

Histomorphometric changes during prenatal development of the spleen in different gestational phases of the dromedary camel (*Camelus dromedarius*)

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Abstract

The spleen functions as the principal secondary lymphoid organ and plays a critical role in filtering blood and initiating immune responses to antigens. The present study examined the prenatal histological and histometric development of the spleen across the three trimesters of gestation. Sixteen camel fetuses were collected and allocated to first, second, and third trimester groups by measuring the crown vertebral rump length (CVRL) of each fetus. Tissue samples were taken from the spleen and processed for histological investigations. In the first trimester, the spleen exhibited a thin capsule, small lymphoid nodules, and a network of developing blood sinuses. In the second trimester, the capsule differentiated into an outer collagenous layer and an inner smooth muscle layer with well-developed and branched trabeculae. The lymphoid nodules in the stroma increased both in number and size. During the third trimester, the trabeculae showed increased branching and contained smooth muscle layer. The white pulp structures appeared spherical, with prominent central arterioles. The thickness of the capsule increased significantly ($p < 0.05$) from $21.3 \mu\text{m}$ in the first trimester to $54.6 \mu\text{m}$ in the second trimester, whereas no significant increase was noted in the third trimester ($55.2 \mu\text{m}$). The diameter of the lymphoid nodules increased significantly ($p < 0.05$) from $133.0 \mu\text{m}$ to $161.6 \mu\text{m}$ in the second and third trimesters, respectively. These observations suggest a progressive increase in the size of these nodules during fetal development. It is concluded that the structural changes in the camel spleen during embryonic development were similar in trend to those of other domestic animals. This study provides novel baseline quantitative data on the morphometry of the splenic capsule and lymphoid nodules during prenatal development of the camel spleen, offering a reference for future studies on lymphoid organ maturation in camelids.

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1. Introduction

The spleen is a secondary lymphoid organ that plays a crucial role in filtering blood and triggering immune responses to antigens in vertebrates. It is divided into two distinct areas: the red pulp and the white pulp, each of which has its own structure and function (Mebius and Kraal 2005). The red pulp filters the blood, removing foreign substances and old or damaged red blood cells. The red pulp also serves as a reservoir of iron, red blood cells, and platelets (Cesta 2006). In contrast, the white pulp and the marginal zone are crucial for both innate and adaptive immune responses (Vondenhoff et al. 2008; Papenfuss and Cesta 2017). In most mammals, the spleen acts as a center for blood cell formation during early stages of development. It then continues to produce lymphoid cells throughout life (Krause and Cutts 1986). In ruminants, the fetal spleen is the main site for producing red blood cells, a process that continues for a few weeks after birth (Eurell and Frappier 2006). Although camel blood is similar to that of other mammals (Vap and Bohn 2015), camels possess unique physiological and immune mechanisms that help them survive in dry environments. Thus, camels are considered a useful model for studying prenatal spleen development (Kulkarni and Falzarano 2021). Morphological changes in the spleen during fetal development have been studied in several mammalian species, including camels (Marwa-Babiker et al. 2023; Bello et al. 2016; Jaji et al. 2019), goats (Gupta et al. 2017; Gautam and Mishra 2015), sheep (Maddox et al. 1987), rodents (Cesta 2006), and humans (Thomas et al. 2017). However, because the

spleen is a vital component of the lymphoid system, further research into its structural development is essential to better understand how the immune system responds to infections.

Comparative histometric investigations of the spleen have been reported in humans and a wide range of animal species (Rahman et al. 2016; Alim et al. 2012; Khalel 2010; Colakoğlu and Selçuk 2020; Verma et al. 2018). Variations related to age in the human spleen, including the fetal stage, have also been documented (Alex et al. 2015). In adult camels, several studies have examined splenic histomorphometry extensively (Zidan et al. 2000; Alshamarri 2010; Maina et al. 2014). Conversely, quantitative data on the histomorphometry of the splenic capsule and lymphoid nodules during prenatal development remains limited. For example, only a few studies have exclusively measured capsule development in prenatal camels, despite its essential role in supporting the spleen and protecting the inner parenchyma from injury (Belic 2015). The present study aimed to investigate the histomorphological features of the camel spleen and to measure the splenic capsule thickness and the diameter of lymphoid nodules during embryonic development.

2. Materials and Methods

2.1 Sample collection

The study was reviewed and approved by the Research Council of the College of Veterinary Medicine, University of Bahri, during its meeting held on 18 March 2018. Sixteen camel fetuses, irrespective of sex, were

collected from slaughterhouses in Khartoum State and Gezira State, Sudan. The crown vertebral rump length (CVRL) of each fetus was measured in centimeters to estimate gestational age, using the formula given by Elwishy et al. (1981). The fetuses were divided into three age groups: first (0.1–23.5 cm, 66–130 days), second (24–71 cm, 131–260 days), and third trimester (71.5–131 cm, 261–424 days) (Table 1).

Table 1. The number of fetuses used for histological investigation in the three trimesters of gestation

Trimester	CVRL (cm)	Age (days)	No. of fetuses
First	0.10 - 23.5	66 - 130	5
Second	24.0 - 71.0	131 - 260	5
Third	71.5 - 131.0	261 - 424	5

CVRL: Crown vertebral rump length

2.2 Histology

The spleen was dissected from the collected fetus and fixed in 10% neutral buffered formalin for at least 48 hours. Fetal samples were taken from the mid-region of the spleen. Specimens were then washed, serially dehydrated in alcohol, cleared in xylene, and infiltrated in wax. The tissue blocks were sliced into 5 μ sections with a rotary microtome (LEICA RM 2125, Germany) and stained with Haematoxylin and Eosin (H&E) for histo-architecture, Van Gieson stain for collagen fibers, and Gordon & Sweet's stain for reticular fibers (Bancroft and Gamble 2008).

2.3 Histo-morphometry

Fifteen of the sixteen collected fetuses were included in the histometric analysis, with five fetuses representing each trimester of gestation (Table 2). The capsule thickness and diameter of white pulp (lymphoid nodules) in each H&E-stained section were recorded using micrometers. Five readings were taken for each parameter in each section. Because lymphoid nodules were difficult to identify and measure during the first trimester of gestation, only 125 measurements were recorded. Images and measurements were obtained using a 40 $\times$ objective lens fitted to a light microscope (Olympus BX51) connected to a digital camera (Olympus DP20) and equipped with an integrated imaging and measurement system.

2.4 Statistical analysis

Statistical analysis was performed using SPSS version 21.0 (IBM

Corporation, New York, USA). Measurements of capsule thickness and lymphoid nodule diameter were tested for normality using the Shapiro–Wilk test. Data that did not meet the assumption of normality were log10-transformed before applying a one-way ANOVA to compare means across the three trimesters. Duncan's multiple range test was subsequently used for post hoc comparison of means. A p-value of < 0.05 was considered statistically significant.

3. Results

The fetal spleen developed in a distinct pattern throughout the gestation period. Fig. 1 shows the timeline of histological changes and main structural developments in the spleen during the three trimesters.

3.1 Histology

3.1.1 First trimester (65–130 days)

In the first trimester, the capsule was generally thin and covered by mesothelial cells. The parenchyma consisted of blood sinuses, small lymphoid nodules, groups of blood-forming cells, and scattered megakaryocytes (Fig. 2a). As the fetal age advanced, the capsule became thicker and distinct trabeculae were observed originating from the inner capsule and extending into the splenic parenchyma (Fig. 2b). Both the capsule and trabeculae displayed prominent reticular fibers (Fig. 2c). Small subcapsular sinuses were also observed in the spleen (Fig. 2a).

3.1.2 Second trimester (131–260 days)

In the second trimester, the spleen displayed trabeculae and a network of reticular fibers, together with the presence of lymphoid nodules. The capsule was thicker than that observed in the first trimester and had two layers: a thick outer layer that was composed mainly of collagen fibers, and a thin inner layer of smooth muscle fibers (Fig. 3a, Fig. 3b). The parenchyma was divided into several compartments by well-developed trabeculae. These trabeculae showed branching patterns, including both primary and secondary branches. The amount of branching increased by the end of the second trimester. Peritrabecular sinuses were also observed at this stage (Fig. 3c). With further development, clear white pulp areas were visible, with distinct lymphoid nodules of either round or irregular shapes. Numerous red blood cells were present in the splenic tissue. By the end of the second trimester, the white pulp was more apparent and had well-developed eccentrically located central arterioles surrounded by periaarteriolar lymphoid sheath (PALS). The central arterioles exhibited abundant reticular fibers, while the lymphoid nodules had fine reticular fibers (Fig. 3d).

3.1.3 Third trimester (261–426 days)

In the third trimester, the spleen continued to develop. The parenchyma showed clear red and white pulp areas. A considerable amount of connective tissue and a greater abundance of smooth muscle were also observed. The splenic capsule remained uniform and appeared thicker than in the second trimester. The inner layer was prominent and contained smooth muscle. The outer layer was composed of collagen and reticular fibers, with adipose tissue. The subcapsular sinuses were lined with endothelial cells (Fig. 4a, Fig. 4b). The branching continued in trabeculae and smooth muscle fibers were also observed in the trabeculae. Distinct white and red pulp zones were observed, and the red pulp contained well-defined blood sinusoids. The lymphoid nodules increased in both size and number and became spherical in

Table 2. The measurements of CVRL and the corresponding age of fetuses used for histometric analysis

Trimester	Fetus	CVRL (cm)	Age (days)
First	1	7	84.7
	2	13	101
	3	14.5	105
	4	18.5	116
	5	19	117.5
Second	1	29	144
	2	31	150
	3	49	199
	4	68	251
	5	70	257
Third	1	72	262
	2	81	287
	3	84	295
	4	90	311
	5	91	314

CVRL: Crown vertebral rump length

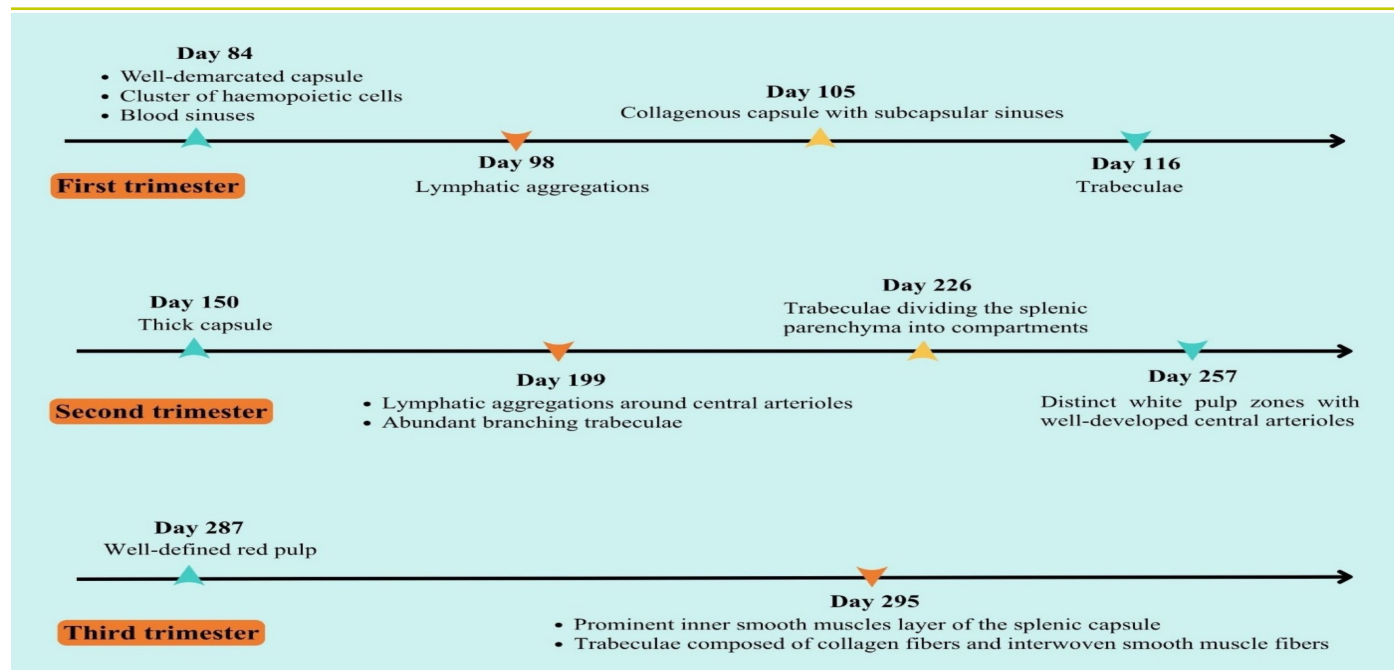


Fig. 1. Chronological timeline illustrating the histological changes and major structural developments in the camel spleen across the three trimesters of gestation

shape. The central arterioles in the white pulp were eccentrically located (Fig. 4c). Reticular fibers were more prominent within the splenic parenchyma, particularly in the trabeculae and white pulp, with abundance in the PALS (Fig. 5a, Fig. 5b). Megakaryocytes were more abundant in the splenic parenchyma (Fig. 5c)

3.2 Histomorphometry

Table 3 shows the histometric analysis of the capsule thickness and lymphoid nodules diameter. Capsule thickness measurements demonstrated a significant increase ($p < 0.05$) of 156.3%, rising from 21.3 μ in the first trimester to 54.6 μ in the second trimester. The further increase in capsule thickness (55.2 μ) during the third trimester was

statistically insignificant ($p > 0.05$) as compared to the second trimester. Lymphoid nodules were rarely observed during the first trimester and hence could not be measured reliably. In the second and third

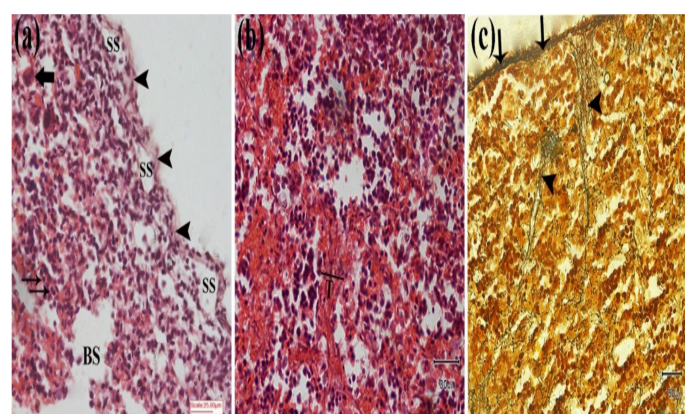


Fig. 2. Light photomicrographs showing histological structures of the camel spleen in the first trimester of gestation

- (a) Fetal spleen at 105 days (14.5 cm CVRL) showing a capsule (arrowheads) composed of loose connective tissue, subcapsular sinuses (SS), blood sinus (BS), a megakaryocyte (thick arrow), and hematopoietic cells (thin arrows) (H&E)
- (b) Fetal spleen at 116 days (18.5 cm CVRL) developing trabeculae (T) (H&E)
- (c) Fetal spleen at 117 days (19 cm CVRL) showing reticular fibres in the developing capsule (arrows) and trabeculae (arrowheads) (Gordon & Sweet's reticulin stain)

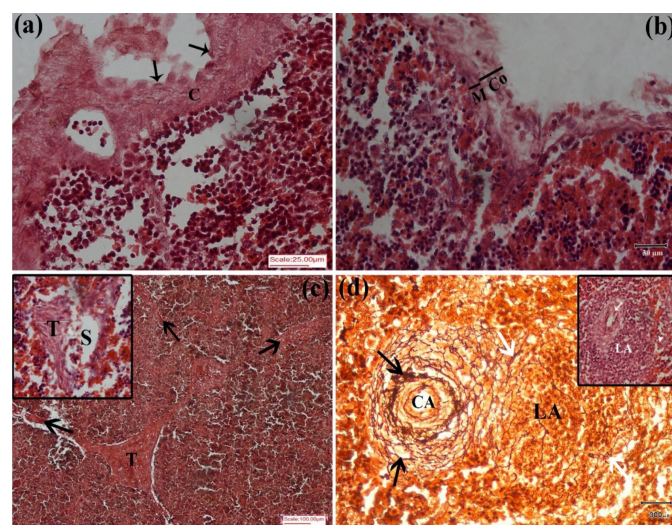


Fig. 3. Light photomicrographs showing histological structures of the camel spleen during the second trimester of gestation

- (a) Fetal spleen at 144 days (29 cm CVRL) showing thick capsule (C) composed of dense collagenous connective tissue and covered by mesothelium (arrows)
 - (b) Fetal spleen at 150 days (31 cm CVRL) showing capsule consisting of a prominent outer collagenous layer (Co) and a thin inner layer of smooth muscle fibres (M)
 - (c) Fetal spleen at 257 days (70 cm CVRL) showing long, branched secondary trabeculae (arrows) extending from the primary trabeculae (T); inset: trabecula (T) with peritrabecular sinus (S)
 - (d) White pulp region showing an eccentrically located central arteriole (CA) surrounded by the periarteriolar lymphoid sheath, exhibiting a distinct network of reticular fibers (black arrows), and a lymphoid aggregation (LA) containing fine reticular fibers (white arrows); inset: lymphoid nodule (LA), central arteriole (arrow), and red pulp (arrowhead)
- (a–b, c inset, d inset: H&E stain; c: Van Gieson stain; d: Gordon & Sweet's reticulin stain)

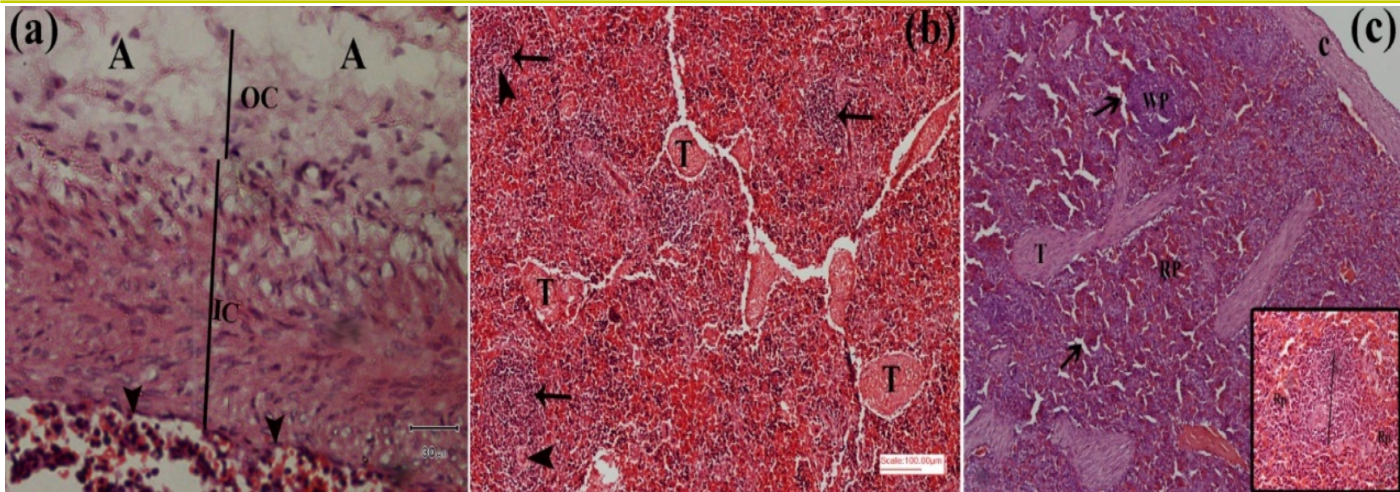


Fig. 4. Light photomicrographs showing histological structures of the camel spleen during the third trimester of gestation
(a) Fetal spleen at 295 days (84 cm CVRL) showing the outer layer of the splenic capsule (OC) composed predominantly of connective tissue fibres with marginal adipose tissue (A), and a well-developed inner layer (IC) of smooth muscle; subcapsular sinuses lined by endothelial cells (arrowheads)
(b) Fetal spleen at 289 days (82 cm CVRL) showing trabeculae (T) dividing the parenchyma into compartments; white pulp areas (arrows) with central arterioles (arrowheads)
(c) Fetal spleen at 314 days (91 cm CVRL) showing well-defined white pulp (WP), red pulp (RP), branching trabeculae (T), and blood sinusoids (arrows)
Inset: spherical white pulp (outlined), red pulp (RP) (H&E stain)

trimesters, the diameter of the lymphoid nodules increased steadily as the pregnancy progressed. A significant increase ($p < 0.05$) was recorded, with the mean diameter increasing from $133.0\ \mu$ in the second trimester to $161.6\ \mu$ in the third trimester.

Table 3. Thickness of the capsule and the diameter of lymphoid nodules means (with 95% CI) of the fetal spleen during the trimester stages of gestation		
Trimester	CVRL (cm)	Age (days)
First	21.3 (18.0 – 27.7) ^a	Not measurable
Second	54.6 (44.6 – 69.3) ^b	133.0 (120.4 – 146.9) ^a
Third	55.2 (41.2 – 76.7) ^b	161.6 (146.0 – 178.9) ^b
Different superscripts within a column indicate significant differences ($P < 0.05$); CI = Confidence interval		

4. Discussion

The histological findings in this study were similar to those reported in previous research on the camel spleen during prenatal development (Bello et al. 2016; Jaji et al. 2019; Marwa-Babiker et al. 2022). There is a consensus among these studies that fetal splenic development in camels follows a common structural pattern, despite variations in the developmental stages. The splenic development in camel, for instance, is characterized by a slower rate of development, most probably attributable to its prolonged gestation and specific adaptive needs (Srivani and Pillai 2019). In the present study, the fetal spleen at early developmental stages exhibited a narrow capsule primarily composed of collagenous fibers. Similar observations have been reported in goat and human fetuses (Asha et al. 2008; Mukhia et al. 2016). With advanced pregnancy, the capsule became stronger due to the increased amount of dense collagen and reticular fibers. This pattern has also been documented in the developing embryos of chickens, humans, and goats (Khalil et al. 2009; Ernst et al. 2019; Gupta et al. 2017).

Moreover, the second trimester was characterized by a noticeable

expansion of subcapsular spaces that were lined with endothelium and occupied by blood components. Similar findings have been reported in human fetal spleen (Halder et al. 2021). Earlier studies on camels demonstrated a gradual thickening of the capsule with advancing fetal age, emphasizing an increased role of smooth muscle in later developmental phases (Bello et al. 2016). Although camels and other mammals have similar developmental sequences, there are significant species variations regarding the timing of stromal differentiation. In goats, collagen and reticular fibers are present at earlier stages as compared to camels (Gupta et al. 2017). Variations are also evident in trabecular development. The present study demonstrated that trabeculae arising from the capsule progressively extended into the parenchyma as reported earlier (Bello et al. 2016; Marwa-Babiker et al. 2022). In contrast, trabeculae in goats exhibited restricted inward growth at similar developmental stages (Asha et al. 2008). The present study revealed a marked increase in capsule thickness between the first and second trimesters. This may indicate vigorous extracellular matrix deposition and structural consolidation during the second trimester. A comparable trend has been documented in classical work conducted by Arey (1954) on human embryos. Although morphologically thicker in the third trimester, capsule thickness did not differ significantly from the second trimester.

During the third trimester, the capsule demonstrated significant development of the inner muscular layer. This observation correlates with the earlier studies who reported that the spleen development is characterized by a distinct capsule consisting of a well-developed inner smooth muscle layer (Zidan et al. 2000; Maina et al. 2014). The absence of a significant increase in capsule thickness during the third trimester in the present study suggests that the prominent muscular layer may result from cellular differentiation and maturation rather than an increase in the number of smooth muscle fibers. Moreover, the marked smooth muscle layer may support the development and functional maturation of the spleen as an effective contractile blood reservoir. The

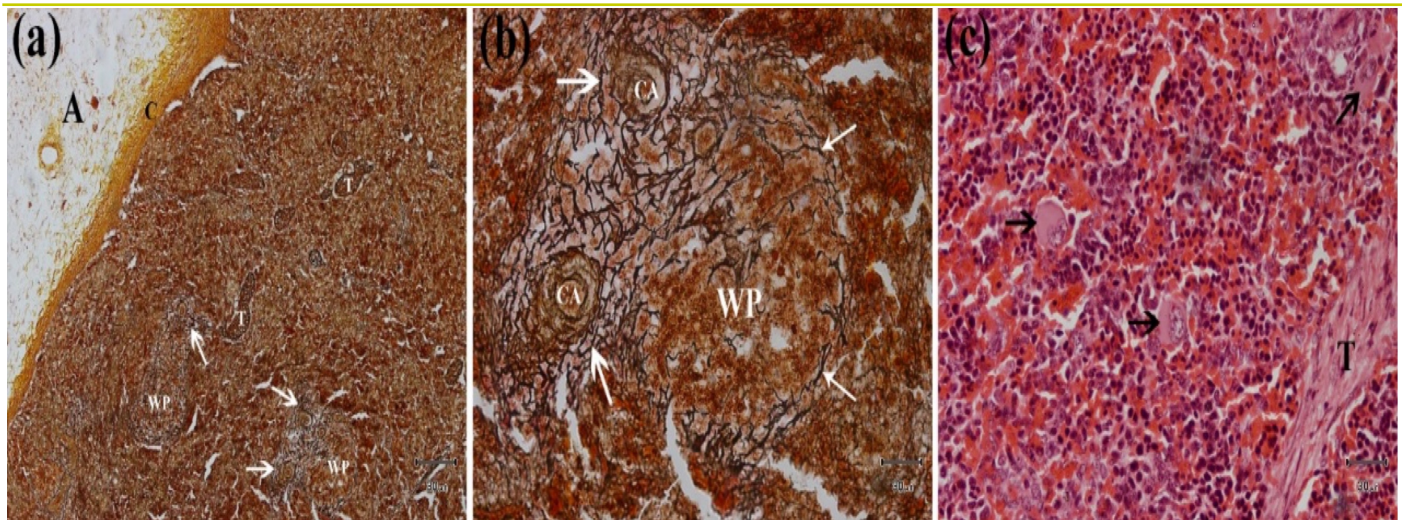


Fig. 5. Light photomicrographs showing histological structures of the camel spleen during the late third trimester of gestation
(a) Fetal spleen at 314 days (91 cm CVRL) showing prominent reticular fibres in the trabeculae (T) and surrounding central arterioles (arrows) within the white pulp; the splenic capsule (C) is covered by adipose tissue (A)
(b) White pulp region (WP) showing an eccentrically located central arteriole (CA) surrounded by the periaarteriolar lymphoid sheath, exhibiting a distinct network of reticular fibers (large arrows), and a lymphoid aggregation containing fine reticular fibers (small arrows)
(c) High magnification of fetal spleen at 262 days (72 cm CVRL) showing megakaryocytes (arrows) within the red pulp and a trabecula (T)
(a, b: Gordon & Sweet's reticulin stain; c: H&E stain)

present investigation showed that lymphoid nodules were either scarce or not easily detectable during early gestation, but their size increased rapidly during the mid and late trimesters. Similarly, connective tissue differentiation in humans and goats has been shown to precede the formation of prominent lymphoid areas (Gupta et al. 2017; Halder et al. 2021). It is plausible that lymphoid cell colonization relies on previous stromal growth.

4. Conclusions

The present study demonstrated a distinct pattern and notable morphological transformations within the camel spleen throughout embryonic development. The histomorphometric parameters of the spleen at different developmental stages reported in this study, including capsule thickness and lymphoid nodules diameter, may serve as a useful reference for future studies on the development of lymphoid organs in camels and other mammals.

Declarations

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Conflict of interest: The author declare no conflicts of interest

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Ethics approval: The study was reviewed and approved by the Research Council of the College of Veterinary Medicine, University of Bahri (Approval letter can be obtained from corresponding author upon request)

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