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AN ANATOMICAL AND HISTOLOGICAL STUDY OF THE RABBIT SPLEEN DEVELOPMENT IN THE POSTNATAL PERIOD IN ALGERIA

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Supporting Information

ABSTRACT: **Our research describes the morphological and histological changes of the rabbit's spleen; local breed of eastern Algeria. In general, the spleen has a rectangular shape with a triangular section, rounded edges, more or less ridged, the surface is smooth, the color is brownish red becomes dark red with age, the capsule and the parietal surface are shiny and smooth. His organ measurement and mass parameters are given. Particular emphasis placed on its microstructure; especially on changes occur during development. Parenchyma histological composition analysis performed using statistical methods. Twenty-month-old rabbits known to have follicles with mantle strongly developed, parenchyma's quantitative analysis components showed significant changes.**

Keywords: **Capsule, Follicles, Lymphoid, Parenchyma, Rabbit, Spleen.**

ORIGINAL ARTICLE

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INTRODUCTION

The mammalian spleen works like a blood deposit. Since birth, red blood cells and lymphocytes are present, also in the postnatal period, the spleen is an organ of lymphopoiesis and protects the body against cells and genetically foreign substances, and also participates in humoral immune responses (Cesta 2006), also, it's a parenchymal organ, unpaired, and participates in the metabolism, especially iron and protein (Tiron et al., 2008). In the field of digestion, the spleen has a physical and chemical role, the blood thickens in the spleen and the elements formed separate to some extent the plasma in which they are suspended (Udroiu et al., 2017). The morphology of the animal and human spleen was carried out by researchers (Udroiu et al., 2017). The morphometry of the white pulp of the spleen in Yorkshire pig has been studied by Shringi et al. (2017).

Spleen studies in goats have been conducted by Hassan et al. (2018). In addition, extensive studies on the human spleen have been conducted by Nerschbach et al. (2016). Like other animals, white and red pulps are distinguished in the spleen of rabbits. The white pulp of the spleen is mainly populated by the corresponding differentiated lymphocytes. The areas of the white pulp include arterial vessels surrounded mainly small lymphocytes (lymph tissue T-dependent), with clear boundaries. Furthermore, in the white pulp of the spleen rabbits, there are lymphoid follicles grouped by lymphoid cells. They are surrounded by a well-defined connective tissue. These lymphoid follicles belong to lymphoid tissue B-dependent (Thomas et al., 1967). The red pulp as white has clear boundaries. Lymphoid follicles are strongly developed according to age and have weak germinal centers. In addition, the white pulp has a distinctive feature: The B-dependent zone is formed after the T-dependent zone and has previously undergone an age-related physiological involution, cited by Nerschbach et al. (2016). Scientific work is devoted to the in-depth study of the normal morphology of different compartments of the spleen, in different species of animals. However, data from the scientific literature regarding the changes in age-related anatomical and histological structure of the rabbit spleen during ontogeny are insufficient.

The objective of this study is to describe the anatomical and histological differences in development of spleen in rabbits during the postnatal period. This work will be of interest for future research in veterinary medicine as well as in biological and medical sciences.

MATERIALS AND METHODS

The ethical approval is not necessary for such type of study. We have used the spleen of rabbits were presented for post-mortem examination. We dissected 50 healthy male rabbits, aged 01-05-10-15 and 20 months, local breed of

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the Souk-Ahras region in Algeria, divided into five age groups according to table 1, whose live weight ranged from 89 to 4200 g. The rabbits were weighed before beginning the experiment in the laboratory of Animal Production, Biotechnology and Health, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria.

A macro-morphometric study (weight gain using a "Tehniprot-WTW" scale with an error point of 0.002 mg) and the measurement of the length and width dimensions of the organs with a GOCT17435-72 ruler set to 1mm. The collected spleens were subjected to a macroscopic and histological study. The following technique was adopted to prepare histological slide; Tissues obtained from rabbits were fixed in 10% formalin for 24 hours and then underwent successive passes through the various compartments; dehydrated in increasing concentration of ethanol, then cleared in xylene and finally soaked in paraffin. The residence time of the fragments in the automate is 24 hours. The blocks were then cut to a thickness of 5 µm with a microtome. The sections were placed into a flotation bath at 37° C. Then, they were placed on the slides and dried on a hot plate. The sections were stained by hematoxylin and eosin to determine the general structures of rabbit spleen and Wiegert hematoxylin and picrofushin (Van Gieson) to study the structural features of the connective tissue stroma. Other frozen sections impregnated with silver nitrate to determine the characteristics of reticular stromal areas of the parenchyma of the spleen. Relative area of tissue components determined by the method of "exact calculation" using S test systems (Dunaievska, 2018) and using optical microscopes Leica DM2000 LED. The photomicrographs made with the digital camera Sony ILCE-6000. Data obtained from our morphometric and histological studies have been performed with the Excel 2016 program.

Table 1 - Rabbit spleen morphometry and mass according to age

Age group, months	1	5	10	15	20
Weight of the animal, g	890±100	1350±100	2400±100	3900±100	4200±100
Mass of the spleen, g	0,8±0,062	1,52±0,026	1,68±0,0688	1,86±0,05	2,11±0,12
Length of the spleen, mm	42,88±0,3	45,8±0,354	61,214±0,95	71,2±1,19	65,28±0,97
Width of the spleen, mm	5,28±0,17	6,66±0,18	7,7±0,19	9,98±0,18	11,3±0,19

RESULTS AND DISCUSSION

After desiccation, the spleen was not directly visible, hidden by the left edge of the stomach, and its axis is in border with the great gastric curvature, in contact with the left costal wall by the external face and in contact with the stomach and the left kidney by the internal surface.

Studies have shown that rabbit spleen may have the following form: oval elongated, with slightly sharp edges, an irregular shape, "baguette", with a process caudate to the back end with a pointed rear end (Figure 1). The oval shape of the spleen has rounded dorsal and ventral ends, the upper and lower edges are smooth. Shaped "drumstick", the dorsal edge of the spleen is transferred and becomes "head" of the "drumstick". The size of this part is nine, six wide and three millimeters thick. Ventral end wider than two millimeters dorsal grows up to eleven millimeters. Smooth sharp edge, wavy and blunt edge.

When the spleen is oval with a caudate process, its dorsal end is six mm in width and one and a half millimeters in length. Next is the three mm extension of the original. The shoot is at a distance of four mm from the dorsal extremity. Its dimensions are four in length, two in width, one and a half millimeters thick, and four millimeters wider than the dorsal in oval ventral extremity. The sharp and blunt edges are smooth. In the case of an irregular shape, the body has a modified rear end of seven centimeters wide, one and a half millimeters thick. The ventral extremity is slightly pointed, with a width equal to six mm. The dull edge is uniform, sharp in the region of the ventral extremity is also smooth, but undulating at the dorsal extremity. In the form with a pointed dorsal extremity, it initially has a width of three, extremity mm wide, still extends three millimeters. Its length is 15 mm, its thickness is five. The ventral end is wider and reaches six mm. Smooth dull edges, wavy sharp edge, the spleen is elongated, with slightly pointed margins, its dorsal extremity has a breadth equal to five mm and a thickness of a one mm and a half, and there is an increase after five mm. The ventral end is seven mm wide and one and a half millimeters thick. Blunt and sharp edges are smooth. In most cases, there is an elongated spleen with some sharp edges. Different forms of the spleen can be explained by the fact that the growth and development of the organ are irregular, and the shape and size of the adjacent organs and the pressure they exert on the formation of internal organs the abdominal cavity.

Morphological changes observed in the hue of the rabbit spleen, the spleen of rabbits aged one month, the hue is red-brown, becomes darker in rabbits which are twenty months old, the lateral edges lose their hue and become less distinct than in the center, this feature was observed in these rabbits with a strong curvature of the arc, a low position and a left shift. The study of the mass of the spleen of the rabbit has revealed a gradual increase of 0.8 g for

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spleens of older rabbits a month and this increase is proportional, reaching 2.11 g for rats aged rabbits 20 months (Figure 2).

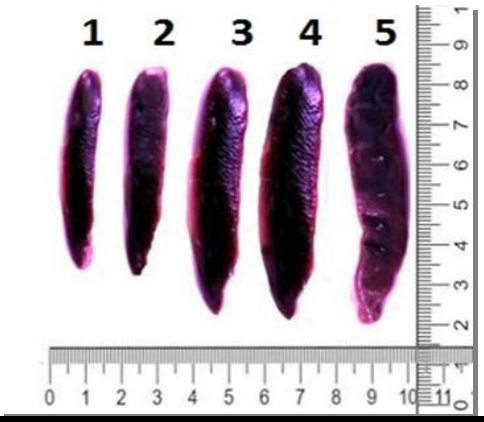

Figure 1. Macroscopic view of different rabbit spleen on negatoscop examination with measurement.

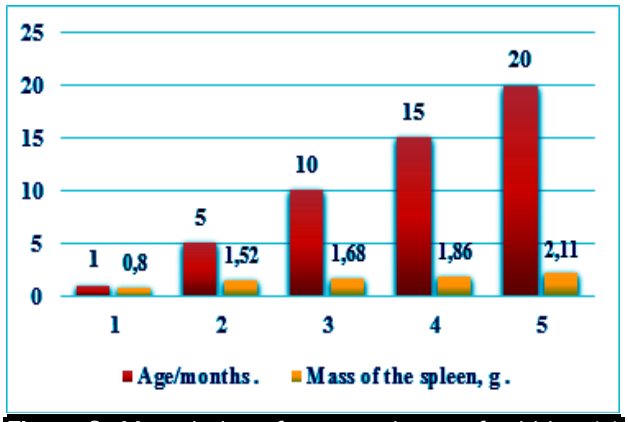

Figure 2. Mass index of some spleens of rabbits, (g) according to age (months)

The length reaches 42.88 mm for the spleen of the animals aged one month and 65.28 mm for the animals aged 20 months, the width was 5.28 mm found in the spleen of rabbits by one month and the value maximum of 11.3 mm for the spleen of 20-month-old rabbits (Figure 3). The length of this portion is nine millimeters, six wide and three millimeters thick. The ventral end is larger than the two-millimeter dorsal. At a distance of seven millimeters from the ventral end, the organ extends to eleven millimeters.

Morphological examination of the spleen of rabbits aged fifteen months revealed a thicker lateral surface than the underneath with three zones of vascular irrigation, wrapped in a thin layer of connective tissue; in particular a fat layer surrounding the end of the spleen, this has also been proved by the researcher (Hristov et al., 2006), the proximal part freely forms a distension curved in height, concerning the spleen of the first group, the fatty layer is minimal and occupies the apical layer of the layer of connective tissue lining the entire splenic zone from the turgor in the ventral part, there has more fixations equal to nine for this age, while the spleen of rabbits aged twenty months, from which the number of supplements reaches 14 according to (Lalić et al., 2018). Thus, the rabbit spleen is characterized by polymorphism and morpho-functional changes that, no doubt, should reflect increasing or decreasing functional processes in the respective developmental periods and affecting the immune system of the animal. The texture, size, weight, color and shape of the spleen vary significantly depending on the duration of its functional activity, age, species and race of the animal (Eberlova et al., 2017).

Stereo-microscopic examination (MBS) revealed rabbit's spleen consists of stroma and parenchyma, stroma formed by capsule and trabeculae that penetrate inside parenchyma. It has been noted that capsule consists of two layers: external connective tissue (elastic) and internal (muscular), allowing the organ to change size and maintain a significant increase in its volume, this was mentioned by Nawal et al. (2018). The muscle layer consists of polymorphic myocytes (Figure 4).

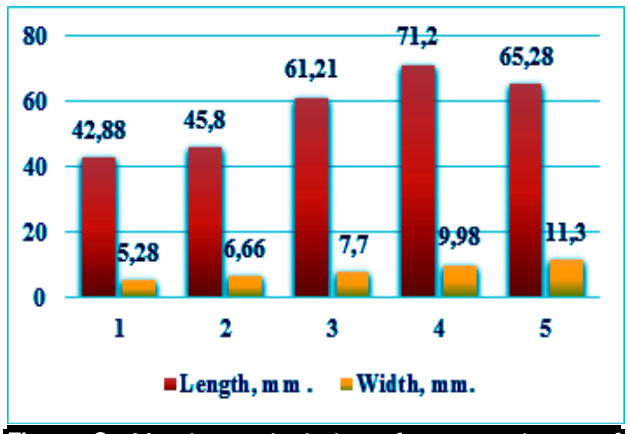

Figure 3. Morphometric index of some spleens of rabbits, (mm) according to the age (month).

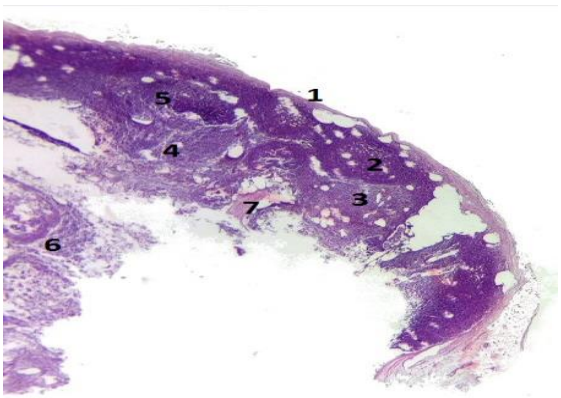

Figure 4. Spleen of one-year-old rabbit. hematoxylin-eosin stain, X 5.7. 1- capsule; 2- red pulp; 3- white pulp. 4 –cortical zone; 5- Cortico-medullary zones; 6- medulla zone; 7- Trabecula.

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At high magnification, it has been observed that red pulp is composed of splenic sinusoidal capillaries and cellular cords. Splenic sinusoids are limited by batch simple epithelium, resting on abasement membrane around sinusoids discontinuously, sinusoidal capillaries contain many blood cells (Figure 5), what has been identified in other spleens by the authors (Medaglia et al., 2017).

It was found that surface of the red pulp is almost identical in spleen of the 1st and 2nd group of age, within maximum is 78.1%, this zone is minimal in the spleen of rabbits aged 10 and 15 months (Figure 5), equal almost 70,78%, deduced that surface of the red pulp decreases with age. These results were consistent with another author research (Fukuta et al., 1969). Concerning white pulp, it should be noted that the surface of the white pulp decreases in the parenchyma of the spleens of the rabbits aged ten months (Figure 6), while 11.56% for spleens of rabbits aged five months. This increase reaches the maximum of 16.04% in the spleen of rabbits aged 20 months. Questions to note about the decrease in spleen value in five-month-old rabbits, probably due to a decrease in antigenic activation.

Capsule trend increase varies from spleen of rabbits aged one month's 10.44% to the spleen of rabbits aged 15 months 14.67%, due to increase in spleen's volume. Also, it has been found that white pulp has several compartments such as peri-arteriolar lymphoid sheaths (P.A.L.S), whose spleen of rabbits aged one month, had a maximum level of P.A.L.S with value of 9.35 ± 0.55%, while the spleen of rabbits aged 20 months, the minimum value was 1.63 ± 0.35%.

Follicles quantitative study shows that rate is almost equal for 2 follicles types (primary and secondary) equal 16.17% 17.38% in spleen of rabbits aged five months. This value also identical in spleen of rabbits aged 15 months 34.45% and 35.82%. It was found that follicles activation is stable probably due to the non-immunization of animals with vaccines, contrary, this finding was not established for rabbits immunized by vaccines, this increase presents change point for secondary lymphoid follicle. Scrutiny optical microscope revealed that mantle secondary lymphoid follicle in marginal areas stand out easily, this was proven by Pinkus et al. (1986), the border zone is transition zone between white and red pulp, also mentioned in research, on mice spleen.

Spleen connective tissue network is highlighted by silver staining, forming precipitates around reticulin fibers. Spleen capsule is crossed by blood vessels, which enter into organ via spans. White and red pulps are clearly visible. Lymphoid follicle has well-defined germinal center and crown (Figure 7). Central artery is also easy to see during this preparation. Reticulin fibers form extensive network through spleen, anchored on capsule and on spans. White pulp comprises lymphoid follicles complex of spleen that fulfill a protective function and produce a larger mass of lymphocytes in the blood. The red pulp has small round growths.

Each site crossed by artery running along periphery, similar research has been demonstrated by other research. Lymphoid tissue silver nitrate impregnation was put in evidence as blood supply to red pulp, which comes from penicillar arteries which give rise arterioles, including endothelial cells and smooth muscle cells well visible in center elucidated also.

Impregnation with silver nitrate, was observed that venous sinuses can be found throughout red pulp, directly adjacent to edge region bordered by endothelial cells loose network lie on basal membrane which sandwiched between endothelial cells and red pulp reticular fibers. In white pulp (Figure 8), it is subdivided into PALS, this research and results conformed with data to Gill et al. (2017), lymphoid follicles and marginal zone. Central arterioles surrounded by PALS in red pulp, composed of lymphocytes and reticular fibers concentric layers and reticular flattened cells.

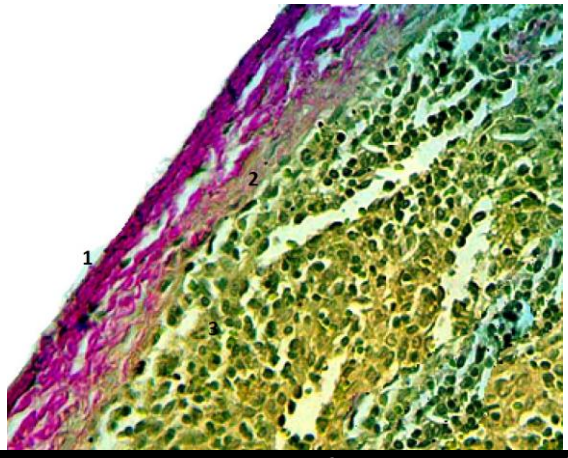

Figure 5. Histological section of rabbit spleen aged 15 months. Van Gieson stain, X40. 1 -layer of connective tissue (elastic). 2 -muscular layers. 3 -splenic parenchyma.

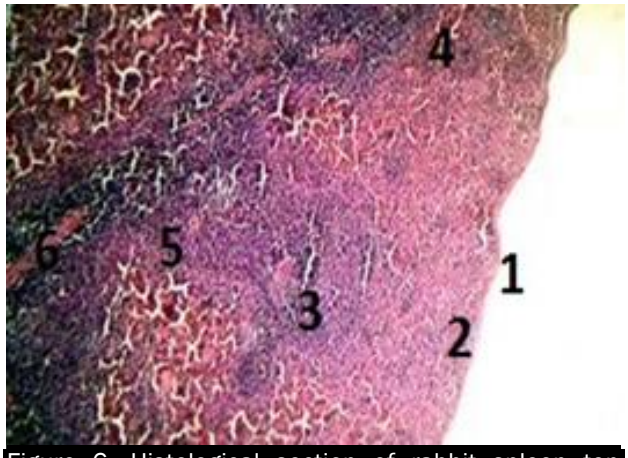

Figure 6. Histological section of rabbit spleen ten months old. Hematoxylin and eosin, × 200: 1 capsule; 2 – subcapsular trabecula; 3 – red pulp; 4 - lymphatic vessels; 5 – white pulp.

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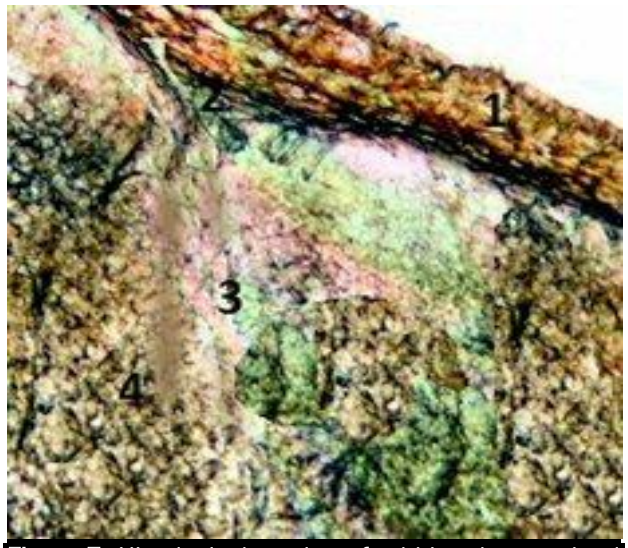

Figure 7. Histological section of rabbit spleen stained with silver nitrate **X 200. 1 - capsule 2 - trabeculae; 3 - pulp artery; 4 – layer of reticular fibers**

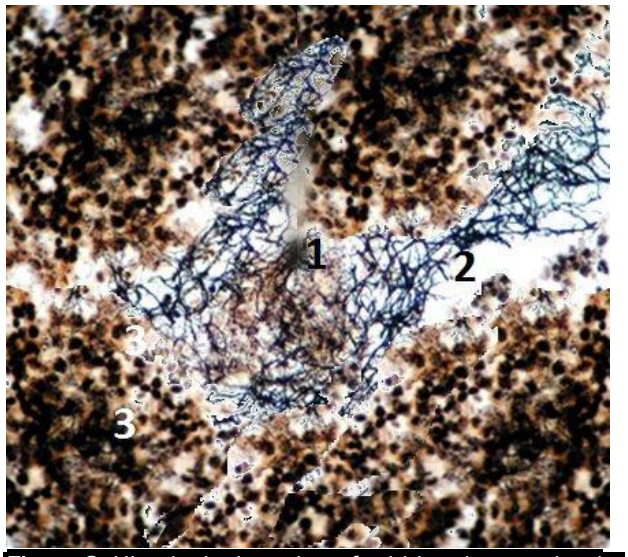

Figure 8. Histological section of rabbit spleen stained with silver nitrate, X400. 1 - lumen of the vessel; 2 - reticular fibers; 3 – lymphocytes.

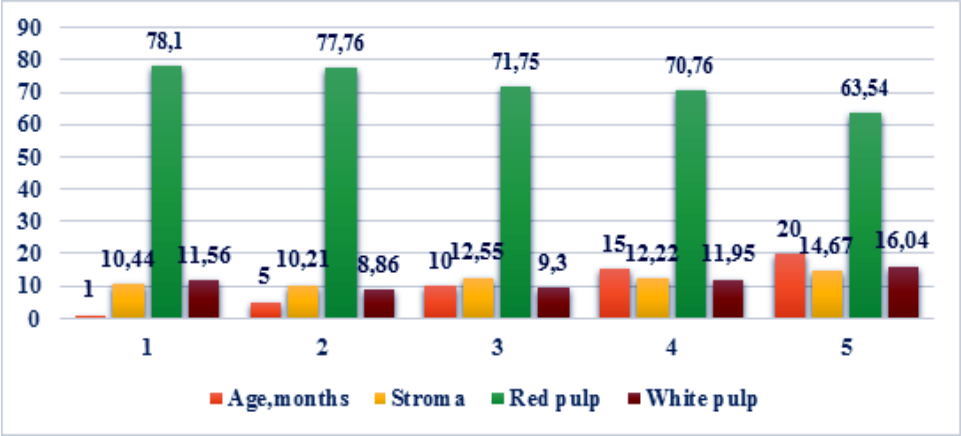

Figure 9. Histogram, rabbit spleen compositions (%) according to age (months). Graph4. White pulp compositions (%) according to age (months).

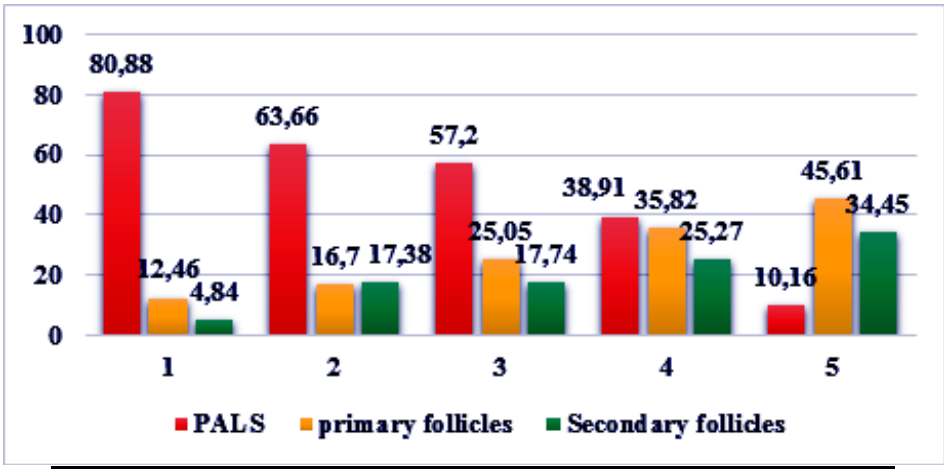

Figure 10. Graph4. White pulp compositions (%) according to age (months).

CONCLUSION

Capsule surface and trabecular meshwork increased by age and form frame for white and red pulp, spleen white pulp shows peri-arteriolar lymphatic system (PALS) and lymphoid follicles, marginal zone separates red to white pulp

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could also be clearly demarcated. In the first group lymphoid tissue still growing, in deformed state, smooth muscle tissue predominates structurally. Twenty months ago, increase in lymphoid follicles percentage and increased smooth muscle tissue. can concluded with animal's age, produces spleen morphological changes. Appearance of reactive centers indicates primary nodules converted into secondary lymphoid follicles, occurs at new locations along blood vessels. Tissue components histogenesis from rabbits spleen during postnatal ontogeny characterized by primary lymphoid follicles intensive development, in context of corresponding significant changes in quantitative parameters other components first stage (up to 30 days), during growth, main parenchyma components tend to decrease, except primary lymphoid follicles in second phase (up to 10 months), accompanied respectively an increase of stroma relative area; Finally, third stage (up to 20 months) absence of significant changes in relationship between stromal and parenchymal components of the organ.

In conclusion according to statistical analysis, 1st group PALS area reaches maximum value and decreases in first age; leaving place for primary and secondary follicular lymphoid.

DECLARATIONS

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Author's contribution

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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