



Morphology of the Spleen in the Camel's Fetus (*Camelus dromedarius*): Gross Anatomy, Histology and Scanning Electron Microscopic Studies

Marwa-Babiker, A.M.^{1,2*}; ALkhdair, K.M.¹; Abdelhay, M. Ali¹; Elseory, A.M.A.^{1,3}; Zolain, H.B.⁴; Al-Ramadan, S.Y.¹ and Althnain, T.A.¹

¹Department of Anatomy, College of Veterinary Medicine, King Faisal University, Saudi Arabia

²Department of Anatomy, College of Veterinary Medicine, University of Bahri, Khartoum-North, Sudan

³Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum, Sudan

⁴Department of Anatomy, Faculty of Veterinary Science, University of Nyala, Sudan

*Corresponding Author: Marwa-Babiker, A.M., E-Mail: mbabakr@kfu.edu.sa

ABSTRACT

The spleen is essential for immunological responses to infections that are carried in the blood. The study aimed to investigate the gross anatomy, topography, histology and scanning electron microscopy of the spleen in the dromedary camel fetus (*Camelus dromedarius*). Fifteen camel fetuses aged 87–418 days were used to examine the prenatal development of the spleen. Standard anatomical, histological, and scanning electron microscopy procedures were applied. Anatomically, the spleen was situated medially to the ribs, caudally to the stomach, and on the caudolateral side of the abdominal cavity. It had C-shaped surfaces with rough surfaces and serrated edges. In the first and second trimesters, the spleen was dark brown and grayish in the third trimester. Histologically, the capsule was made of very thin mesenchyme connective tissue, and the parenchyma had a sporadic distribution of many cell types in the first trimester. The capsule displayed thick, dense, irregular connective tissue with descending trabeculae made up of collagen fibers and bundles of smooth muscle fibres in the second and third trimesters. The parenchyma in the second and third trimesters was presented as white and red pulps. Megakaryocytes were detected in all samples. Scanning electron microscopy revealed that the spleen is lined with mesothelial cells separated by reticular connective tissue fibers. The reticular cells in the cordal gaps, marginal zones, and parietal sheath were relatively small in the first trimester of gestation. In the second and third trimesters, the reticular cells formed a network where dendritic macrophages and lymphoblasts were present. Medium-sized and tiny lymphocytes were seen. The results revealed that the spleen of the dromedary camel fetus was shown to have well-developed characteristics in the second and third trimesters of gestation. Also, the development of the camel's spleen was comparable to that of other mammalian species but with unique traits and characteristics.

Keywords: Anatomy; Camel fetus; Histology; Scanning electron microscopy; Spleen.

Original Article:

DOI:<https://dx.doi.org/10.21608/javs.2023.219602.1250>

Received : 23 June, 2023.

Accepted : 13 August, 2023.

Published in October, 2023.

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INTRODUCTION

The immune system is comprised of two principal interconnected components called innate and adaptive immunity (Marcoux *et al.*, 2021). The innate immune system mounts a nonspecific response that protects against the spread of foreign pathogens, and the adaptive immune system has developed to specifically recognize a given pathogen and lead to immunological memory (Marcoux *et al.*, 2021). The spleen is a major secondary lymphatic organ implicated in filtering the blood and storing erythrocytes in the red pulp and platelets in the

splenic cords. In addition, the spleen filters blood from old or damaged red blood cells (Cheung and Nadakavukaren, 1983; Mebius and Kraal, 2005; Cesta, 2006; Eurell and Frappier, 2006; Khalil *et al.*, 2009; Bello *et al.*, 2019; Gnanadevi *et al.*, 2019; Lewis *et al.*, 2019; Xu *et al.*, 2020; Abdellatif, 2021; Al-Ramadan, 2022).

Mammals developed a new function for the spleen: the storage of red blood cells. In addition, mammalian species evolved different features of the red pulp increasing, the specialization of the filtration function (pulp sinuses) and/or storing function,

(Udroiu, 2017). Research concerning the development of the spleen of the camel fetus in the available literature is scant for instance as reported by **Taher, et al., (1967); Bello et al., (2016); Bello et al., (2019); Jaji et al., (2019) and Marwa-Babiker et al., (2022)**. This may be due to the paucity of material available for investigation; therefore, this current study aimed to study the gross anatomy, topography, histology and, scanning electron microscopy of the spleen in the dromedary camel fetus (*Camelus dromedarius*).

MATERIALS AND METHODS

Fifteen camel fetuses were used in this study. The fetuses were obtained from Tamboul, Sudan, and, Al-Omran, Saudi Arabia, slaughterhouses, where the animals were slaughtered under official license and supervision for meat production. The age of the fetuses was determined using the equation of crown vertebral-rump length (CVRL).

$$GA = \frac{CVRL + 23.99}{0.366}$$

Where GA is the mean gestational age in days (**Elwishy et al., 1981**), the age of the fetuses ranged between 87 and 418 days of gestation (8 cm to 129 cm CVRL). Fetuses were fixed with 10% formalin and dissected to study the spleen's gross anatomy and topography. For the histological study,

specimens were taken and then fixed in 10% buffered formalin. Specimens were processed by routine histological procedures and stained with H&E and Masson's trichrome (**Bancroft and Stevens, 2008**).

Immediately after slaughter, small pieces of tissue were taken for electron microscopic investigation. First, samples were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 48 hours at 4°C. Then, samples were washed in 3 changes of cacodylate buffer and post-fixed in osmic tetroxide for 90 minutes. They were then washed in 3 changes of cacodylate buffer for 4 hours and dehydrated in ascending grades of alcohol (30%, 50%, 70%, and 90%) for 2 hours each and in absolute alcohol for two days (**Bancroft and Stevens, 2008**). Then the specimens were immersed in amyl acetate for 1-2 days and dried using the critical point drying process. Specimens were further dehydrated using a graded series of amyl acetate and absolute alcohol followed by absolute amyl acetate for 5 minutes each. The soaked specimens were then quickly moved to a cooled high-pressure chamber (**Polliack et al., 1973**). Finally, the specimens were coated with a very thin layer of gold and examined by a JEOL JSM-6390LA analytical scanning electron microscope.

RESULTS

Gross Anatomy and Topography of the Spleen

At the late stages of the first trimester (22 cm CVRL camel fetus, 125 days of gestation), the spleen of the camel fetus was relatively large (Fig. 1). It was located in the lateral and caudal parts of the abdominal cavity. It was related laterally to the ribs and lateral abdominal wall and caudally to the first compartment of the stomach. Medially it was related to the first compartment of the stomach and large intestine and caudally to the left kidney and gonads . The spleen at this stage of pregnancy had a C-shape; it was dark brown in color (Fig. 1).



Fig.1: A photograph of a 22-cm CVRL camel fetus (125 days of gestation), showing the spleen (S) related, laterally to the ribs and the lateral abdominal wall, caudally to the first compartment of the stomach (St.), medially to the first compartment of the stomach and large intestine (L.I): spiral loop of ascending colon, and caudally to the left kidney and gonads. D; diaphragm, L: liver.

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In the second trimester, the spleen was related laterally to the last rib and lateral abdominal wall and caudally to the first compartment of the stomach. It was related medially to the first compartment of the stomach and colon and caudally to the pancreas and metanephros. At this stage, the spleen had a C-shape; it was dark brown in color (Fig.2, a and b).

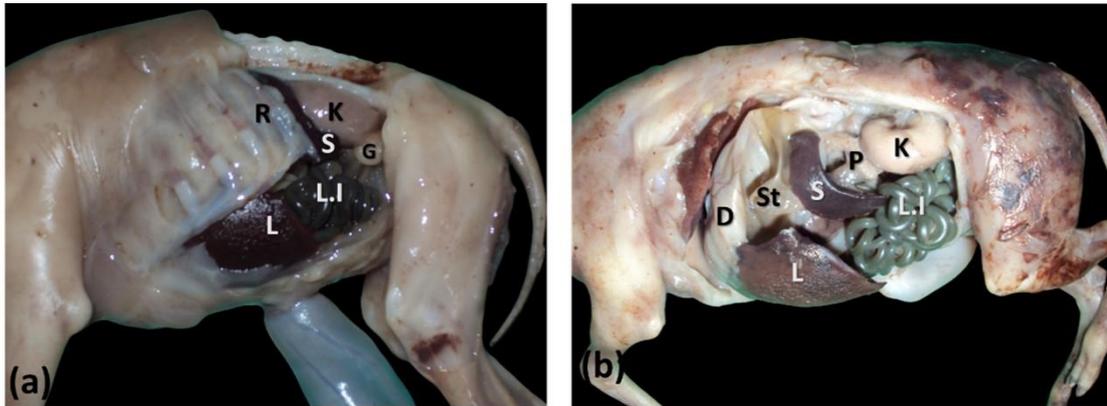


Fig. 2: (a): a photograph of a camel fetus 37cm CVRL (166 days of gestation) and (b): a camel fetus 56cm CVRL (218 days of gestation); at the second trimester the spleen, (S) is related laterally to the last rib (R) and lateral abdominal wall, caudally to the first compartment of the stomach (St). Medially to the first compartment of the stomach (St), pancreas (P) and, large intestine (L.I): (jejunum and colon) and caudally to the kidney (K) and gonads (G).

In the third trimester, the spleen was related laterally to the last rib and lateral abdominal wall (Fig. 3a). It was related cranially to the first compartment of the stomach, medially and, caudally to the large intestine (jejunum and colon) (Fig. 3b). It has a C-shape, and the edges are serrated, blunt, and rough. The thickness was not uniform; it appeared thick at the midpoint and the hilus but thin at the edges. The surface of the camel spleen was rough and grayish in color (Fig.3, a and b).

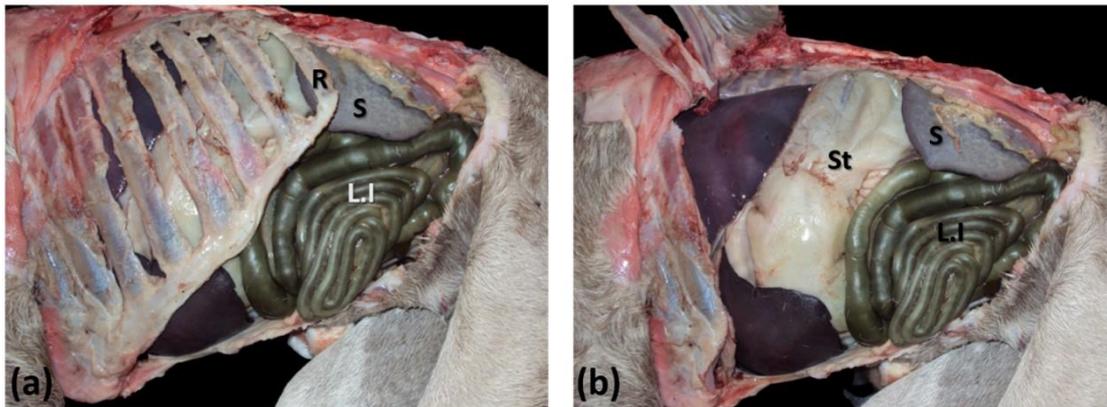


Fig.3: (a) and (b): photographs of a camel fetus 129cm CVRL (418 days of gestation): at the third trimester the spleen, (S) is related laterally to the last rib (R) and lateral abdominal wall, caudally to the first compartment of the stomach (St). It was related medially to the first compartment of the stomach (St) and large intestine (L.I): (colon).

Histology of the spleen

In the first trimester, the capsule of the spleen was composed of fine mesenchymal connective tissue (Fig. 4a). In the second trimester of gestation, the capsule and trabeculae showed thick, dense, irregular connective tissue with large amount of collagen fibers and smooth muscle fibres (Fig. 4b). At the third trimester, the capsule of the spleen showed thick dense irregular connective tissue with large amount of collagen fibers (Fig. 5a). In addition, the capsule also showed smooth muscle fibers and adipose tissue.

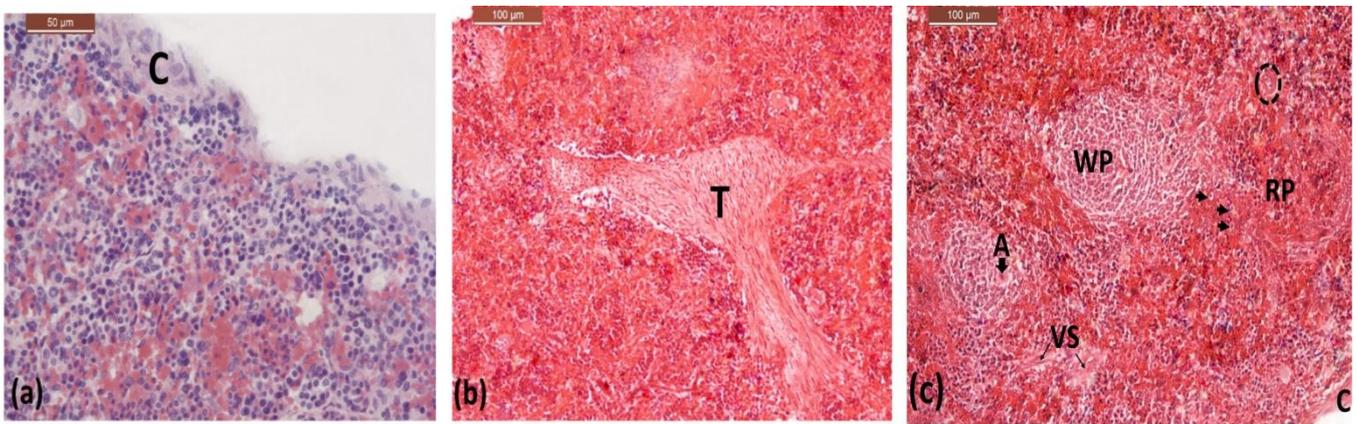


Fig.4: (a): Photomicrograph of the spleen of a camel fetus 23 Cm CVRL (128 days of gestation) showing, the capsule with very thin mesenchymal connective tissue (C). (b) and (c) Photomicrographs of the spleen of a camel foetus 78 cm CVRL (278 days of gestation) showing, the trabeculae (T) made of dense connective tissue with smooth muscle fibers on the photomicrograph (b); capsule (C), artery (A), white pulp (WP), red pulp (RP), venous sinuses (VS), megakaryocytes (dotted circle), macrophages (arrowheads). H&E. 40X, 20X, 20X respectively.

The parenchyma, in the first trimester consisted of randomly distributed lymphocytes, macrophages, erythrocytes and megakaryocytes (Fig. 4a). In the second and the third trimesters, it was arranged as white and red pulps (Fig. 4b&c). The white pulp (lymphoid tissue) was formed of lymphoid nodules (splenic corpuscles) and nonnodular (periarterial sheath) lymphoid tissue. The red pulp is formed of venous sinuses that fill the spaces between the capsule, the trabeculae, and the white pulp (Fig. 4b&c)". Splenic cords between sinuses that were formed of reticular nets fixed to reticular cells and suspended on blood cells and macrophages. Megakaryocytes observed in the red pulp at the first, second and, third trimesters of gestation (Fig. 4c ; 5 a, b & c).

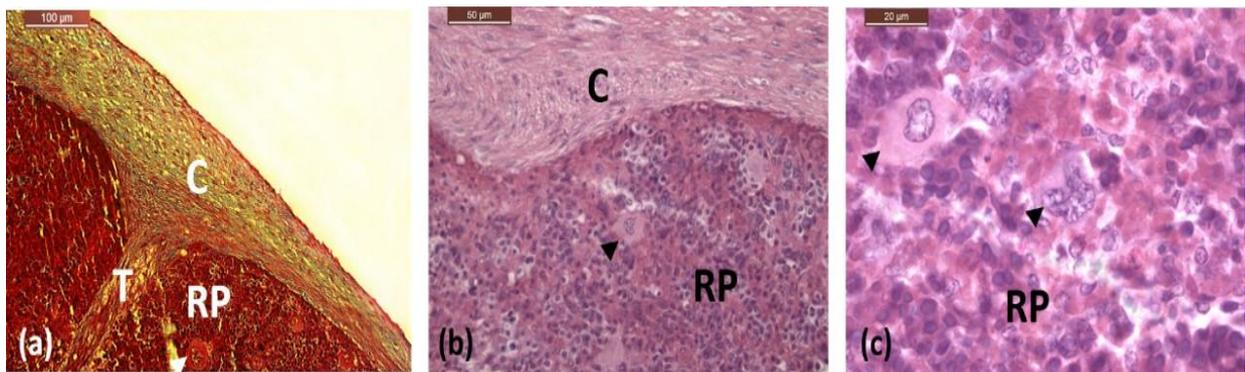


Fig.5: Photomicrographs of the spleen of a camel fetus 90 Cm CVRL (311 Days of Gestation) showing the capsule (C) and trabeculae (T) made of collagenous dense connective tissue and smooth muscle. Note the megakaryocytes (arrowheads) in the red pulp (RP). Masson's trichrome 10X. H&E. 40X, 100X respectively.

Scanning electron microscopy of the spleen

Scanning electron microscopy revealed that the spleen in the first trimester was lined with close mesothelial cells (Fig. 6a). Whereas the second and third trimesters of gestation, reticular connective tissue fibers separated the mesothelial cells (Fig. 6b). The reticular cells in the cordal gaps, marginal zones, and parietal sheath were relatively small in the first trimester of gestation (Fig. 7a & b). In the second and third trimesters, the reticular cells formed a network where dendritic macrophages and lymphoblasts were located. In addition, medium-sized and tiny lymphocytes were seen as well. The white pulp of the spleen'

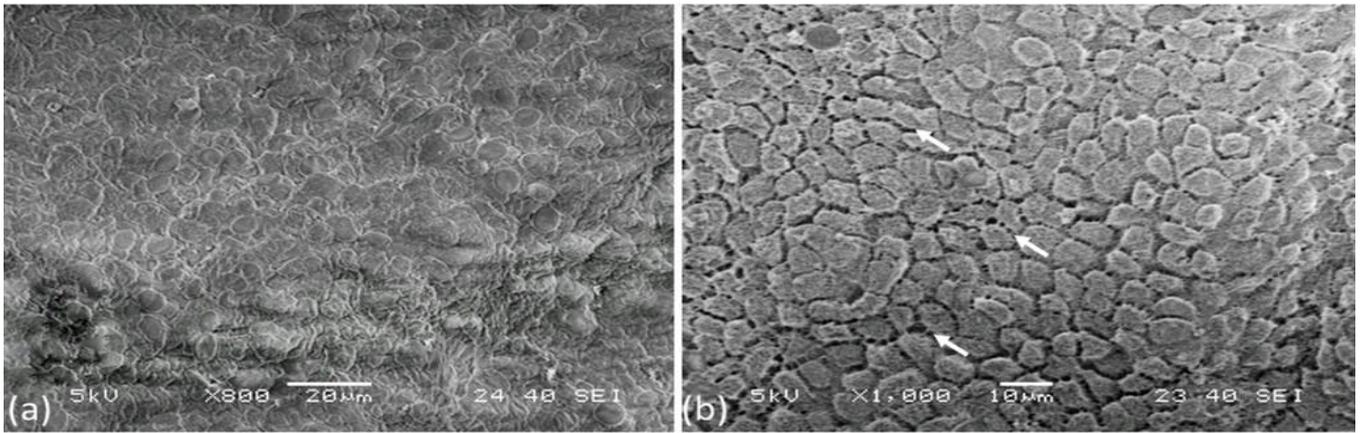


Fig. 6: Scanning electron micrograph of the spleen of a camel fetus (a): 8cm (87days of gestation) showing, mesothelial cells lining the spleen. (b): 98cm (332days of gestation) showing mesothelial cells lining the spleen with reticular connective tissue fibres in-between (arrows).

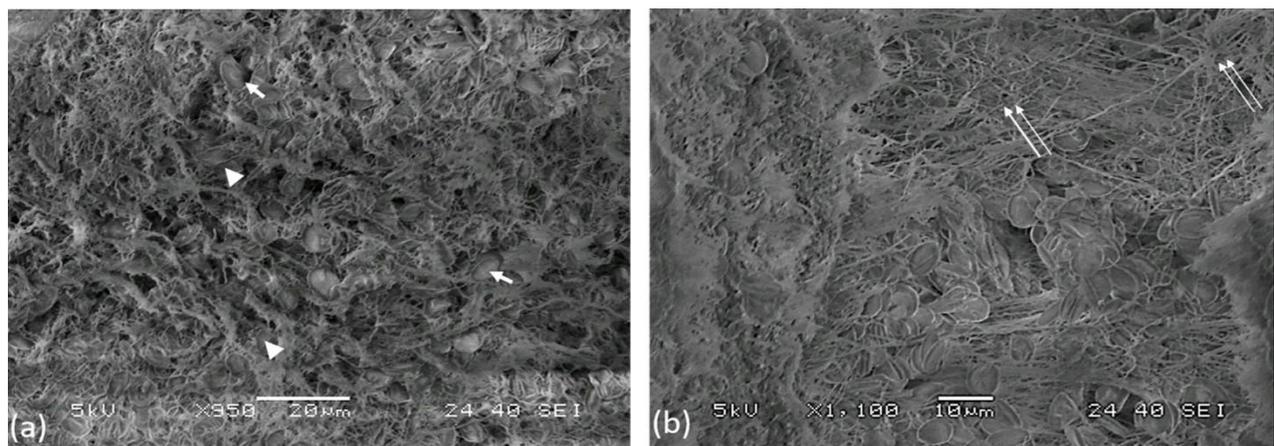


Fig.7: (a) and (b) Scanning electron micrograph of the spleen of a camel fetus 8cm (87days of gestation) showing, erythrocytes (short arrows), reticular cells (arrowheads) and reticular connective tissue fibres (long arrows).

In the second and third trimesters, the reticular cells formed a network where dendritic macrophages and lymphoblasts were located. In addition, medium-sized and tiny lymphocytes were seen as well. The white pulp of the spleen's reticular framework was clearly visible at the late stages of the gestational period (Fig. 8).

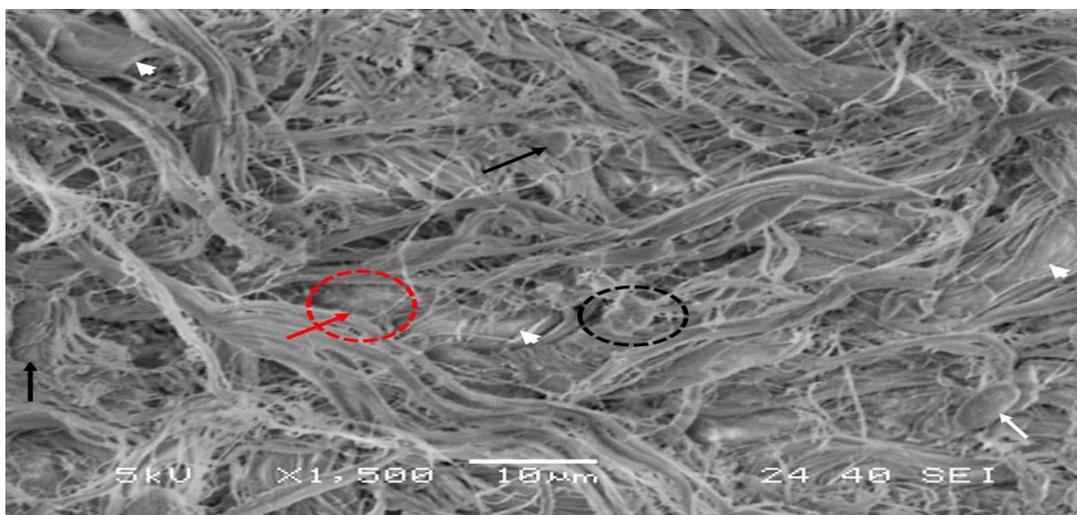


Fig. 8: Scanning electron micrograph showing a 115 cm CVRL (379days of gestation) camel fetus showing; erythrocytes (white arrows), reticular cells (white arrowheads), dendritic macrophages (black dotted circle), lymphoblasts (red arrow in dotted red circle) and, medium and tiny lymphocytes (black arrow) in a network of reticular connective tissue fibres.

DISCUSSION

Topography and Gross Anatomy of the spleen

The present study showed that the spleen of the camel fetus in the first trimester of gestation was located under the ribs and lateral abdominal wall at the lateral and caudal parts of the abdominal cavity: the spleen in the camel fetus was C-shaped. Similar findings have also been stated by **Maina et al., (2014)**; **Bello et al., (2016)** ; **Bello et al., (2019)** in the adult and fetus of dromedary camels, respectively. The spleen in other domestic animals has different shapes; it appears triangular with rounded angles in sheep (**Khalel, 2010**) and quadrangular in goats (**Suri, et al., 2017**; **Gnanadevi, et al., 2019**). This study showed that spleen of a camel fetus had two rough surfaces and serrated edges. This finding may be similar to that of the adult camel which was mentioned by **Maina et al., (2014)**; **Nawal and Maher (2018)**. In addition, these findings are similar to those stated by **Rajani, et al., (2021)** in the Asian elephant. The present study showed that the thickness of the spleen was not uniform; it appeared thick at the midpoint and the hilus but thin at the edges. The spleen of the sheep also has two surfaces: the parietal surface, which appears convex, and a visceral concave surface and it has a hilus. There were two ends in the sheep spleen: the base (dorsal end) which, appears broad and thick, and the ventral end, which appear, narrower and thinner than the base (**Khalel, 2010**).

The current study revealed that the color of the spleen in the camel fetus was different depending on the gestational stage. In the first and second trimesters of gestation, it was dark brown in color and grayish in the third trimester of gestation. This could be due to an increase in connective tissue and capsule thickness. The latter observation was not noticed before.

Histology of the spleen

The current histological results revealed that in the first trimester, the spleen capsule was a thin layer of mesenchymal connective tissue. This finding is similar to that stated by **Rahman et al., (2016)** in the rat. In addition to thickness increasing with age in the second and third trimesters, collagen fibers and smooth muscle also increased. This agrees with that in many species (Human, Goat, Buffalo, Rabbit) mentioned by **Rahman et al., (2016)**. This study also agrees with **Thanvi et al., (2020)**, who stated the capsule of the spleen of sheep (*Ovis aries*) was composed of smooth muscle fibers, collagen, and elastic and reticular fibers. The current result on the contrary in that mentioned by **Zidan et al., (2000)** in the adult camel; a thick capsule with an internal layer

mainly made of smooth muscle cells and an external layer mainly made of connective tissue.

The current investigation showed that the parenchyma of the spleen in the first trimester of gestation consisted of randomly distributed different types of cells. This agrees with **Marwa-Babiker et al., (2022)** in the camel fetus. In the second and third trimesters, the parenchyma is arranged as white and red pulp. This agrees with **van Krieken, (1997)**; in human; **Zidan, et al., (2000)** in adult camel; **Thanvi, et al., (2020)** in sheep; **Rajani, et al., (2021)** in Asian elephants; and **Marwa-Babiker et al. (2022)** in the camel fetus.

The current study showed that the megakaryocytes were in the red pulp at the three gestational stages: this agrees with **Marien and McFadden, (1970)** stated that the megakaryocytes were determined in representative fields of the liver, spleen, and bone marrow. In addition, in camels **Zidan et al., (2000)** discovered megakaryocytes in the red pulp of adult dromedary camels and **Marwa-Babiker et al., (2022)** in the camel fetus.

Scanning electron microscopy of the spleen

Several researchers examined the spleen's ultrastructure in various species (**Burke and Simon, 1970**) albino rabbits: (**Polliack et al., 1973**); humans; (**Weiss, 1974**) rats; (**Zapata, et al., 1981**), turtle; (**Hataba et al., 1981**), mice (**Polák et al., 2009**), humans. The current investigation showed the scanning electron microscopy of the spleen of a camel fetus lined with mesothelial cells. The reticular cells in the cordal gaps, marginal zones, and parietal sheath were relatively small in the first trimester of gestation. In the second and third trimesters, the reticular cells formed a network where dendritic macrophages and lymphoblasts were present. In addition, medium-sized and tiny lymphocytes were seen. The white pulp of the spleen's reticular framework was clearly visible at the late stages of the camel's gestational period. This research agrees with **Kashimura and Fujita, (1987)** and **Fujita and Ushiki, (1992)**. Unfortunately, transmission electron microscopy was not available during the conduct of the study; therefore, further investigations are needed to show the application of transmission electron microscopy of spleen development in camels.

CONCLUSION

Anatomically, the spleen was large size in the first trimester. It was C-shaped, with serrated, blunt, and rough edges. Histologically, the spleen of the camel fetus consists of stroma and parenchyma. In the first trimester of pregnancy, the reticular cells in the cordal gaps, marginal zones, and parietal sheath

were small. At the end of the gestational period, the reticular structure of the spleen's white pulp was clearly apparent. In conclusion, the development of the camel's spleen reveals that it has special characteristics features compared to other domestic animals.

Competing interes

There is no conflict of interests of any sort between authors or elsewhere.

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How to cite this article:

Marwa-Babiker, A.M., ALkhodair, K.M., Abdelhay, M. Ali; Elseory, A.M.A., Zolain, H.B., Al-Ramadan, S.Y. and Althnaian, T.A., 2023. Morphology of the Spleen in the Camel's Fetus (*Camelus dromedarius*): Gross Anatomy, Histology and Scanning Electron Microscopic Studies. Journal of Applied Veterinary Sciences, 8 (4): 54-61. DOI: <https://dx.doi.org/10.21608/jav.2023.219602.1250>