

Histomorphology and histochemical study of the spleen in postnatal puppies

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ABSTRACT

Background and aim: The spleen is an immune organs the main function have been attributed to the spleen it stores and concentrates erythrocytes and releases them when need ,it filtered the blood and remove worn out erythrocyte from circulation .the study aims to identify the morphometries of the postnatal' spleen in dogs and to add information about the spleen of puppies to the data in clinical and academic examinations.

Materials and Methods: Nine puppies were taken after birth and dissected after euthanasia.These puppies were divided into two groups: the first group for macroscopic study and the second group for microscopic

Results: The macroscopic results revealed that the spleen in puppies is situated beneath the diaphragm on the left side. The spleen was oblong, crescent-shaped, reddish-brown in color, related to the jejunum ventrally, the liver and stomach dorsally, and the abdominal wall laterally and had two surfaces. The microscopic result in the postnatal spleen, surrounded by a thin capsule, extended the trabeculae to stroma and became thick near to hilus. The parenchyma of the spleen consists of an aggregation of lymphoid cells around the arterioles while the red pulp consists of the cord of sinuses lined by endothelium cells.

Conclusions: no demarcated structure of the spleen between the three weeks of age.

Introduction

The spleen grows throughout life, with growth of animals [1]. The lymphatic system is divided into cellular and vascular parts. The cellular part includes lymphoid organs such as the spleen, thymus gland and, lymph nodes [2], while the lymph vessel. In higher mammals, the thin capillaries collect the lymph from limited area. the immune system is composed of lymphoid organs and lymphoid vessels were collect lymph from all these in the body [3]. There are two types of the immune system: primary lymphoid organs (spleen, thymus, bone marrow), and In addition to Peyer's patches in the ileum of ruminants. These organs play a role in the immune response [4,5]. The spleen is the largest hemal node situated in the left upper part of the abdominal cavity. It plays a role in filtering the blood [6,7]. The red pulp is responsible for filtering the blood (the filtering system) and plays a role in iron recycling by spleen macrophages in the red pulp [8,9]. The venous system in the red pulp of the spleen gives this area its unique capacity to filter the blood and remove erythrocytes [10]. and it is a secondary lymphoid organ responsible for hematopoietic stem cell residence, and it plays a significant role in immunity to produce immunoglobulin (IgG) and remove abnormal erythrocytes [11], in acute anemia, the erythropoiesis develops rapidly in the spleen, and very little is known about the response of this extramedullary erythropoiesis [12]. The spleen is one of the important immune system organs that consist of numerous lymphocytes. B-lymphocytes undergo development and maturation. They respond from the mediated immunity, the antigen-presenting cells (APCs) play a key role in regulating the immunity of the spleen (immune reaction) [13]. The complete removal of the spleen leads to local systemic complications [1]. The study aims to identify the morphometric of the puppies' spleen and to add information about the spleen of puppies to the data in clinical and academic examinations.

Material and Method:

Ethical approval

"The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (215); August 2025, H and H)".

Statistical Analysis:

The Statistical Packages of Social Sciences - SPSS [14] program was used to detect the effect of difference age groups in parameters study. Least significant difference-LSD test ANOVA

one way) was used to significant compare between means (0.05 probability) in this study.

Anatomical study

Bitches after parturition in the farm of Veterinary College of Diyala University take the puppies at 2, 8 weeks, and are weighed using an electronic sensitive scale; the weights were $1499,3 \pm 1.22$ g , $2132,1 \pm 0.130$, 3901 ± 0.30 g, respectively. This study was conducted from July to August 2025. The puppies were dissected after euthanasia by intramuscular injection with xylazine (0.30 mg/kg) and ketamine (0.15 mg/kg) IM [15,16], and were divided these puppies two groups first group for anatomical study and the second group for histology The first group to determine the anatomical study of the spleen includes measurement and parameters of the spleen and was photographed using a digital Camera Fujifilm (Japan\ 5Mega megapixels). The puppies were opened up the abdominal cavity, and the spleen was removed from this cavity, then the weight, length, and thickness of the spleen were measured using a sensitive vernier (Chain).

Histological study

The second group, after removing the specimens, washed with distilled water then the small pace of the spleen fixed by 10% formalin and Bouin solution (Al-jubail-Saudi Arabia) [17,18] to prepare the block of spleen tissue by used the processing (made in china), cutting the block to use a section of tissue by the rotary microtome (R M 2135 Leica ,china) and finally prepare the slide and stain with H&E (routine stain) some slides stained by Masson's trichrome stain depending to the protocol of Bancroft [19]. Finally examined under light microscope (Genex, USA).

Results and Discussion

Anatomical results

The spleen in puppies is located in the anterior abdominal cavity on the left side in the lateral side of the abdominal wall and related with the liver and stomach dorsally, jejunum ventrally, and the abdominal wall laterally (Fig.1), it was situated on the parietal surface of the stomach and was observed the caudal end of the spleen on the visceral surface of the stomach in left side (fig.1)

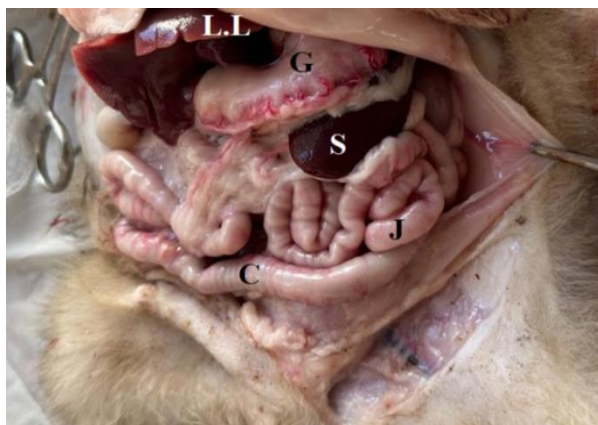


Figure 1. Photography of the Abdominal cavity of puppies at 2 week (A): illustrated the position spleen (S), in left side, stomach (G) liver lobes (LL), Jejunum (J), colon (C)

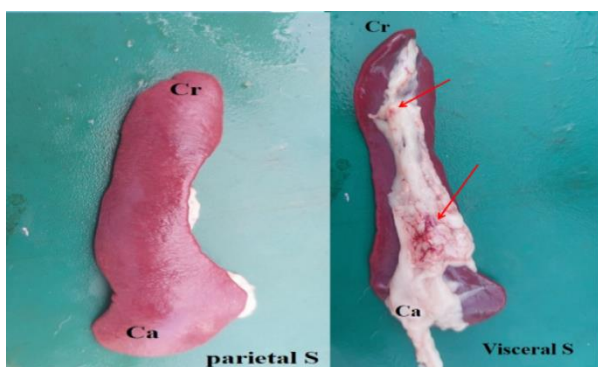


Figure 2. Photography of the spleen (S) of puppies at 4 weeks as illustrated the parietal surface and visceral surface, hilus (red arrow)

The spleen was elongated, Crescent in shape, brown-reddish in color, it had two surfaces (parietal and visceral) (Fig.2), The parietal surface was concave, curved, and smooth (Fig.2 A), whereas the visceral surface had the hilus. (Fig.2

B), the weighted $10.87 \pm 2.77g$, $12.16 \pm 2.67g$ and 16.60 ± 3.94 . The body of the spleen has two ends (cranial and caudal), the wide cranial ends were 12.39 ± 3.55 mm, 12.99 ± 2.74 mm, and 13.12 ± 4.76 mm, respectively, whereas the caudal ends 19.78 ± 2.25 mm, 20.6 ± 4.98 mm, and 25.2 ± 0.57 mm, respectively. The caudal end is wider than the cranial. But there were no differences in shape and color between the spleens at three weeks of age. Whereas the thickness of the spleen was not uniform in all body, it is thicker in caudal end the cranial endless the body (Fig.2), Table (1)

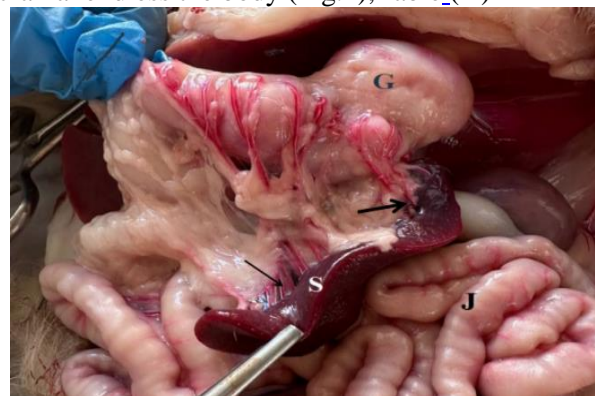


Figure 3. Photography of the spleen (S) of puppies at 4 weeks s related with jejunum (J), stomach (G) illustrated the visceral surface, hilus (black arrow)

The branched inside the parenchyma, which subdivided into multiple branches at the hilum of the spleen, characterize have hilus along the body receive the blood through splenic artery which raise from celiac artery, the spleen related with the jejunum, stomach (Fig.3).

Table 1: The measurement of the spleen of the puppies at three (2,4 and 8) weeks of ages

Parameters	Mean \pm SD			L.S.D.
	2 weeks	4 weeks	8 weeks	
Length of spleen (mm)	8.20 ± 0.67 b	9.32 ± 2.67 b	18.072 ± 4.45 a	2.901 *
Weight of the spleen (g)	10.87 ± 2.77 b	12.16 ± 2.67 b	16.60 ± 3.94 a	3.047 *
Thick of body (mm)	4.52 ± 1.32 b	6.55 ± 2.95 a	6.70 ± 2.66 a	1.895 *
Thick carinal end (mm)	12.39 ± 3.55 a	12.99 ± 2.74 a	13.12 ± 4.76 a	2.077 NS
Thick caudal end (mm)	19.78 ± 2.25 a	20.6 ± 4.98 a	25.2 ± 0.57 b	4.029 *

Means having with the different letters in same row differed significantly. * ($P < 0.05$).

Histological results:

The spleen in puppies is enclosed by a thick capsule, consisting of two layers outer layers consist of connective tissue, consisting of collagen, elastic, and reticular fibers and the inner layers are composed of smooth muscle fibers

(Fig.4). The capsule sends short trabeculae extended into the stroma of the spleen (Fig, 4 A). it has a thin and becomes thick at the hilus, resulting in continuity with tissue of the blood vascular system.

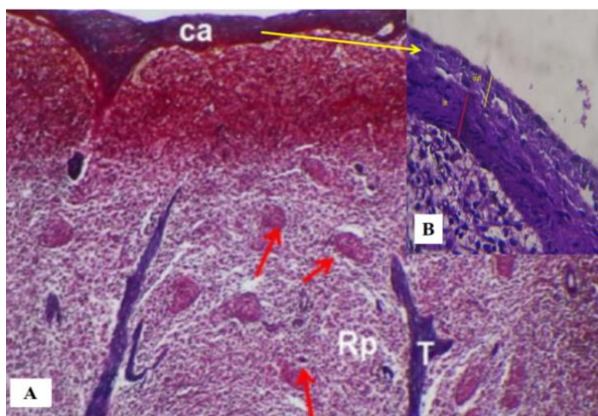


Figure 4. Microscopic section illustrated spleen of the puppies at the 2 weeks white pulp (WP), sinuses (S), central arteriole ,trabeculae (T),inner muscular (In) ,outer fibrous (out) A:(Masons trichrome stain x100),B:PAS stain x400.

The parenchyma of the spleen is similar to most mammalian spleens, consisting of white nodules (white pulp) (Fig. 5), which is embedded red with matrix known as red pulp. Red pulp is a highly vascular tissue that receives blood by the splenic artery enters the spleen through hillus and anastomosis. A few branches (arterioles) are surrounded by fibrocollagenus sheath. These vessels known as central arterioles, the tissue around them known as periarterioles and demonstrate the sinusoids with different sizes, lined by endothelial cells (Fig.6) and containing numerous cells such as macrophage, plasma cells, cells of periaarterial zone , White pulp, composed of the lymphoid aggregate of T-cells, surrounds the fibrocollagenous sheath these vessels know central arterioles, which form the periarteriole lymphoid sheath (PALS)

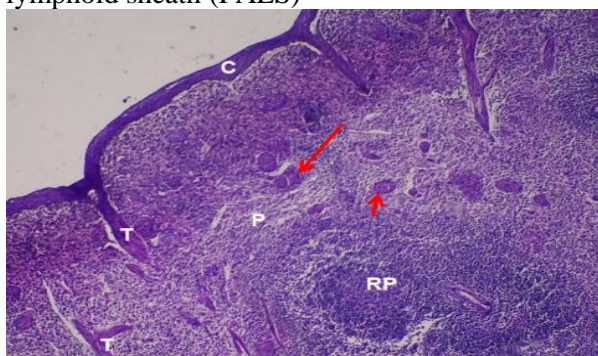


Figure 5. Microscopic section illustrated spleen-of the puppies at (4 weeks) shows, capsule(C), parenchyma (p), septa(T), red pulp (RP), white pulp (red arrow) (H&E X 40).

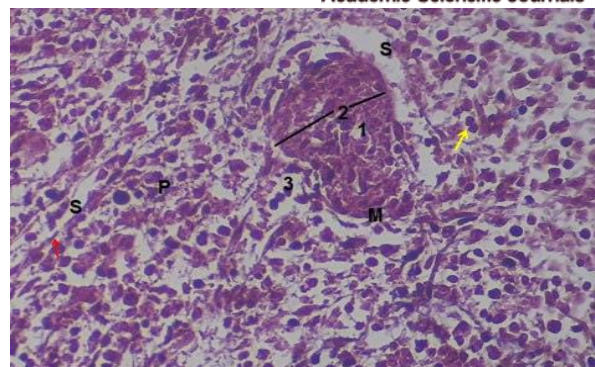


Figure 6. Microscopic section illustrated spleen at the 8 weeks white pulp) embedded in stroma (p), periarteriole lymphoid sheath (1) (germinal center,(2), venous sinuses(3), sinusoid (S),marginal zone(M), endothelial cell (red arrow);macrophage (yellow arrow). (Masson trichrome stain x400).

Discussion:

Anatomy

In the current study, the spleen crescent (fusiform) in shape is similar to that of the cat [20] and golden Hamster [21]. These results differ from those of previous studies in sheep [22,23] in gazelle [24].

The spleen is located on the left side dorsally in the parietal surface of the stomach and curves the caudal end roastery and is fixed by the gastrosplenic ligament. This is similar to a cat [20] Different from in sheep (awasi) [22] ,gazall located in laterally to rumen [24] related with the jejunum and stomch.In recent study illustrated that the cranial end was wider than, caudal end and observed when examining the carcass in camel [6].

Histological

In current study the capsule of the spleen was thick consist of two layers out connective tissue and inner smooth fibers these result finding in camel [6], in gazelle [24].the capsule extending the trabecule from the inner layer in to paranchyma some them orginated from the hillus [25] which containing the major vessels and poor smooth muscle these results finding in north eastern nigerian camel [6] :in goat and sheep [23].

The present study appears the spleen in puppies there are no demarcation between white pulp and red pulp similar finding was observed in sheep and goat [23],The white pulp consist of lymphoid center of lymphatic center tissue and involve the lymphocyte and plasma cells and macrophage which responsible the protect this organs and form the lymphoid nodules and particular lymphatic sheath similar into red pulp these areas situated between the white pulp and trabecular [21],These consist of the sinusoids that

is irregularly distributed between pulp cord of the cellular tissue and the lymphoid follicle with germinal centers, these results was findings in human, the lymphoid tissue is less wall-organised than in other animals [26] in prenatal goat these follicles appeared as splenic nodules and ellipsoids and irregularly scattered lymphoid masses, but differ in sheep Awasi [21], in golden hamsters [2,20].

Conclusion:

The spleen in puppies is similar to the spleen in farm animals; there is no present any demarcating between the structures. The spleen is characterized by highly vascular tissue (blood sinusoids containing sinusoids) and consists of lymphatic tissue responsible for Immune defence mechanism for the viscera of abdomen.

Conflict of interest

The authors assert the absence of any conflicts of interest.

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Declaration of interests

The authors have no conflicts of interest to declare.

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Publication consent

The authors agree to publication.

Data and material availability

All data analyzed during this study are included in this article.

Author contribution

The authors contributed equally to the article.

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دراسة مورفولوجية وكيميائية نسيجية للطحال في الجراء بعد الولادة

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الملخص

خلفية البحث والهدف: الطحال هو أحد أعضاء الجهاز المناعي، وظيفته الرئيسية فهو يخزن ويركز كريات الدم الحمراء ويطلقها عند الحاجة، كما أنه يقوم بتصفية الدم وإزالة كريات الدم الحمراء البالية من الدورة الدموية. تهدف هذه الدراسة إلى تحديد القياسات المورفومترية للطحال بعد الولادة لدى الكلاب، وإضافة معلومات جديدة حول طحال الجراء إلى البيانات المتوفرة في الفحوصات السريرية والأكاديمية.

المواد وطرق العمل: تم أخذ تسعة جراء بعد الولادة وتشريحها بعد القتل الرحيم. قُسمت هذه الجراء إلى مجموعتين: المجموعة الأولى للدراسة العيانية، والمجموعة الثانية للدراسة المجهرية.

النتائج: كشفت نتائج الفحص العياني أن الطحال لدى الجراء يقع أسفل الحجاب الحاجز على الجانب الأيسر. كان الطحال مستطيلاً، هلالياً الشكل، بني محمر اللون، ويرتبط بالصائم من الجهة البطنية، والكبد والمعدة من الجهة الظهرية، وجدار البطن من الجانبين، وله سطحان. أما نتائج الفحص المجهرية، فقد أظهرت أن الطحال بعد الولادة محاط بمحفظة رقيقة، وأن الحزم العضلية تمتد إلى النسيج الضام، وتصبح سميكة بالقرب من سرة الطحال. يتكون نسيج الطحال من تجمع للخلايا اللمفاوية حول الشرايين الصغيرة، بينما يتكون اللب الأحمر من سلسلة من الجيوب المبطنة بخلايا بطانية.

الاستنتاجات: نستنتج من هذا لا يوجد بنية محددة للطحال بين عمر ثلاثة أسابيع.

الكلمات المفتاحية: التشريح، علم الأنسجة، الطحال، الجراء.