, ungulates) or a protective type (humans, rodents) (Dunajevska, 2016). According to another classification, the spleen is divided into the following types: metabolic (humans and rodents), in which the white splenic pulp predominates over the red pulp in terms of quantity, and the reservoir, in which, on the contrary, the red pulp predominates over the white (ungulates and carnivores) (Komakhydze, 1971; Hruzdeva, 2000). Differences in the structure of organs in different animal species depend mainly on the predominance of the function performed. However, there is a lack of scientific information on the morphological and functional characteristics of the spleen in productive rabbits. In particular, there is a lack of information on the normal morphology of the spleen in rabbits of new meat breeds, especially on the timing and stages of its formation. Additional information on the morphogenesis of the rabbit spleen during its postnatal adaptation, especially in the conditions of intensive rabbit breeding, is extremely important for further solving the problems of breeding, feeding, keeping, diagnosis, treatment and, above all, prevention of diseases of this species (Nikitina et al., 2021; Nikitina, 2022).

It is known that the lymphoid tissue of the white pulp forms periarterial lymphoid sheaths (PALS), primary and secondary lymphoid nodules and the marginal zone separating them from the red pulp. Identification and characterisation of each splenic compartment, including assessment of the relative size and cellular composition of the PALS and marginal zone, the size and maturation of the lymphoid nodules as manifested by the formation and expression of the major functional zones (periarterial, mantle and light centre), are key to establishing full morphological and functional maturity and accurately assessing the immunological impact on the spleen (Haley, 2017).

The aim of this study was to determine the developmental characteristics and timing of morphological and functional maturity of the spleen of rabbits from the early maturing Hyplus crossbreed.

Materials and methods

The conditions of keeping and feeding the experimental animals, selection of age groups of rabbits, and their withdrawal from the experiment were provided in accordance with generally accepted methods and requirements in compliance with bioethics. The research protocol was reviewed and approved by the local ethics committee of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University (Dnipro, Ukraine). The study was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, France, 18 March 1986, ETS No. 123) and the Law of Ukraine "On Protection of Animals from Cruelty" (Kyiv, 21 February 2006, No. 3447-IV).

Morphological studies were carried out at the Department of Animal Anatomy, Histology and Pathomorphology of the Dnipro State Agrarian and Economic University. The study used 36 meat rabbits (early maturing crossbreed Hyplus) divided into six age groups (1, 10, 20, 30, 60, 90 days old) of 6 animals each. The animals were bred in a private enterprise (Dnipro region, Ukraine). According to technological standards, rabbits are slaughtered at 90 days of age when they reach a weight of 3.5–4.0 kg. The conditions under which the animals were housed met zoohygienic standards; the rabbits were constantly provided with high quality, well-balanced food (fed twice a day) and had constant access to fresh drinking water. The experimental animals were not prevaccinated or treated against ecto- and endoparasites.

For the morphological study, the spleen was removed by anatomical dissection, its topography and general appearance were determined, and the absolute weight was determined using an analytical balance (Metrinco AB224, China) with an accuracy of 0.1 mg. The relative weight of the organs was calculated in relation to the body weight of the animals.

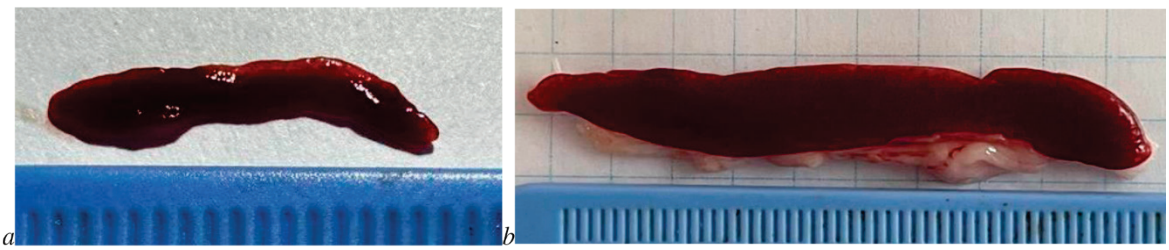


Fig. 1. Spleen of Hyplus rabbit (gross specimen): *a* – day-old rabbit; *b* – 90-day-old rabbit

Midline segments were prepared from the selected organs in a plane perpendicular to the splenic gate and fixed in a 10% aqueous solution of neutral formalin for 3 days. After fixation, they were washed for 3–6 hours to remove the fixative. To obtain overview histological specimens, some of the segments were embedded in paraffin according to generally accepted methods (Horalskyi et al., 2019) and some were used for whole frozen sections on a cryostat microtome. Serial histological sections (6–8 μm thick) were prepared from paraffin blocks and stained with haematoxylin and eosin. Total median frozen sections were impregnated with silver nitrate according to Foot's method modified by Gavrilin (1999). The obtain-

ned microslides were examined with a light microscope Micromed XC-3330 (Ningbo Zhanjing Optical Instruments Co., Ltd., Yuyao, Zhejiang, China) (eyepiece ×10, objectives ×4, ×10, ×40), microphotographs were taken with a digital camera Micromed MDC500 (Ningbo Zhanjing Optical Instruments Co., Ltd., Yuyao, Zhejiang, China) and a personal computer. Qualitative and quantitative parameters of the spleen were determined using ImageJ software (Research Services Branch of the National Institute of Mental Health, USA). The relative areas of connective tissue stroma, red pulp, functional zones of white pulp (periarterial lymphatic sheath (PALS), splenic lymphoid nodules, marginal zones were quantified.

Spleen imprint smears were used for cytological examination. These were prepared by cutting a fresh spleen with a blade, removing excess moisture with filter paper, and applying the cut surface to a degreased slide. The resulting sections were air dried and stained with Pappenheim (Hemacolor, Merck, Germany). The relative number of cells (cytograms) of individual structural and functional zones of the splenic white pulp was determined by differential counting of 100 cells in five fields of view of each area on five preparations of each organ (Horalskyi et al., 2019). The relative number of cells (cytogram) of individual structural and functional zones of the splenic white pulp was determined by differential counting of 100 cells in five fields of view of each area on five slides of each organ (Horalskyi et al., 2019).

All the data were analysed using Statistica 8.0 program (StatSoft Inc., USA). Results in the tables are demonstrated as x ± SD (mean ± standard deviation). Differences between the control and experimental groups values were determined by using the Tukey test (with consideration of Bonferroni's correction), where the differences were considered significant at $P < 0.05$.

Results

In day-old rabbits, the spleen is soft in texture and yellowish-red to light brown in colour. It lies closely adjacent to the dorsal surface of the stomach, in the left cavity between the posterior edge of the stomach and the left kidney, and is suspended from the omentum. Anatomically, the visceral surface of the spleen is slightly concave towards the stomach. The inferior edge is blunt and faces ventrally and posteriorly, whereas the superior edge is sharp and faces dorsally and anteriorly (Fig 1a). The ventral end is directed inferiorly and anteriorly towards the left costal arch and the dorsal end is directed superiorly and posteriorly towards the spine. The splenic gate is located on its visceral surface and in this area a significant thickening of the organ is observed. The absolute weight of the spleen in day-old rabbits is 0.03 g, which is only 0.044% of the animals' body weight (Table 1).

Table 1

Absolute and relative spleen weight of rabbits aged 1 day – 90 days (x ± SD, n = 6)

Tissue elements	Age, number of days					
	1	10	20	30	60	90
Absolute weight, mg	0.033 ± 0.005[a]	0.116 ± 0.025[b]	0.158 ± 0.020[b]	0.491 ± 0.035[c]	0.728 ± 0.069[d]	1.102 ± 0.103[e]
Relative weight, %	0.044 ± 0.011[a]	0.063 ± 0.007[b]	0.045 ± 0.004[a]	0.081 ± 0.002[c]	0.032 ± 0.002[d]	0.035 ± 0.002[d]

Note: different letters indicate values that differed one from another reliably within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction; if within a line the numbers do not have letter indices, then no significant differences were found within one line of the Table.

Histologically, the spleen of day-old rabbits is already differentiated into connective tissue stroma and parenchyma. The stroma is composed of unformed connective tissue with the appropriate cellular composition and structure of the intercellular substance; its relative area does not exceed 7.7% (Table 2). At this age, only the capsule is clearly defined in the stromal component of the spleen. The parenchyma of the organ is composed of reticular tissue with different cellular elements, among which haematopoietic and lymphoid cells can be distinguished. The relative area of the parenchyma is 92.3%. From a morphological and functional point of view, there is no clear differentiation between white and red pulp, but a concentration of lymphoid cells is already noted around the pulp vessels (Fig. 2a).

Table 2

Changes in relative area (%) of rabbit spleen stroma and parenchyma (x ± SD, n = 6)

Tissue elements	Age, number of days					
	1	10	20	30	60	90
Stroma (capsule, trabeculae)	7.69 ± 0.75[a]	9.77 ± 0.98[b]	11.92 ± 0.97[c]	11.64 ± 1.06[c]	11.10 ± 1.08[c]	9.82 ± 1.03[c]
Parenchyma	92.31 ± 0.75[a]	90.23 ± 0.98[b]	88.09 ± 0.97[c]	88.36 ± 1.06[c]	88.91± 1.08[c]	90.18 ± 1.03[b]

Note: see Table 1.

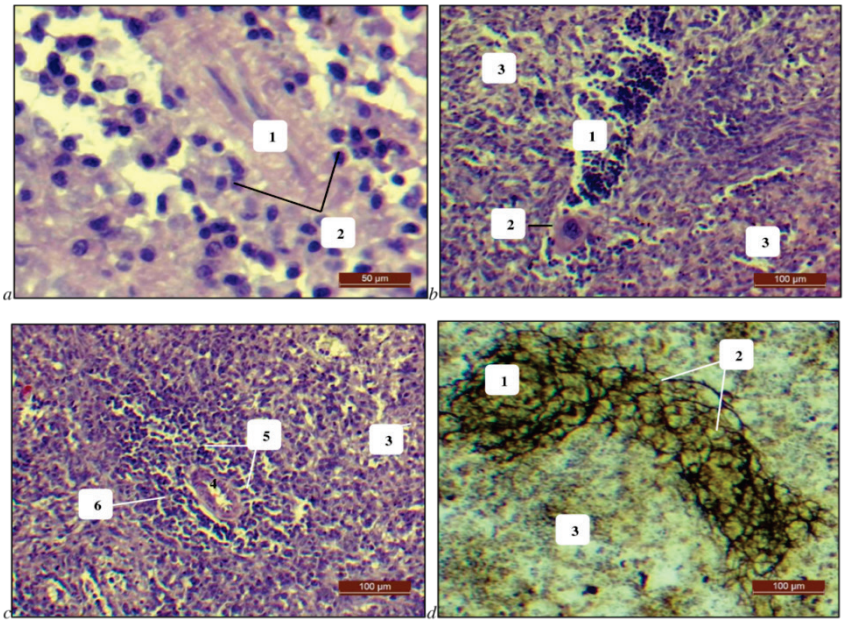


Fig. 2. Microscopic structure of the spleen: *a* – splenic parenchyma of a day-old rabbit: *1* – pulmonary artery, *2* – concentration of lymphoid cells; haematoxylin and eosin; *b–c* – splenic parenchyma of a 10-day-old rabbit: *1* – sites of myelopoiesis, *2* – megakaryocyte, *3* – red pulp, *4* – pulp artery, *5* – periarterial lymphatic vein, *6* – marginal zone; haematoxylin and eosin; *d* – architecture of periarterial lymphatic vein reticular fibres in 10-day-old rabbit spleen: *1* – pulp artery, *2* – reticular fibres, *3* – red pulp; silver nitric acid impregnation

In 10-day-old rabbits, the spleen remains topographically fixed by a ligament to the stomach greater curvature and greater omentum and is displaced deeper into the left hypochondrium due to enlargement of the surrounding organs. The shape changes to elongated-rounded and the colour changes to bright red. The absolute weight of the spleen increases significantly by 3.51 times and the relative weight by almost 1.43 times (Table 1). Histologically, the stroma is more clearly expressed in the spleen of rabbits of this age, and its relative area has increased to 9.8%. The connective tissue capsule is well developed, its thickness is not the same in different areas, it is greatest in the splenic hilum. In the spleen of animals of this age, trabeculae are already well expressed, consisting of dense, unformed connective tissue with individual smooth muscle cells between the collagen fibres. The trabeculae are clearly connected to the blood vessels that grow into the organ from the hilum, and in the parenchyma they branch and form numerous thin-walled vessels.

The spleen parenchyma of 10-day-old rabbits occupies 90.2% of the area and is already differentiated into red and white pulp (Table 3). The red pulp occupies 86.7% of the organ area, its base is formed by reticular tissue and contains a significant number of sinusoids. The cavities of the sinusoids contain clusters of haematopoietic cells, including both erythroid cells and megakaryocytes. Between the sinusoids there are splenic cords with a heterogeneous cellular composition (Fig. 2b).

Table 3

Changes in relative area (%) of white and red pulp of rabbit spleen (x ± SD, n = 6)

Tissue elements	Age, number of days				
	10	20	30	60	90
Red pulp	86.69 ± 0.90^{a}	82.40 ± 0.89^{b}	79.43 ± 2.02^{b}	73.45 ± 2.68^{c}	70.31 ± 2.96^{c}
White pulp, total	3.54 ± 0.62^{a}	5.68 ± 0.53^{b}	8.92 ± 1.42^{c}	15.45 ± 2.35^{d}	19.88 ± 2.57^{e}
marginal zone	1.83 ± 0.33^{a}	2.95 ± 0.28^{b}	4.63 ± 0.74^{c}	7.66 ± 1.12^{d}	9.22 ± 0.73^{d}
periarterial lymphatic sheath	1.71 ± 0.30^{a}	1.35 ± 0.13^{a}	2.13 ± 0.34^{b}	3.56 ± 0.56^{c}	5.57 ± 0.79^{d}
lymphoid nodules	–	1.38 ± 0.13^{a}	2.17 ± 0.34^{b}	4.23 ± 0.67^{c}	5.09 ± 1.05^{c}

Note: see Table 1.

The splenic white pulp consists of lymphoid tissue located along the arterial bed, with a relative area of no more than 3.5% (Table 3). In 10-day-old rabbits, the white pulp is represented by periarterial lymphoid sheaths (PALS) and marginal zones (Fig. 2c). No clearly formed lymph nodes were detected, but at the bifurcation of the pulp arteries, accumulations of lymphocytes were observed, indicating the beginning of their formation, but none of them had light centres. PALS are located around the pulp arteries, formed by reticular fibres and lymphoid cells, their percentage in animals of this age is only 1.7%. Reticulocytes are arranged circularly around the artery and reticular fibres have the same orientation (Fig. 2d). The marginal zone is located at the periphery of the PALS and separates the white pulp from the red pulp. The marginal zone is separated from the red pulp by the marginal sinus and its relative area does not exceed 1.8% in the spleen of 10-day-old rabbits (Table 3). Lymphocytes (small, medium, large), macrophages, plasma and reticulocytes represent the cytoarchitecture of the functional zones of the white pulp at this age. Lymphocytes and reticulocytes are the most abundant cellular elements. Thus, the content of small lymphocytes in PALS is 45.2%, medium – 20.9%, large – 3.3%, while reticulocytes make up 27.9% and the relative number of macrophages does not exceed 1.3%. Plasma cells can also be detected among the lymphoid cells of the PALS, but their number is insignificant – 1.5% (Table 4).

In the marginal zone, the cytoarchitecture is similar, but is distinguished by the fact that there are fewer medium and large lymphocytes. Plasma cells are found only as single cells in some fields of view. The number of macrophages and reticulocytes is significantly higher (Table 5).

Table 4

Changes in relative number (%) of periarterial lymphatic sheath cells in the white pulp of rabbit spleen (x ± SD, n = 6)

Cells	Age, number of days				
	10	20	30	60	90
Small lymphocytes	45.25 ± 0.50^{a}	46.08 ± 0.34^{a}	47.32 ± 0.57^{b}	48.72 ± 1.32^{b}	49.47 ± 2.43^{b}
Medium lymphocytes	20.87 ± 0.33^{a}	19.63 ± 0.61^{b}	18.37 ± 0.55^{c}	16.54 ± 1.12^{d}	15.75 ± 1.18^{d}
Large lymphocytes	3.29 ± 0.19^{a}	3.13 ± 0.23^{a}	2.73 ± 0.45^{a}	2.55 ± 0.35^{a}	2.49 ± 0.25^{a}
Macrophages	1.26 ± 0.14^{a}	1.38 ± 0.20^{a}	1.44 ± 0.21^{a}	1.55 ± 0.27^{a}	1.35 ± 0.27^{a}
Plasma cells	1.49 ± 0.10^{a}	1.66 ± 0.11^{a}	1.73 ± 0.12^{a}	1.85 ± 0.08^{a}	1.88 ± 0.21^{a}
Reticulocytes	27.83 ± 0.29^{a}	28.12 ± 0.69^{a}	28.41 ± 0.66^{a}	28.79 ± 0.81^{a}	29.07 ± 1.35^{a}

Note: see Table 1.

Table 5

Changes in relative number (%) of cells in the marginal zone of rabbit spleen white pulp (x ± SD, n = 6)

Cells	Age, number of days				
	10	20	30	60	90
Small lymphocytes	45.38 ± 1.07^{a}	48.97 ± 0.45^{b}	51.81 ± 1.05^{c}	56.07 ± 1.49^{d}	61.12 ± 0.68^{e}
Medium lymphocytes	16.22 ± 0.94^{a}	15.35 ± 0.63^{a}	14.65 ± 0.82^{a}	13.45 ± 0.79^{a}	12.57 ± 0.50^{a}
Large lymphocytes	1.346 ± 0.064^{a}	1.411 ± 0.089^{a}	1.281 ± 0.140^{a}	1.160 ± 0.089^{a}	1.025 ± 0.028^{b}
Macrophages	3.660 ± 0.389^{a}	3.820 ± 0.074^{a}	3.463 ± 0.064^{b}	3.173 ± 0.288^{b}	2.523 ± 0.108^{c}
Plasma cells	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.273 ± 0.042^{b}	0.286 ± 0.040^{b}
Reticulocytes	33.39 ± 0.95^{a}	30.45 ± 0.64^{b}	28.80 ± 1.21^{b}	25.88 ± 1.07^{c}	22.48 ± 0.87^{d}

Note: see Table 1.

In 20-day-old rabbits, the spleen becomes elongated and its colour changes to deep red. During this period, the absolute weight of the spleen increases by 33% and the relative weight decreases by 1.40 times. Histologically, thickening of the capsule and trabeculae, the percentage of which in the spleen increases significantly to 11.9% (Table 2), indicates further development of the connective tissue structure of the organ. The splenic parenchyma becomes more structured, its division into red and white pulp is pronounced. Indicators of the area of spleen parenchymal components changed significantly. For example, the area of the red pulp decreases by almost 5.0%, and the area of the white pulp, on the other hand, increases by a factor of 1.60 times (Table 3). Foci of erythroid haematopoiesis are still detectable in the red pulp (Fig. 3a). However, they are mainly localised in the sinusoids located under the capsule and along the trabeculae. The white pulp is represented by formed PALS, marginal zones and single lymphoid nodules (Fig. 3b). The relative area of the marginal zone increases to 2.9% and the PALS does not exceed 1.4%. Lymphoid nodules are predominantly primary, do not have a pronounced germinal centre (Fig. 3c), and in this age group their relative area is minimal and only accounts for 1.4% (Table 3).

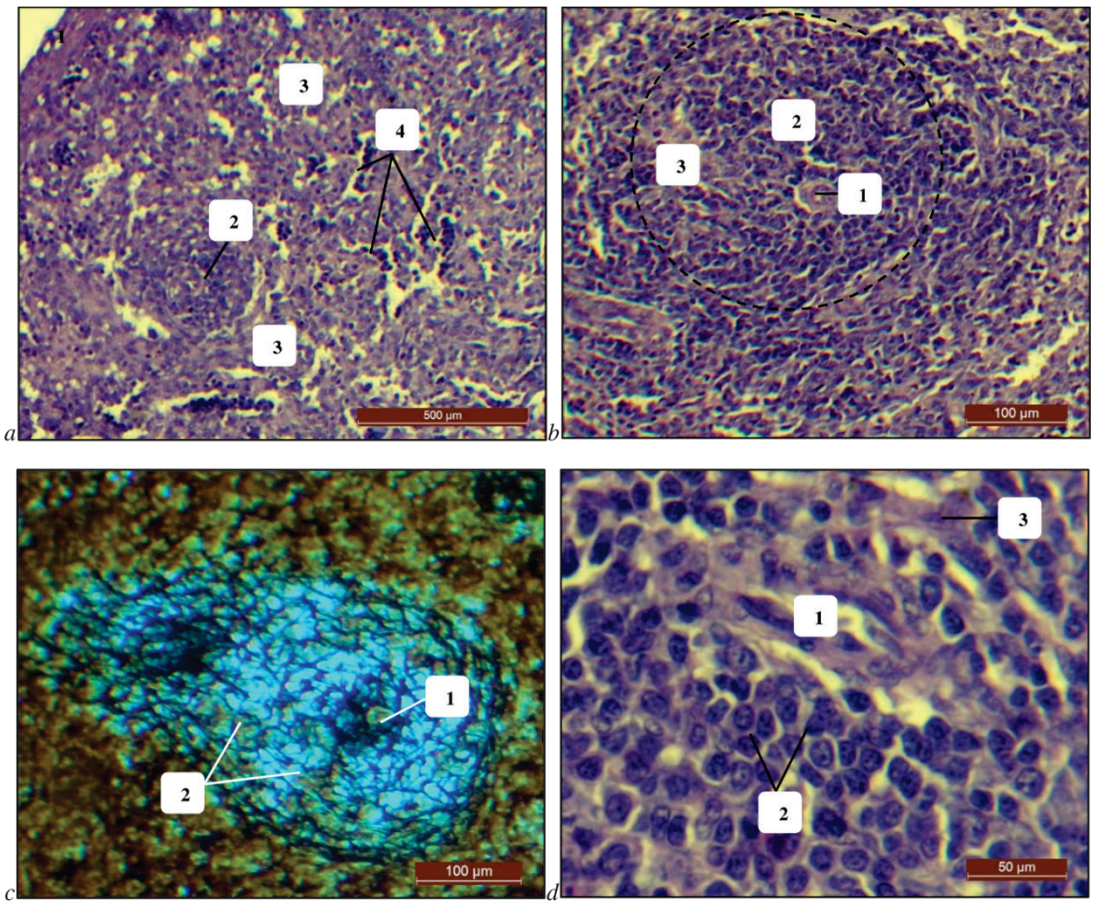


Fig. 3. Microscopic structure of the spleen in 20-day-old rabbit: *a* – histological structure of the spleen: *1* – connective tissue capsule, *2* – white pulp, *3* – red pulp, *4* – sites of myelopoiesis; haematoxylin and eosin; *b* – primary lymphoid nodule: *1* – central artery, *2* – mantle zone, *3* – marginal zone; haematoxylin and eosin; *c* – architecture of the primary lymphoid nodule reticular fibres: *1* – central artery, *2* – reticular fibres; silver nitrate impregnation; *d* – cellular composition of the lymph node: *1* – central artery, *2* – small lymphocytes, *3* – reticulocytes; haematoxylin and eosin

As in the previous group, the cytoarchitecture of the functional zones of the white splenic pulp of 20-day-old rabbits is represented by the same cell types (Fig. 3d). There was a significant change in the relative number of lymphoid cells in the PALS, particularly an increase in the number of small lymphocytes and plasma cells and a decrease in the number of medium lymphocytes (Table 4). In the marginal zone, an increased number of small lymphocytes was also observed, accompanied by a decreased relative number of reticulocytes (Table 5).

In rabbits at 30 days of age, the spleen is elongated with rounded edges, has a predominantly dark red colour, and is elastic in consistency. The absolute weight of the spleen increases sharply and significantly by a factor of 3 and the relative weight by a factor of 1.80 times (Table 1). Splenic histoarchitecture is characterised by having all the structural components formed. Quantitative indicators are characterised by a significant change in the relative area of parenchyma, with the area of red pulp decreasing (by 3.6%) and that of white pulp increasing by one and a half times (Table 3). The red pulp of the spleen is clearly differentiated into sinusoidal capillaryes and cellular splenic cords (Fig. 4a). There are no islands of erythroid haematopoiesis. The white pulp has all the major structural and functional zones, including formed lymphoid nodules with light centres and mantle zones (Fig. 4b). The marginal zone remains the most developed functional zone of the splenic white pulp of this age rabbits, and its relative area increasing sharply to 4.7% compared to the previous age (Table 3). The cellular composition changed only by increasing the relative number of small lymphocytes (by 5.8%) and decreasing macrophages and reticulocytes (Table 5). PALS also increased to 2.1%. This zone is located around the central artery. Its base is made up of reticular fibres and the cellular elements are dominated by small and medium-sized lymphocytes and reticulocytes. By the age of 30 days, the number of small and medium-sized lymphocytes in this zone had changed significantly, with an increase in the relative number of small lymphocytes and a decrease in the number of medium-sized lymphocytes (Table 4). The splenic lymphoid nodules are mostly round or oval in shape, located near the central artery, their total relative area reaches 2.2%, which is 0.8% more than in the previous age group. The central part of the lymph node is occupied by a germinal centre. It is characterised by a loose arrangement of cellular elements and rare reticular fibres. Among the cellular elements, the majority are lymphoid cells, their total number is almost 94.2%, while the number of reticulocytes does not exceed 5.1%. Plasma cells are also found in some fields of view, and the number of macrophages is only 0.7% (Table 6). The mantle zone surrounds the light centre and is characterised by a dense distribution of cellular elements, particularly small and medium-sized lymphocytes (91.7%), and the number of reticulocytes does not exceed 7.2%.

At 60 days of age, the shape of the spleen does not change and the colour varies from dark red to brown. The absolute weight of the spleen increases significantly by 34% and the relative weight decreases by 2.53 times (Table 1). In the spleen, the relative area of the stroma does not change considerably, but the capsule and trabeculae thicken, and smooth muscle cells surrounded by collagen fibres are clearly visible (Fig. 4c). The parenchymal water content increases due to the 1.73-fold increase in the white pulp, while the water content of the red pulp decreases (Table 3). The relative area of all the structural and functional zones of the white pulp increases, but not uniformly. The relative area of the marginal zone increased by 1.65 times, the PALS by 1.67 times and the lymph nodes by half. All lymph nodes are clearly divided into a light centre and a mantle zone (Fig. 4d). The cytoarchitecture of the functional zones of the white pulp of the spleen continues to be represented by lymphoid and reticulocytes as well as macrophages. In all zones, the content of small and medium lymphocytes underwent quantitative changes compared to the previous age, with a significant increase in the number of small lymphocytes and a decrease in the number of medium lymphocytes. There was also a decrease in the number of macrophages and reticulocytes in the marginal zone.

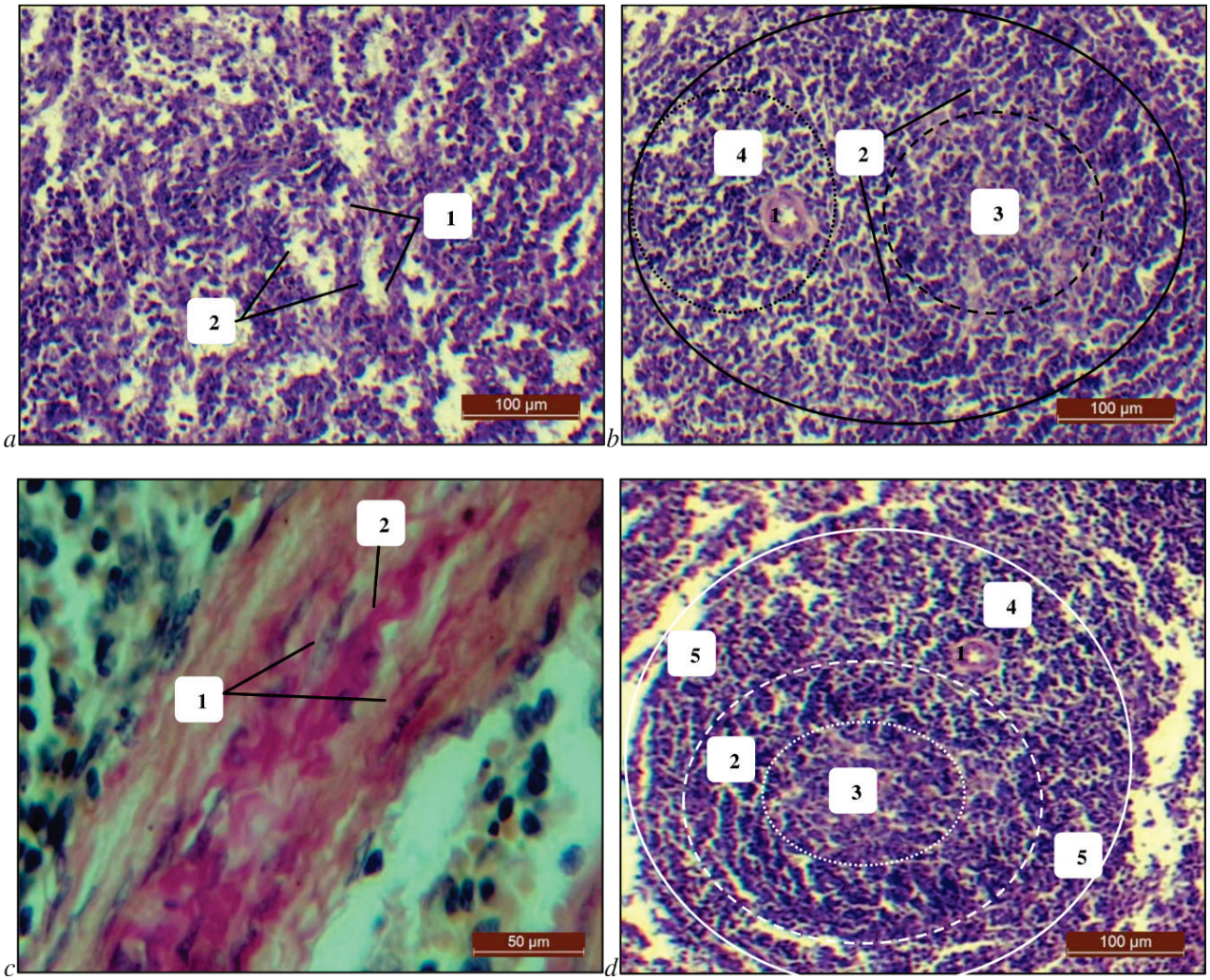


Fig. 4. Microscopic structure of spleen: *a* – red pulp of spleen from 30-day-old rabbit: *1* – splenic cords, *2* – sinusoids; haematoxylin and eosin; *b* – secondary spleen lymphoid nodule from 30-day-old rabbit: *1* – central artery, *2* – mantle zone, *3* – germinal centre, *4* – periarterial zone; haematoxylin and eosin; *c* – spleen connective tissue capsule from 60-day-old rabbit: *1* – smooth muscle cells, *2* – collagen fibres; Van Gieson's stain (picric acid and fuchsin); *d* – secondary lymphoid nodule of the spleen in 60-day-old rabbit: *1* – central artery, *2* – mantle zone, *3* – germinal centre, *4* – periarterial zone, *5* – marginal zone; haematoxylin and eosin

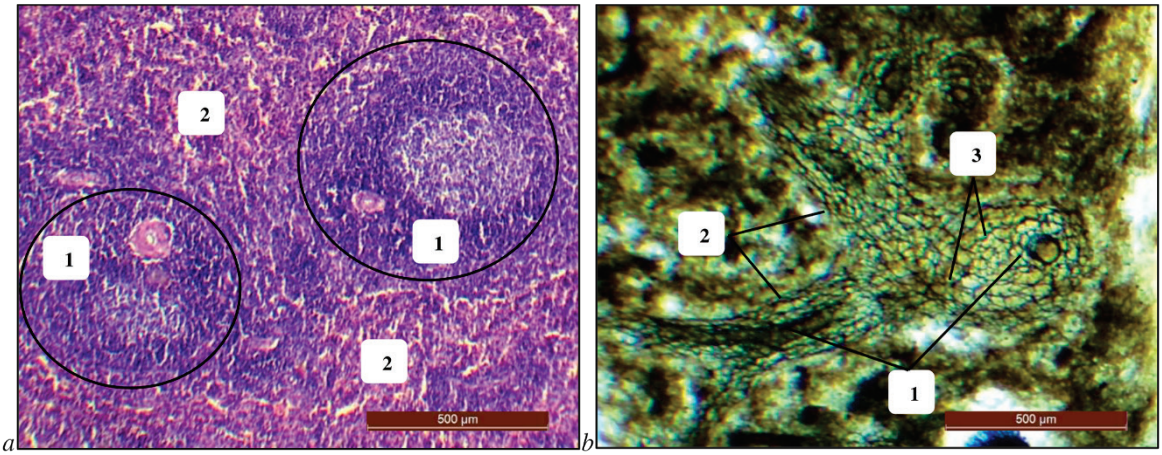
Table 6
Changes in relative number of cells (%) in lymphoid nodules of splenic pulp in rabbits aged from 1 day to 90 days (x ± SD, n = 36)

Cells		Age, number of days		
		30	60	90
Mantle zone	Small lymphocytes	71.48 ± 0.70^{a}	72.99 ± 1.18^{a}	73.56 ± 1.64^{a}
	Medium lymphocytes	20.20 ± 0.38^{a}	18.58 ± 0.64^{b}	17.48 ± 1.38^{b}
	Large lymphocytes	0.973 ± 0.083^{a}	0.868 ± 0.104^{a}	0.785 ± 0.097^{a}
	Macrophages	0.171 ± 0.026^{a}	0.193 ± 0.035^{a}	0.205 ± 0.041^{a}
	Plasma cells	0.000 ± 0.000^{a}	0.121 ± 0.022^{b}	0.133 ± 0.019^{b}
	Reticulocytes	7.18 ± 0.42^{a}	7.25 ± 0.88^{a}	7.84 ± 0.63^{a}
Germinal centre	Small lymphocytes	70.06 ± 0.99^{a}	72.04 ± 0.93^{b}	74.06 ± 1.18^{b}
	Medium lymphocytes	22.24 ± 1.47^{a}	20.33 ± 0.59^{a}	18.90 ± 1.14^{a}
	Large lymphocytes	1.905 ± 0.164^{a}	1.711 ± 0.259^{a}	1.286 ± 0.050^{b}
	Macrophages	0.728 ± 0.138^{a}	0.763 ± 0.166^{a}	0.833 ± 0.033^{a}
	Plasma cells	0.000 ± 0.000^{a}	0.334 ± 0.033^{b}	0.366 ± 0.034^{b}
	Reticulocytes	5.07 ± 0.77^{a}	4.84 ± 0.41^{a}	4.57 ± 0.45^{a}

Note: see Table 1.

In 90-day-old rabbits, the spleen is elongated, slightly flattened at the sides, with a pointed end dorsally and a rounded end ventrally (Fig. 1b). The colour ranges from brownish red to dark purple. The position of the spleen depends on the size and filling of the stomach, but it is always adjacent to the left abdominal wall, with the cranial edge remaining in the left hypochondrium. Its absolute weight increases by almost 51% and its relative weight by 9.4%. Histologically, there is a tendency for the stromal component to decrease in volume and the parenchymal component to increase (Table 2). The red pulp of the spleen is represented by well-formed splenic cords of reticular tissue and various cells, including macrophages and blood cells (erythrocytes, granulocytes, lymphocytes). The reticular fibres form a delicate, fine-meshed network (Fig. 5b). The sinusoids of the red pulp are thin, mostly shrunken or contain a small amount of blood. The water content of the red pulp did not change significantly compared to the previous age of the animals (Table 3). The white pulp of the spleen has the highest relative area rate throughout the observation period, almost 20%, which is 4% more than in the previous age group. Among the functional zones, the marginal zone has the highest relative area (9.2%). The PALS and lymph nodes are fully formed (Fig. 5a), with relative area of 5.6% and 5.1% respectively.

The cellular composition changes significantly in the marginal zone and the germinal centre of the lymph nodes compared to the previous age group. In the marginal zone, the number of small lymphocytes increases significantly, while the proportion of medium and large lymphocytes, reticulocytes and macrophages decreases (Table 5). In the light centre of the lymph node, the quantitative changes affect only lymphocytes, with a significant increase in the number of small lymphocytes and a decrease in the number of medium and large ones (Table 6). The number of cellular components in the splenic PALS of 90-day-old rabbits does not change compared with 60-day-old rabbits (Table 4).



a *b*

Fig. 5. Microscopic structure of the spleen in a 90-day-old rabbit: *a* – histological structure of the spleen: *1* – white pulp lymphoid nodules, *2* – red pulp; haematoxylin and eosin; *b* – architecture of white pulp reticular fibres: *1* – pulp artery, *2* – fine-mesh reticular fibres of the periarterial lymphatic sheath; *3* – rough mesh of lymphoid nodule reticular fibres; silver nitrate impregnation

Discussion

The spleen is the largest lymphoid parenchymal organ of the abdominal cavity, which is a characteristic feature of all vertebrates. Independently of the animal species, the organ consists of the support-contractile apparatus and the pulp (Dunajevska, 2016). At the organ level, the rabbit spleen is an unpaired, compact organ located on the left side of the dorsolateral surface of the great curvature of the stomach. It is relatively small compared to other parenchymal organs. According to Dănacu & Bogdan (2013) and Willaert (2022), in domestic rabbits, the spleen shows no macroscopic differences compared to crossbreeds, maintaining similarity in colour, shape and size, and the variations that exist depend mainly on age, body weight and housing conditions. We have confirmed that in productive Hyplus rabbits the spleen is attached to the left curvature of the stomach by a long ligament. During ontogenesis, the position of the spleen changes slightly due to its enlargement and filling, but its cranial edge remains in the left hypochondrium. It is known that the shape of the spleen varies not only with species and age, but also within the same species and age group (Rahmoun et al., 2020; Fares et al., 2023). For example, in neonates, of 20 spleens examined, 44% were wedge-shaped, 24% were tetrahedral and 32% were triangular (Musleh et al., 2022). Dunajevska (2016) states that the shape of the rabbit spleen is mostly oval-elongated, sometimes with pointed edges, and quite often irregular in shape and may have a caudal extension. Grigorev & Moljanova (2009) observed cases of atypical rabbit spleens (tetrahedral or tongue-shaped). The elongated and crescent shape of the rabbit spleen was described in the study by Altaey et al. (2025). Cases of splitting of the rabbit spleen into two unequal lobes in the hilum region have also been reported (Hristov et al., 2006; Dimitrov et al., 2012; Huynh & Berry, 2017). However, this was not observed in our study. Ikegami (2016) reported a flat and elongated shape of the spleen in domestic rabbits. A number of researchers agree that indicators such as the colour, size, shape or consistency of the spleen are not constant even within the same species and largely depend on the age of the animals, functional load and the condition of surrounding organs and systems (Vishnevskaya & Abramova 2015; Ikegami et al., 2016). We found that the spleen of Hyplus rabbits has a soft consistency with significant variations in shape: elongated, rounded and pointed. The colour of the organ is bright red (from birth to 10 days of age), dark red (from 20 to 30 days of age), and brownish red to dark purple (from 60 to 90 days of age), depending on the intensity of the blood filling and the age of the animals. Changes in its size and shape during postnatal ontogenesis are associated with the active development and shaping of the surrounding internal organs. Because the rodent spleen shrinks slowly, it tends to change its overall shape less (Valli et al., 2002). According to Rahmoun et al. (2019), in adult 15-month-old rabbits of the local breed from the Souk Ahras region (Algeria), the absolute weight of the spleen was 1.86 g with an average rabbit weight of 3900 g. In adult New Zealand rabbits, the spleen weight of males is 1.54 g with an average weight of 3276 g and that of females 1.10 g with a weight of 2714 g (Selcuk, 2022). Dimitrov (2012) found that at the age of 8 months, when rabbits weigh 2800–3200 g, the length of the spleen is 56.2 mm, the thickness was 5.6 mm and the width was 9.8 mm. In rabbits of the same breed with an average weight of 800–1000 g, the weight of the spleen did not exceed 0.547 g (Qasem er et al., 2015). In 6-month-old Californian rabbits, the absolute weight of the spleen was 1.7 g, with a length of 7.45 cm (Dunajevska, 2016). Our studies have shown that both the weight and morphometric parameters of the spleen in crossbreed rabbits at the time of reaching marketable weight (3138 g) were the highest for the entire observation period, in particular, the absolute weight was 1.10 g (Myroshnychenko & Lieshchova, 2022). The older age of the rabbits studied may explain the higher organ weights found by other investigators. Therefore, it can be assumed that at 90 days of age, the spleen of Hyplus rabbits has not yet reached its maximum weight and morphometric parameters. According to Jeklova et al. (2007), the relatively small size of rabbit spleen is determined by the fact that the intestine-associated tissue contains up to 50% of the total mass of lymphoid tissue. It is also known that the spleen of the rabbit is of a protective type and not of a depot type, which also explains the small morphometric parameters of this organ, even in the adult (Haley, 2017).

The formation of the spleen takes place in the prenatal period of ontogenesis. It develops from mesenchymal tissue in the peripheral part of the dorsal mesentery of what will become the great omentum. In the human foetus it can be detected as early as the fourth week in the form of clusters of mesenchymocytes, from the eighth week blood vessels with erythroid nuclei can be detected in it, and from the ninth to tenth week this organ functions with a predominant blood storage function. At 13–14 weeks, white pulp (lymphoid nodules) begins to form in the spleen, and only then is there a clear division into red and white pulp (Musleh et al., 2022). One of the functions of the spleen is myeloid haemopoiesis in the prenatal period of ontogenesis (Cesta, 2006). It varies in intensity depending on how other haematopoietic organs are developing (Cenariu et al., 2021). In most animals, haematopoiesis in the spleen is thought to cease before birth. The exception is rodents, where this process can occur in the postnatal period. Extramedullary haemopoiesis is more common in the mouse spleen than in the rat. In the rabbit, prenatal haemopoiesis occurs in the gallbladder during the first trimester and in the liver at the beginning of the second trimester. During the third period of gestation, the embryonic liver continues to be the best donor organ in order to obtain an active cell transplant (Salutin, & Palianytsia, 2012). In our study, we detected few foci of myeloid haematopoiesis in the spleen up to day 20 of postnatal ontogeny, which may be a functional feature of the early maturing cross. The morphological and functional maturity of the spleen is assessed by the degree of stromal and parenchymal components and the differentiation of the main structural and functional zones of the lymphoid tissue with the corresponding histo- and cytoarchitecture. Important criteria in this case are the presence, size, degree of formation and cellular composition of the PALS, lymphoid nodules and marginal zone (Haley, 2017).

In newborn Hyplus rabbits, the spleen is a well-formed parenchymal organ surrounded by a capsule. However, the parenchyma is not clearly

divided into red and white pulp. The absence of lymphoid nodules in the spleen of rabbits at birth has been reported in other studies (Jeklova et al., 2007). Marasulov (2011), who examined the spleens of rabbits of different ages but without specifying the breed, found that lymphoid tissue was already detectable at one day of age in the form of compact groups of cells around the arteries, but without a clear division into T- and B-dependent cell zones. The time of complete morphological and functional maturation of the spleen, based on the separation of T- and B-dependent zones, has been set at one month of age. This was also confirmed in our study. In humans, the neonatal spleen is structured like the adult spleen. The lymphoid tissue around the arterial bed forms PALS and lymphoid nodules without germinal centres (Musleh et al., 2022). The study by Kholodkova et al. (2011) showed that in the newborn, the white pulp of the spleen has all the characteristic features of the definitive structure. The lymphoid nodules are oval in shape but without germinal centres, the border zone is not clearly defined, and the PALS is formed by 3–5 layers of small lymphocytes. Maximum development of the lymphoid component of the spleen (in relative area) in humans is determined in early childhood. In newborn laboratory rats, the spleen is incompletely formed. Functional zones of the white pulp are not clearly expressed, and germinal centres in the lymph nodes are not detected. The highest functional (immune) activity of the spleen was found only at the age of 6 months, which was manifested by an increase in the number of lymphoid nodules with germinal centres and a higher content of lymphocytes in them (Khasanova, 2022). We found that the relative area of the spleen's lymphoid component is minimal in 10-day-old rabbits. With age, this indicator gradually increases due to all functional zones. The white pulp of the spleen reaches its maximum size in 90-day-old animals, reaching almost 20% of the total parenchymal area. Sadyikova (2016) showed that the relative area of white pulp of spleen in domestic rabbits is 35%, which differs significantly from our experimental results. Dunaievska (2016) shows that the area of white splenic pulp of rabbits is no more then 17.7%. In New Zealand rabbits, the volume of white spleen pulp is 16.7% in females and 13.1% in males, with no statistical significance between them (Selcuk, 2022). Rahmoun et al. (2019) determined the relative area of tissue components in the spleen of local rabbits. It was found that the relative area of white pulp was 11.56% in 1-month-old rabbits, decreased during the 5th and 10th months of development (to 8.9% and 9.3%, respectively), and reached its maximum value of 16.0% only in 20-month-old rabbits. The differences in the results can be explained by the different breeds and age groups of the animals studied, as well as the rearing and feeding conditions. The relative area of white pulp in newborn white laboratory rats averages only 17.2%, increasing to 22.2% at 3 months of age and up to 20.5% at 6 months of age (Khasanova, 2022). Herbut (2005) found the relative area of white pulp in outbred rats at pre-reproductive age (immature) to be 16.5%. Small lymph nodes and bright centres were found in only 16.6%. The highest white pulp area was found in reproductive age rats (20.3%).

The cytoarchitecture of mammalian splenic white pulp is represented by cells of different origin, but mainly lymphocytes, reticulocytes and macrophages. The proportion of these cells varies depending on the white pulp functional zone, animal age and the level of antigenic stimulation (Cesta, 2006). According to Dunajevska (2018), the cellular composition of the white pulp of the spleen of 6-month-old European rabbits is represented by lymphocytes, which account for 89.7% of the total number of cellular elements, among which small (68.0%) and medium (18.7%) are predominant. The number of macrophages does not exceed 1.5% and the smallest number of large lymphocytes (1.4%) and cells with signs of destruction (up to 0.9%). It should be noted that with increasing age of animals, the number of lymphocytes in all structural components of the spleen decreases (Dunajevska & Goralskiy, 2018). We found that in productive rabbits, the cellular composition of the white pulp is represented by lymphocytes (small, medium and large), reticulocytes, macrophages and plasma cells. During the postnatal period of ontogenesis, the number of small lymphocytes increases in all functional zones of the white pulp. It reaches its maximum values in 90-day-old animals. On the contrary, the relative number of medium and large lymphocytes decreases. At the end of the study, the number of reticulocytes did not change significantly in the PALS and lymphoid nodule zones and decreased significantly in the marginal zone. It is known that the splenic white pulp PALS is classified as a T-dependent zone due to the predominance of T-lymphocytes. The lymphoid nodules are a B-dependent zone (Cesta, 2006). A common feature of the splenic pulp of birds and mammals is the location of a subpopulation of lymphocytes with CD4+, CD8+, CD19+, CD20+ clusters, singly and diffusely, most often in the form of chains or clusters.

The main differences between representatives of these classes refer to the number and location of these populations (Goralskiy et al., 2018). In his scientific studies of the spleen of domestic rabbits from day-old to 1.5 years of age, Marasulov (2011) found that the cellular composition of the T-zone is represented by a predominant number of T-lymphocytes and individual macrophages. Even in day-old rabbits, lymphocytes with the surface marker of differentiation CD3+ (T-lymphocytes) are found in significant numbers around the vessels and as a diffuse infiltration in the red pulp. Instead, CD79αcy+ cells (B-lymphocytes) were found around the vessels among the T-lymphocytes, localised in the periphery. A clear division of the white splenic pulp into T-dependent and B-dependent zones is not achieved in rabbits until one month of age. According to Marasulov (2011), at 1.5 years of age, the white pulp in domestic rabbits has clearly formed lymphoid tissue, with a slight decrease in B-zone. It has been found that in the European rabbit, the number of CD4+ lymphocytes in the PALS of the splenic white pulp is almost twice as low as in the chicken and the pigeon from the total number of pulp populations. The relative number of CD8+ lymphocytes in the spleen was 24.5% in rabbits and 38.1% in pigs. CD19+ lymphocytes were present in the splenic lymphoid nodules of all the animals in the study, but they were most abundant in the rabbit (Goralskiy et al., 2018). The first cells to appear in the rodent spleen at birth are T cells, which initially accumulate in the area of the PALS (Losco, 1992). By the 5th day of life, dendritic cell precursors appear, followed by the development of B-cell nodules. Active immunological function begins at 14 days of age due to stable intercellular contacts of antigen-presenting dendritic cells (Van Rees et al., 1996). In rats, the spleen reaches peak development during puberty (2–3 months, depending on the sex of the animal), after which it gradually becomes involved (Losco, 1992). Apt & Talanova (2011), in their study on white rats, found that the cell population of the T-dependent zones of the spleen is characterised by an increase in the proportion of medium-sized lymphocytes (from 37% to 60%) and a decrease in the proportion of large lymphocytes (from 21% to 9%) by the 7th day of life. There was also a statistically significant decrease in the relative number of reticulocytes in the splenic PALS at this age. From day 14, the proportion of medium lymphocytes decreases and the proportion of small lymphocytes increases in the T-dependent zones of the rat spleen. Miller (1991) found that the highest mitotic activity was characteristic of the first (1.2%), 5th (1.2%) and 30th days of life, which, according to him, reflects the process of population formation of the cytotoxic lymphocyte pool in the rat spleen during this period. The number of PNA+ lymphocytes increases from birth to day 5, decreases until day 7 and then increases again.

Conclusions

The topography of the spleen of the Hyplus rabbit follows the general anatomical principles of organ localisation in this mammalian species. Its position is constant and does not change during postnatal ontogenesis. The rabbit spleen at birth is an anatomically and histologically shaped organ, elongated and flattened in shape with minimal mass, length and width. Histologically, it is already represented by stroma and parenchyma, without a clear division into red and white pulp. During the first month of life, there is a rapid development of the major tissue structures of the spleen. This is accompanied by an increase in mass and morphometric parameters. In 30-day-old rabbits, all functional zones of the white pulp can be detected: PALS located along the arterial bed, lymph nodes with distinct germinal centres and a mantle zone, and a marginal zone at the border with the red pulp. This shows the complete formation of lymphoid structures and the organ's readiness for an immune response. During the next period of life (second or third month), the spleen gradually increases the relative area of all the functional zones of the white pulp, reaching its maximum value at 90 days of age.

The cytoarchitecture of the splenic white pulp of Hyplus rabbits is characterised by high lymphocyte content in all structural and functional

zones. Different types of lymphocytes (small, medium, large, plasma cells), reticulocyte cells and macrophages make up the cellular composition of the white pulp of the rabbit spleen. Small and medium lymphocytes predominated in all functional zones, and reticulocytes predominated among the non-lymphoid cells. In most functional zones of the white pulp, the relative numbers of reticulocytes, macrophages and plasma cells increased with age.

Authors declare no conflict of interests with respect to this paper.

References

Altaey, O. Y., Hasan, A. A., & Alhaaik, A. G. (2025). Early-life development of spleen in white rabbit (*Oryctolagus cuniculus*): A morphometric and histochemical analysis. Veterinary Integrative Sciences, 23(1), e2025012-1-13.

Apt, O. A., & Talanova, O. S. (2011). Osoblyvosti morfohenezu biloyi pulpy selezinky shchuriv u rannomu pislianatalnomu periodi ontohenezu v normi ta pislia vnutrishnoplidnoho vvedennia antyheniv riznoyi pryrody [Peculiarities of the morphogenesis of the white pulp of the spleen of ruminants in the early postnatal period of ontogenesis in normal conditions and after intrauterine administration of antigens of various nature]. Pharmacy and Medicine: Science and Practice, 24(1), 18–21 (in Ukrainian).

Avilova, O. V., Prykhodko, O. O., Trach, O. O., Yarmolenko, O. S., & Bumeister, L. V. (2017). Morfo-funktsionalna orhanizatsiia selezinky laboratornykh tvaryn [Morpho-functional organization of the spleen of laboratory animals]. World of Medicine and Biology, 13(59), 175–179 (in Ukrainian).

Cenariu, D., Iluta, S., Zimta, A. A., Petrushev, B., Qian, L., Dirzu, N., Tomuleasa, C., Bumbea, H., & Zaharie, F. (2021). Extramedullary hematopoiesis of the liver and spleen. Journal of Clinical Medicine, 10(24), 5831.

Cesta, M. F. (2006). Normal structure, function, and histology of the spleen. Toxicologic Pathology, 34(5), 455–465.

Di Ianni, M., Del Papa, B., De Ioanni, M., Terenzi, A., Sportoletti, P., Moretti, L., Falzetti, F., Gaozza, E., Zei, T., Spinozzi, F., Bagnis, C., Mannoni, P., Bonifacio, E., Falini, B., Martelli, M. F., & Tabilio, A. (2005). Interleukin 7-engineered stromal cells: A new approach for hastening naive T cell recruitment. Human Gene Therapy, 16(6), 752–764.

Dimitrov, R. S. (2012). Comparative ultrasonographic, anatomotopographic and macromorphometric study of the spleen and pancreas in rabbit (*Oryctolagus cuniculus*). Notulae Scientia Biologicae, 4(3), 14–20.

Dunaievska, O. (2018). Features of the cellular composition of the mammals splenic pulp. Notes in Current Biology, 381, 49–55.

Dunaievska, O. F. (2016). Morfolohichni osoblyvosti selezinky teplokrovnykh tvaryn [Morphological features of the spleen of warm-blooded animals]. Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6(3), 399–406 (in Ukrainian).

Dunaievska, O. F. (2017). Morfometrychni osoblyvosti selezinky zhuinykh u vikovomu aspekti [Morphometric features of the spleen in the age aspect]. Scientific Bulletin of the Lesya Ukrainka Volyn National University, Series: Biological Sciences, 362, 104–110 (in Ukrainian).

Dunaievska, O. F., Horalskyi, L. P., & Sokulskiy, L. P. (2021). Markerni oznaky selezinky tvaryn v onto- ta filohenezi [Marker signs of the spleen of animals in onto- and phylogenesis]. Polissia National University, Zhytomyr (in Ukrainian).

Dunaievska, O., Sokulskyi, I., Radzykhovskii, M., Gutyj, B., Dyshkant, O., Khomenko, Z., & Brygadyrenko, V. (2024). Immunophysiological state of dogs according to the immunoregulatory index of their blood and spleens. Animals, 14(5), 706.

Endo, A., Ueno, S., Yamada, S., Uwabe, C., & Takakuwa, T. (2014). Morphogenesis of the spleen during the human embryonic period. The Anatomical Record, 298(5), 820–826.

Fares, M. A., Khenenou, T., & Rahmoun, D. E. (2023). Anatomo-topographic and histo-cytological study of dromedary's spleen in Algeria. Folia Morphologica, 82(1), 137–146.

Gavrilin, P. N. (1999). Modification of the silver impregnation method according to fut histotopograms of hemopoietic organs made on a microtome cryostat. Bulletin of Morphology, 5(1), 106–108.

Goralskiy, L. P., Dunaievska, O. F., & Yaroshenko, T. Y. (2019). Porivnialna anatomo-imunohistokhimichna kharakterystyka selezinky predstavnykiv klasiv ptakhy i ssavtsi [Comparative anatomic and immunohistochemical characteristics of the spleens of representatives of the bird and mammal classes]. Medical and Clinical Chemistry, 4, 72–78 (in Ukrainian).

Haley, P. J. (2017). The lymphoid system: A review of species differences. Journal of Toxicologic Pathology, 30(2), 111–123.

Herbut, A. O. (2005). Porivnial'na kharakterystyka vidnosnykh ploshch strukturnykh komponentiv selezinky bilykh shchuriv-samtsiv u normi ta vikovomu aspekti [Comparative characteristics of the relative areas of the structural components of the spleen of white male rats in normal and age-related aspects]. Scientific Bulletin of Uzhhorod University: Series Medicine, 24, 9–11 (in Ukrainian).

Hogenesch, H., & Hahn, F. F. (2001). The lymphoid organs: Anatomy, development, and age-related changes. Pathobiology of the Aging Dog, 1, 127–135.

Ikegami, R., Tanimoto, Y., Kishimoto, M., & Shibata, H. (2016). Anatomical variation of arterial supply to the rabbit spleen. The Journal of Veterinary Medical Science, 78(2), 199–202.

Jeklova, E., Leva, L., & Faldyna, M. (2007). Lymphoid organ development in rabbits: Major lymphocyte subsets. Developmental and Comparative Immunology, 31(6), 632–644.

Jeklova, E., Leva, L., Kudlackova, H., & Faldyna, M. (2007). Functional development of immune response in rabbits. Veterinary Immunology and Immunopathology, 118(3–4), 221–228.

Kashchenko, S. A. (2004). Budova orhaniv imunnoyi systemy statevonezrilykh shchuriv pislia vvedennia tymohenu [The structure of the immune system organs of immature rats after administration of thymogen]. Problems of Environmental and Medical Genetics and Clinical Immunology, 58, 49–56 (in Ukrainian).

Khasanova, D. A. (2022). Morphological features of spleen at different stages of postnatal ontogenesis. Central Asian Journal of Medical and Natural Science, 3(1), 154–159.

Kholodkova, O. L., Marasych, A. P., & Neskoromna, N. V. (2011). Morfofunktsionalni osoblyvosti biloyi pulpy selezinky ta yiyi hemomikrotsyrkuliarnoho rusla v ontohenezi [Morphofunctional features of the white pulp of the spleen and its hemomicrocircular channel in ontogenesis]. Odesa Medical Journal, 127, 17–20 (in Ukrainian).

Lewis, S. M., Williams, A., & Eisenbarth, S. C. (2019). Structure and function of the immune system in the spleen. Science Immunology, 33, 6085.

Liu, D., Winer, B. Y., Chou, M. Y., Tam, H., Xu, Y., An, J., Gardner, J. M., & Cyster, J. G. (2023). Dynamic encounters with red blood cells trigger splenic marginal zone B cell retention and function. Nature Immunology, 25(1), 142–154.

Losco, P. (1992). Normal development, growth, and aging of the spleen. In: Mohr, U., Dungworth, D. L., & Capen, C. C. (Ed.). Pathobiology of the aging rat. ILSI Press, Washington. Pp. 75–94.

Marasulov, A. A. (2011). Morfologiya organov i tkaney immunnoy sistemy u krolikov v vozrastnom aspekte [Morphology of organs and tissues of the immune system in rabbits in the age aspect]. Bishkek, Kyrgyz National Agrarian University (in Russian).

Marasulov, A. A., Irgashev, A. S., & Aldajarov, N. S. (2007). Voprosy izucheniya morfologii limfoidnykh organov u krolikov [The issue of studying the morphology of lymphoid organs in rabbits]. Bulletin Kyrgyz National Agrarian University, 1(7), 162–165 (in Russian).

McAnulty, P. A., Dayan, A. D., Ganderup, N. C., & Hastings, K. L. (2012). The minipig in biomedical research. 1st ed. CRC Press.

Mebius, R. E., & Kraal, G. (2005). Structure and function of the spleen. Nature Reviews Immunology, 5(8), 606–616.

Miller, J. F. (1991). The discovery of the immunological function of the thymus. Immunology Today, 12(1), 42–45.

Musleh, A. H., Ali, A. M., & Shalal, M. S. (2022). Anatomical and histological study of neonatal human spleen. NeuroQuantology, 20(4), 52–57.

Myroshnychenko, I. I., & Lieshchova, M. A. (2022). Topography and dynamics of spleen and lymph nodes' morphometric parameters in rabbits. Theoretical and Applied Veterinary Medicine, 10(3), 21–26.

Nikitina, M. (2022). Slice area assessment of the gut-associated lymphoid tissue of sacculus rotundus in Hiplus rabbits. Bulgarian Journal of Veterinary Medicine, 25(2), 200–210.

Nikitina, M., Lieshchova, M., & Kokarev, A. (2021). Morphogenesis and structure of immune formations of rabbits' intestines in the period of post-natal adaptation. Baltija Publishing, Riga.

Qasem, H. H., Rabee, F. O., & Al-A, A. S. (2015). A comparative anatomical and morphological study of spleen in rabbit (*Oryctologus cuniculus*) and guinea pig (*Cavia porcellus*). Journal of Kerbala University, 13(4), 147–155.

Rahmoun, D. E., Fares, M. A., Bouzebda-Afri, F., & Ben Driss, K. (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. Online Journal of Animal and Feed Research, 9(2), 44–50.

Rahmoun, D. E., Fares, M. A., Haffiene M., Messai, K., & Lieshchova, M. A. (2020). Morpho-histological study of spleen ontogenesis in lambs during antenatal and posnatal period. Online Journal of Animal and Feed Research, 10(1), 12–16.

Salutin, R. V., & Palianytsia, S. S. (2012). Characteristic of embryonal hematogenesis in rabbits. Bukovinian Medical Herald, 16(63), 91–94.

Selcuk, M. L. (2022). Stereological and morphometric analysis of spleen on New Zealand white rabbits (*Oryctolagus cuniculus*). Journal of Animal and Plant Sciences, 32(6), 1562–1568.

Seymour, R., Sundberg, J. P., & Hogenesch, H. (2006). Abnormal lymphoid organ development in immunodeficient mutant mice. Veterinary Pathology, 43(4), 401–423.

Steiniger, B. S. (2015). Human spleen microanatomy: Why mice do not suffice. Immunology, 145(3), 334–346.

Tarantino, G., Savastano, S., Capone, D., & Colao, A. (2011). Spleen: A new role for an old player? World Journal of Gastroenterology, 17(33), 3776–3784.
Valli, V. E., McGrath, J. P., & Chu, I. (2002). Hematopoietic system. In: Haschek, W. M., Rousseaux, C. G., & Wallig, M. A. (Ed.). Handbook of toxicologic pathology. Academic Press, San Diego. Vol. 2. Pp. 647–679.
Van Rees, E. P., Sminia, T., & Dijkstra, C. D. (1996). Structure and development of the lymphoid organs. In: Mohr, U., Dungworth, D. L., Capen, C. C., Caldron,W. W., Sundberg, J. P., & Ward, J. M. (Ed.). Pathobiology of the aging mouse. ILSI Press, Washington. Pp. 173–187.
Willaert, W. (2022). Chapter 4: Anatomy and embryology of the lymphatic system of the colon and rectum. In: Ceelen, W. (Ed.). The lymphatic system in colorectal cancer: Basic concepts, pathology, imaging and treatment perspectives. Academic Press, Cambridge. Pp. 57–72.