

HISTOLOGICAL STUDY OF PRENATAL DEVELOPMENT OF THE SPLEEN IN CAMEL (*Camelus dromedarius*)

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ABSTRACT

Spleen development in the camel foetus was studied during the 1st, 2nd and 3rd trimesters of gestation using histological techniques. Ten spleens of camel foetuses were collected from Omran slaughterhouse, Al-Ahsa, Saudi Arabia. The samples were prepared by routine histological procedures and stained by the general histological stain (H and E) and some other special stains including Van Geison's for collagenous fibres, Verhoff's for elastic fibres, Gordon and Sweet for reticular fibres. At the 1st trimester, the spleen capsule was composed of fine connective tissue fibres, in the 2nd trimester the capsule and trabeculae showed thick connective tissue, while in the 3rd trimester the capsule also showed smooth muscle fibres and surrounded with large amount of adipose tissue. The parenchyma, at the 1st trimester consisted of randomly distributed lymphocytes and macrophages. At the 2nd and 3rd trimesters, it was arranged as white and red pulps. Megakaryocytes observed previously in the red pulp of adult dromedary camel were seen in the red pulp at the 1st, 2nd and 3rd trimesters of gestation. It was concluded that the spleen showed very important histological developmental changes throughout the three gestational stages.

Key words: Camel, development, foetus, gestational stage, histology, spleen

The mammalian spleen develops as gathering of mesenchymal cells in the dorsal mesogastrium. The mesenchymal cells differentiate and form the splenic capsule and connective tissue (McGeady *et al*, 2017). The histomorphological structure of the spleen has been reported by many authors (Bello *et al*, 2019; Jaji *et al*, 2019; Zidan, 2000a, Maina *et al*, 2014).

It has been stated that the spleen is surrounded by a capsule from which fibrous trabeculae emerge (Dijkstra and Veerman, 1990; Alshamarry, 2010). The spleen of the one humped camel consists of a thick capsule and parenchyma consists of splenic pulp, which is composed of white pulp packed with lymphocytes and red pulp consisted of three-dimensional reticular network and splenic sinuses (Zidan *et al*, 2000a).

Bello *et al* (2016) stated that the spleen of dromedary foetus had red and white pulps of variable shape and size and special feature of connective tissue inter-digitations of variable sizes into the parenchyma along the whole surface at second and third trimester. However, there was paucity in the literature on the development of the spleen in camel

foetus. Therefore, the aim of this study was to provide more information about the histological structure of the prenatal development of spleen of the dromedary camel foetus.

Materials and Methods

Ten spleens of camel foetuses were collected from Omran slaughterhouse, Al-Ahsa, Saudi Arabia. The Research Ethics Committee, Deanship of scientific research, King Faisal University approved all experiments number: KFU-REC-2022-JAN-EA000343. Depending on the gestational age (GA), the foetuses were divided into three groups: first (0-130 days), second trimester (131-260 days) and third trimester (261-423.5 days). The age of the foetus was determined using the equation of crown vertebral-rump length (CVRL) $GA = (CVRL + 23.99)/0.366$

Small pieces (about 1cm³ thick) were taken, then were fixed in 10% buffered formalin. The specimens (5 µm thick) were processed by routine histological procedures and stained with (H&E) and some special stains including Van Geison's for collagenous fibres, Verhoff's for elastic fibres, Gordon and Sweet for reticular fibres (Bancroft and Stevens, 2008).

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Results and Discussion

The histological details of foetal spleen obtained at different GA were noted. The foetal spleen obtained at 1st trimester was surrounded by a thin capsule of fine loose connective tissue fibres or mesenchymal connective tissue from which connective tissue trabeculae extended in the parenchyma (Fig 1). At the second trimester, the connective tissue capsule and the trabeculae became thickened in foetal spleen. The diffuse collagen connective tissue into the parenchyma was observed at the stage of 2nd trimester (Fig 2). At the third trimester, the foetal spleen capsule consisted of dense regular connective tissue with few smooth muscle fibres that also extended in the trabeculae and the spleen was surrounded with large amount of adipose tissue (Fig 3). However, at the late stages of the third trimester, the smooth muscle fibres were clearly visible in the trabeculae (Fig 3, d).

At the first trimester, the white and red zones of the foetal spleen were not clearly differentiated and the parenchyma showed large amount of randomly distributed B & T lymphocytes and large spherical macrophages with oval nuclei and eosinophilic cytoplasm (Fig 1). Giant cells (Megakaryocytes) with lobulated nuclei and highly eosinophilic cytoplasm were also observed (Fig 1).

At the 2nd trimesters, the foetal spleen parenchyma began to differentiate in a white and a red pulp. The megakaryocytes were clearly shown in the red pulp with lobulated nuclei (Fig 2).

At the 3rd trimesters, the foetal spleen parenchyma was differentiated in white and red pulps. The white pulp showed splenic corpuscles which consisted of lymphatic nodules (B-dependent zone) surrounding the central artery and periarterial sheath of non-nodular lymphoid tissue (T-dependent zone). The red pulp fills the spaces between the capsule, the trabeculae and the white pulp. The red pulp was formed of splenic venous sinuses and splenic cords in-between the sinuses that was composed of reticular net fixed to it, reticular cells and suspended aggregates of red blood cells, B & T lymphocytes and macrophages (Fig 3). In addition, the megakaryocytes were observed at the 3rd trimesters of gestation in the red pulp only (Fig 3). The number of megakaryocytes cells were increased at the 3rd trimester and some of them showed binucleated nuclei (Fig 3, c).

The present study showed that the foetal spleen at the 1st trimester of GA was surrounded with

capsule which start with fine connective tissue fibres and send fine connective tissue trabeculae in the parenchyma, then at the 2nd trimester capsule and trabeculae appeared thicker. At the stage of the 3rd trimester of GA the capsule became thick and the smooth muscle fibres were surrounded with large amount of adipose tissue. The present study agreed with some previous studies (Bello *et al*, 2016) which stated that trabecular connective tissue and capsular thickness increase depending on the developmental stages. According to Jaji *et al* (2019) the capsule in the spleen of the dromedary camel foetus had an inner smooth muscle and an outer predominant connective tissue layer. However, in present study the most of the salient features of the postnatal spleen were already evident in the first growth phase and became more developed by the second phase.

In the present study, the splenic corpuscles were observed clearly at the 3rd trimester of GA where lymphatic nodules surrounded the central artery and a non-nodular zone was seen. The red pulp is formed of splenic venous sinuses, splenic cords, reticular cells, blood cells and macrophages. The splenic cords were suspended by reticular cells net with aggregates of blood cells and macrophages. According to Veerman and van Ewijk (1975) and Steiniger (2015) the composition of the white pulp of some animal species consisted of periarteriolar lymphatic sheath (PALS), follicles and marginal zone. There were three compartments, each had their specific lymphoid and non-lymphoid cells. Reticular cells and reticulin fibres, although found in all three compartments, they formed a characteristic pattern in each compartment.

The arrangements of the spleen parenchyma was also described previously in some species (Eurell and Frappier, 2006 and Steiniger, 2015). The spleen consisted of the white pulp embedded in the red pulp. In the white pulp, T and B lymphocytes formed accumulations, the periarteriolar lymphatic sheaths located around intermediate-sized arterial vessels, the central arteries. The red pulp was a reticular connective tissue containing all types of blood cells (Steiniger, 2015). In this study a large amount of randomly distributed B and T lymphocytes, macrophages and erythrocytes, megakaryocytes were shown in 1st trimester. In the 3rd trimester splenic corpuscles the white pulp consisted of lymphoid tissue formed of lymphatic nodules was surrounded the central artery.

The number of the megakaryocytes in the red pulp of adult dromedary camel decreased with

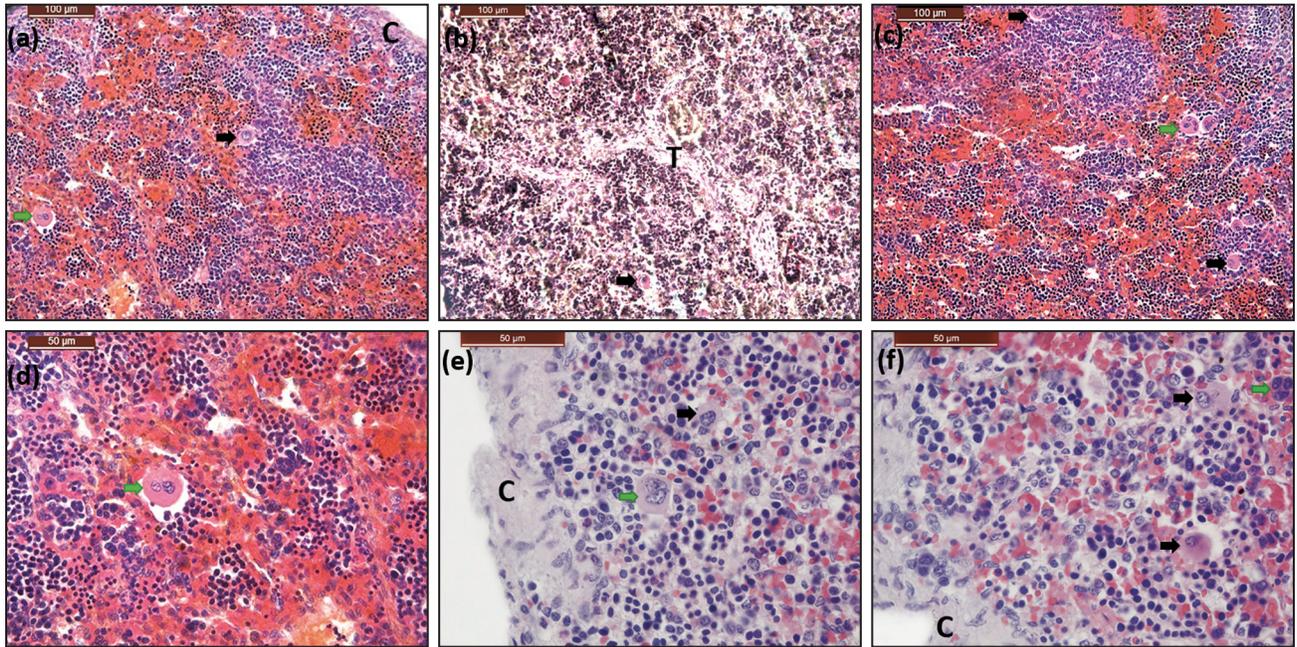


Fig 1. Photomicrographs showing spleen of camel foetus during the 1st trimester of gestation; connective tissue capsule (C) and fine connective tissue trabeculae (T), randomly distributed lymphocytes, macrophages and megakaryocytes were seen (arrows), binucleated megakaryocyte were present (green arrow). (a & c) Haematoxylin and Eosin (X20) (b) Gordon and Sweet (X20), (d) Haematoxylin and Eosin (X40), (e & f) Haematoxylin and Eosin (X100).

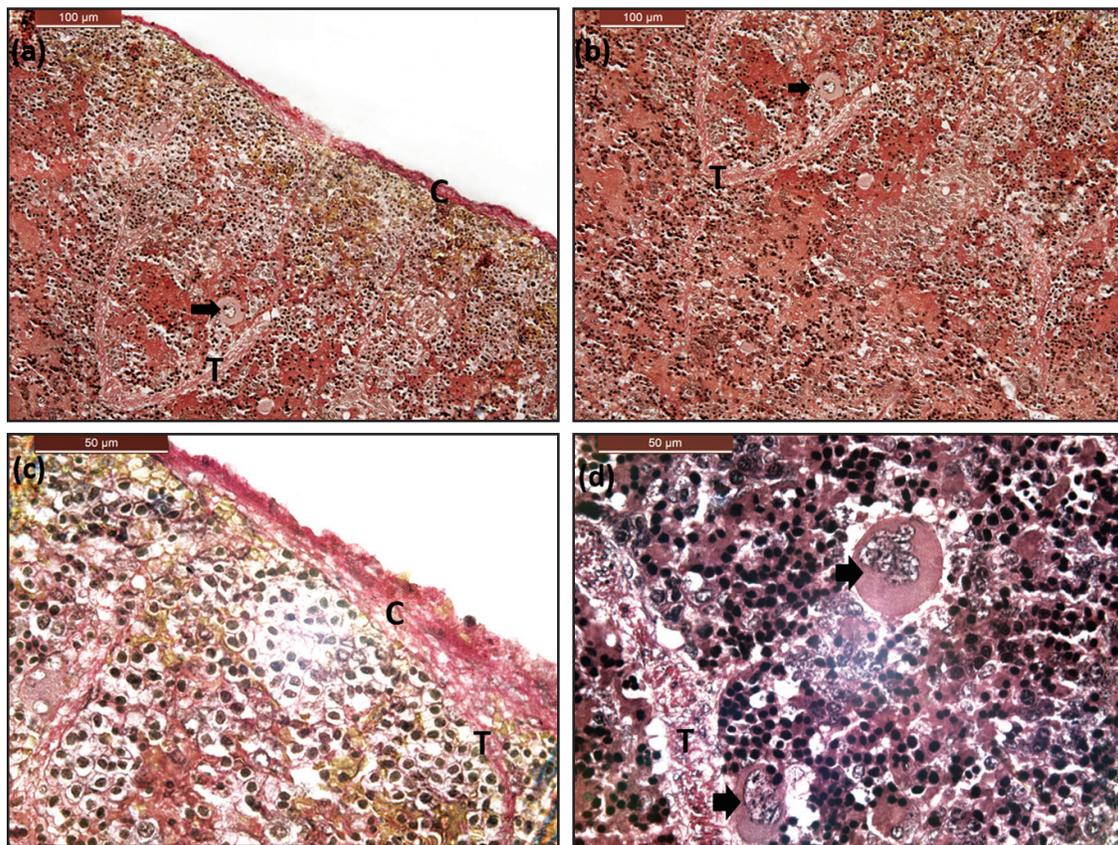


Fig 2. Photomicrographs showing spleen of camel foetus during the 2nd trimester of gestation; connective tissue capsule (C) connective tissue trabeculae (T) Especially collagen fibres. (a), (b) and (c); Megakaryocytes (arrows). (a), (b) and (c) Van Gieson's (X20, X63 respectively), (d), Gordon and Sweet (X63).

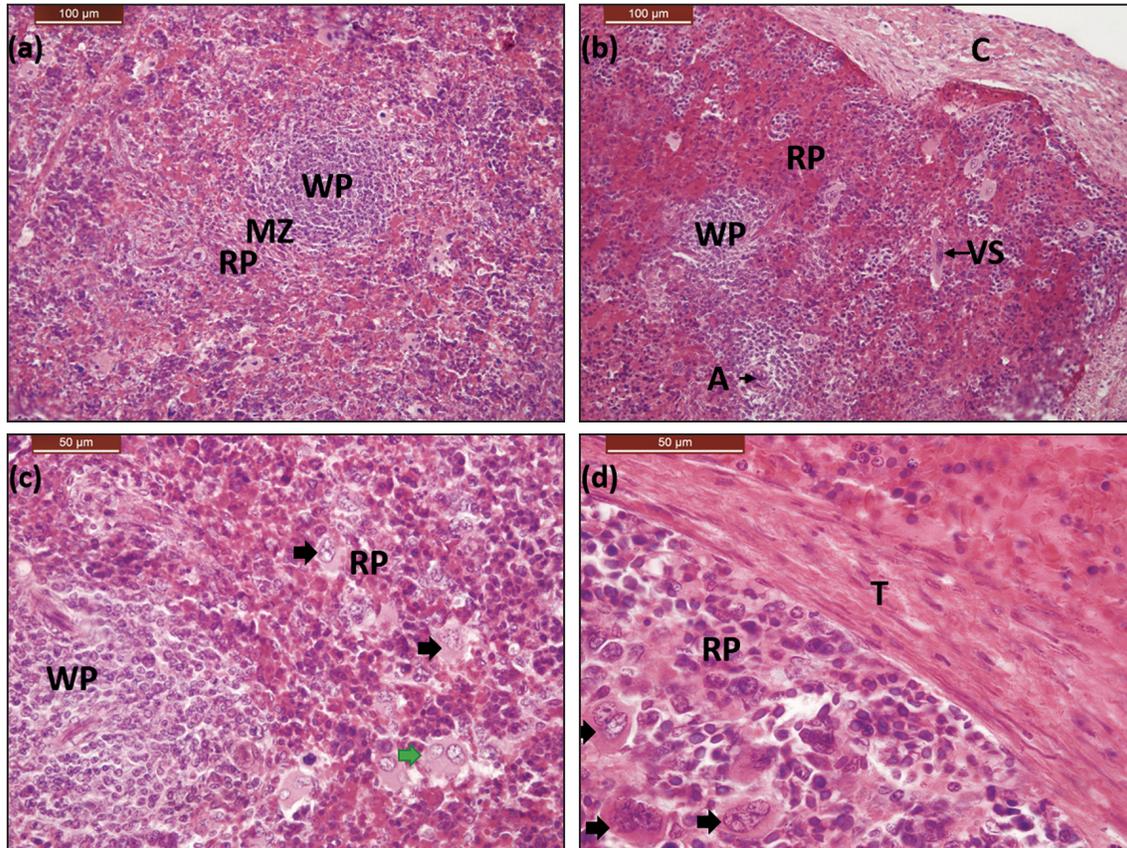


Fig 3. Photomicrographs showing spleen of camel foetus during the 3rd trimester of gestation; (a) white pulp (WP) surrounded central artery (A). (b), red pulp consists of venous sinuses (VS) at the margin of white pulp, marginal zone (MZ). Capsule (C) and trabeculae (T) consisted of smooth muscle fibres. (c and d); Megakaryocytes had lobulated nuclei (black arrows), binucleated megakaryocyte were present (green arrow). Haematoxylin and Eosin. (a) and (b): (X20), (c): (X40), (d): (X100).

age (Zidan *et al*, 2000b). However, megakaryocytes observed in the red pulp of foetal spleen of present study at the 1st, 2nd and 3rd trimesters increased in their numbers during the three GA. Binucleated megakaryocytes were observed at the 3rd trimester of GA.

The results of present study showed that the development of the spleen in 1st GA showed ordinary mesenchymal cells and undifferentiated zones of white and red pulps which later developed into differentiated zones of white and red pulps. In addition, the capsule and trabeculae developed from fine connective tissue to well-developed connective tissue. This development followed the advancement in subsequent GA from 1st to 3rd trimester.

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