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Developmental dynamics of spleen morphogenesis and histogenesis in local Iraqi Awassi sheep fetuses: Insights from light and scanning electron microscopy

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Abstract

Objective

Spleen plays an important role in the hemopoietic function in embryonic life. During the hepatic growth stage, spleen produces blood cells along with liver while in myeloid stage, it produces the blood cells along with liver and bone marrow. Destruction of older erythrocytes, lymphocytes and thrombocytes take place in spleen. The aim of this study was to investigate the prenatal morphological and histological development of the spleen in local Awassi sheep used light and scanning electron microscopy.

Materials and methods

Thirty spleen specimens were collected from healthy pregnant Awassi ewes at different stages of gestation. Fetal age was determined using the fetal crown rump length (CRL) equation and the specimens were classified into three groups: Group 1: 50-60 days, Group 2: 90-100 days, and Group 3: 130-140 days. For scanning electron examination, small spleen blocks (~1 mm^3) were fixed. SEM was used to observe the splenic surface at multiple magnifications. Data analysis of histological and morphological parameters was performed using SPSS version 24.

Results

In group 1, spleen a tiny reddish bloody patch then appeared reddish in color more cohesive while, by scanning electron microscopy and histologically revealed an immature surface structure with incomplete development of the cortex and the trabeculae were undeveloped yet reflecting early splenic differentiation. In group 2, the spleen showed an increase in size and the trabecula extend from capsule to parenchyma, clear differentiation of white pulp and red pulp, indicating progressive structural maturation. In group3, spleen was more development with advancement of gestation and become relatively similar to that in postnatal periods and consist of the splenic capsule, trabeculae, red pulp, white pulp with present of cellular components and increased in trabecular network by light and scanning microscope examination.

Conclusion

These findings highlight the progressive prenatal maturation of the spleen and underscore its pivotal role in immune system development. Deviations from these normal microstructural patterns may indicate pathological or immunological disorders.

Keywords: Awassi sheep, Fetuses, Prenatal development, SEM, Spleen

Paper Type: Research Paper.

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Introduction

The spleen, the largest organ in the lymphatic system, is usually situated in the cranial part of the abdominal cavity on the left of the stomach (in ruminants on the left lateral wall of the reticulum) and contains the largest collection of reticuloendothelial cells in the body with no afferent lymphatics vessels (Aughey & Frye, 2001). Spleen plays an important role in the hemopoietic function in embryonic life. During the hepatic growth stage, spleen produces blood cells along with liver while in myeloid stage, it produces the blood cells along with liver and bone marrow. Destruction of older erythrocytes, lymphocytes and thrombocytes take place in spleen. When the erythrocyte becomes old (120 days), the cell membrane becomes more fragile. Destruction occurs mostly in the capillaries of spleen because the splenic capillaries have a thin lumen. So, the spleen is known as ‘graveyard of RBCs. In animals, spleen stores large amount of blood and the spleen filters the blood by removing the microorganisms (Sembulingam & Sembulingam, 2012). Histologically, the spleen is surrounded by a thick connective tissue capsule invested by the peritoneum. The capsule composed of two layers: dense irregular and smooth muscle layers. The total thickness and relative amount of smooth muscle vary with the species. Trabeculae composed of collagen and elastic fibers with smooth muscle cells extend from the capsule and hilus into the parenchyma. The capsule, trabeculae, and reticular fibers support the splenic parenchyma composed of a red pulp involved in the storage of red blood cells and a white pulp rich in lymphocytes and active in immune responses. The red pulp is composed of venous sinuses or venules and splenic cords. Two main types of red pulp are present in mammalian spleens, depending on the type of postcapillary vessels: sinusal or non-sinusal. White pulp is lymphatic tissue that is distributed throughout the spleen and is comprised of lymphatic nodules and diffuse lymphatic tissue called periarterial lymphatic sheaths (PALS). The marginal zone lies

between the white pulp and the red Pulp (Figueiredo & Turek, 2025). Moreover, Small ruminants, particularly native breeds, play a crucial role in the livelihoods of a significant portion of the human population in tropical regions from socio-economic perspectives (Molaei Moghbeli et al., 2013; Alhasoon et al., 2026; Saadatabadi et al., 2023; Mohammadabadi et al., 2024). These animals are essential sources of meat, milk, wool, and hides, contributing to food security and rural incomes. Furthermore, they are well-adapted to harsh environmental conditions, making them vital for pastoral and small-scale farming systems (Hajalizadeh et al., 2021). Given their importance, combined efforts that focus on both effective management strategies and genetic improvement are crucial to enhancing animal productivity and ensuring sustainable development (Mohammadipour Saadatabadi et al., 2022; Vahabzadeh et al., 2020; Amirteymoori et al., 2021; Mohammadabadi et al., 2022). Genetic improvement programs, such as selective breeding, molecular marker-assisted selection, and genomic approaches, can significantly boost desirable traits like growth rate, milk yield, and resistance to diseases (Nejad et al., 2024). The economic and biological efficiency of small ruminant production enterprises generally improves by increasing both productivity and reproductive performance in these animals (Zamani et al., 2011; Safaei et al., 2022; Barazandeh et al., 2016; Mohammadinejad, 2016; Shokri et al., 2023). Enhanced reproductive performance can be achieved through improved nutrition, strategic breeding practices, and advanced reproductive technologies such as artificial insemination and embryo transfer (Noori et al., 2017). By integrating these approaches, small ruminant breeders can improve flock productivity, ensure food security, and contribute to the economic well-being of rural populations (Mohammadabadi et al., 2022). Thus, the present study aimed to investigate the prenatal morphohistological and ultrastructural development of spleen in local Awassi sheep fetuses using light and scanning electron microscopy to correlate these changes with fetal age to provide baseline data for normal splenic maturation. This study provides the first detailed SEM-based ultrastructural description of prenatal spleen development in local awassi sheep foetuses and these findings contribute original baseline data that may serve as a reference for future developmental, immunological, and pathological studies.

Materials and methods

Thirty splenic samples were collected from fifty fetuses of pregnant Awassi ewes slaughtered at Karbala and Babylon Province abattoirs in middle of Iraq. The fetuses were selected based on their normal morphological appearance, absence of congenital abnormalities and availability at the desired gestational ages. Only healthy fetuses with intact membranes and no signs of autolysis were included in the study and fetal age was estimated using the crown-rump length (CRL) formula: X=2.1(Y+17), Where X is the age of fetus in days and Y is crown rump length in centimeters (Noakes et al., 2019; Mohassen and Al-Jebori, 2020). Fetuses were categorized into three prenatal groups (ten fetuses for each group): Group I (50-60 days), Group II (90-100 days), and Group III (130-140 days of gestation). spleen samples were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Serial sections were cut at 5-6 µm using a rotary microtome and the staining included Hematoxylin & Eosin (H&E) for general morphology, Masson's Trichrome for connective tissue,

Periodic Acid-Schiff (PAS) for basement membranes and glycoproteins and Tolidine blue for enhance visualization of splenic architecture, lymphocyte and reticular cells in spleen. For scanning electron examination, small spleen blocks (~1 mm^3) were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) at 4°C for 24 hours, washed, and post-fixed in 1% osmium tetroxide for 2 hours. Samples were dehydrated in a graded ethanol series (30-100%), dried using critical point drying, mounted on SEM stubs, and sputter-coated with gold-palladium. SEM was used to observe the splenic surface at multiple magnifications. All procedures were performed following ethical standards for the use of animal tissues in research (Niyf and Al-Jebori, 2024; Suvarna et al., 2018). Data analysis of histological and morphological parameters was performed using the Statistical Package for the social sciences (SPSS) version 24. A one-way analysis of variance was performed, and differences were considered significant at $P < 0.05$.

Results and discussion

Observation during first trimester (days 50-60): During early gestation, sheep foetuses exhibited an average body weight of fetuses (82.6±4.45 gram) and a crown-rump length (10.4±1.14 mm) (Table 1). Macroscopically, at 50 days of gestation, spleen a tiny reddish bloody patch appears on the dorsal surface of left side of the developer gut tube in the upper left region relatively nearest to liver and contact with two true last ribs without clear splenic hilus (Figure 1).

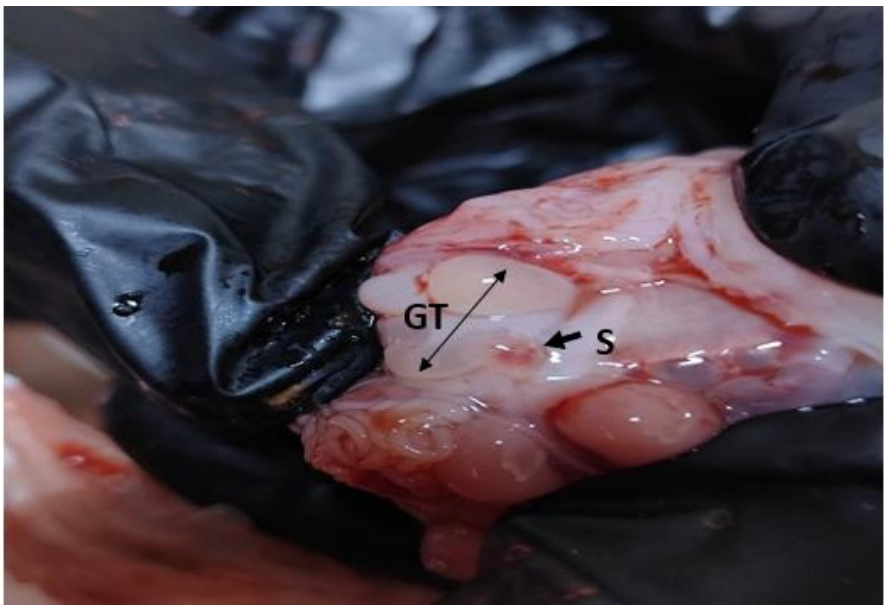

Figure 1. Photograph at 50 days of gestation show the spleen (SP) as tiny reddish bloody patch on the dorsal surface of left side of the developer gut tube (GT)

The results agreement with Gupta et al. (2017) observed the splenic primordium at 46 days of gestation in goat fetuses as white thickening on the dorso-medial surface towards the cranial end of the stomach tube until 50 days of gestation. While, at 60 days of gestation, spleen appeared reddish in color more cohesive and acquired the shape of thin umbrella or oyster shell like shape structure located on left side of abdominal cavity inside of ribs cage and highly relationship with rumen, diaphragm, ribs and have two surfaces (visceral and parietal), the visceral surface concave

and parietal surface convex with thin translucent whitish borders. The splenic hilus begging clearer and the splenic ligaments poorly development (Figure 2). These findings partially agree with Chaurasia et al. (2019) in gout. They reported a progressive change in splenic coloration toward a reddish-brown appearance with advancing gestation. In contrast, Malik et al. (2014) described the spleen at a comparable stage in goats as having an irregular triangular shape, which differs from the present findings. Similarly, Jaji et al. (2019) reported that the dromedary spleen during the first trimester appeared dark brown and semilunar in shape, indicating species-related variations in splenic morphology. Morphometric measurements of current study showed spleen weight (0.12±0.01 gram), volume (0.06±0.015 cm^3), length (5.12±0.10 mm), width (4.42±0.39 mm), and thickness (2.14±0.074 mm) (Table 1).

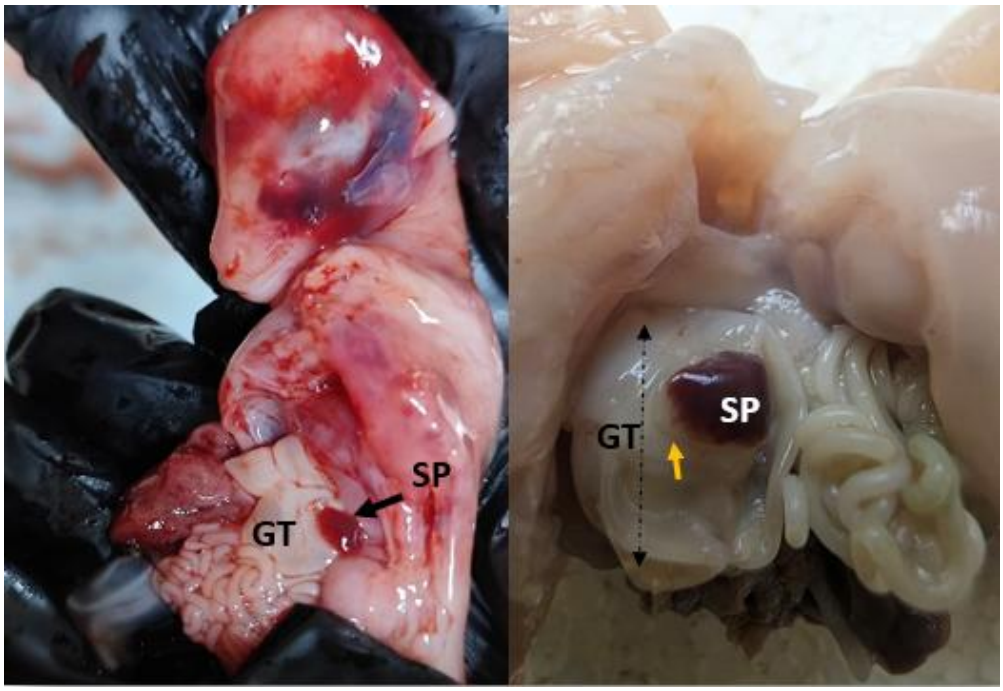

Figure 2. Photograph at 60 days of gestation show spleen (SP) location as on the dorsal surface of left side of the developer gut tube (GT) translucent whitish borders (yellow arrow)

Microscopically, the early development of the spleen consisted primarily of the stroma (splenic capsule) and the primordium of the parenchyma (anlage of the red and white pulp). The external surface of the organ was enveloped by mesothelium composed of simple squamous epithelial cells with low cuboidal epithelial cells observed using hematoxylin and eosin (H&E) staining. The splenic parenchyma was composed of large aggregations of cells, including primitive lymphoid cells (lymphoblasts) and small lymphocytes, which were dispersed throughout the parenchyma and organized into randomly distributed cellular aggregates (Figure 3). These cells were more densely distributed in the subcapsular region and around major blood vessels forming the periarteriolar lymphoid sheath (PALS) compared to the remaining splenic tissue. The nuclei exhibited marked variation in size and were darkly stained with uniform basophilia (Figures 3 and 4).

Table 1. Morphometric Changes of Fetal Growth and Splenic Development During Prenatal Stages in Sheep (Mean ± SE)

parameters Gestational age	Body Weight (g)	Crown-Rump Length (mm)	Spleen Weight (g)	Length (mm)	Width (mm)	Thickness (mm)	Volume (cm^3)
50-60 days pregnant	82.6 ± 4.45[a]	10.4 ± 1.14[a]	0.12 ± 0.01[a]	5.12 ± 0.10[a]	4.42 ± 0.39[a]	2.14 ± 0.07[a]	0.06 ± 0.02[a]
90-100 days pregnant	1311 ± 60[b]	259 ± 6.67[b]	0.84 ± 0.05[b]	24.7 ± 0.42[b]	15.7 ± 0.78[b]	8.44 ± 0.98[b]	0.91 ± 0.04[b]
130-140 days pregnant	4870 ± 121[c]	403 ± 9.40[c]	2.59 ± 0.09[c]	30.5 ± 0.36[c]	20.9 ± 0.80[c]	9.97 ± 1.10[c]	2.35 ± 0.24[c]

* Values are presented as Mean ± Standard Error (SE). Different superscript letters (a, b, c) within the same column indicates statistically significant differences ($P \leq 0.05$) based on one-way ANOVA followed by Tukey's post hoc test.

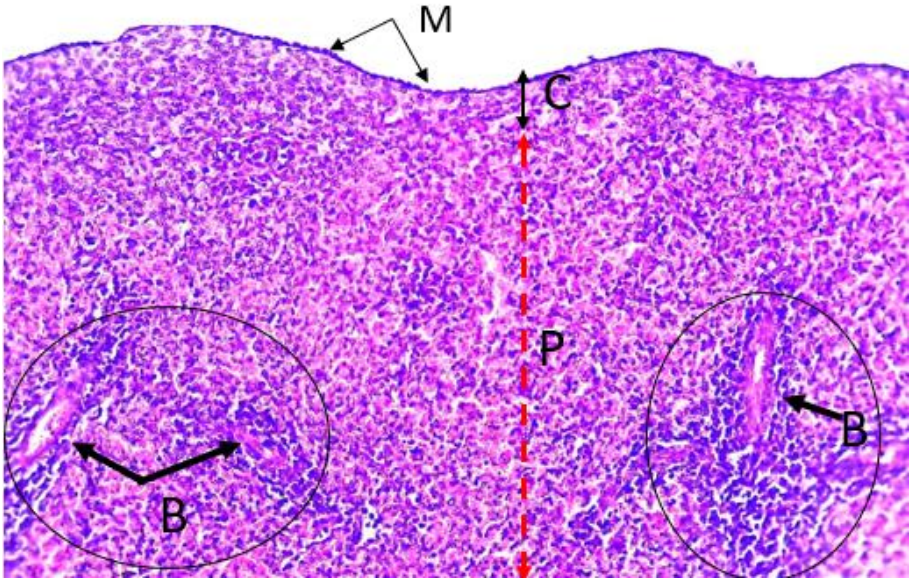

Figure 3. Cross histological section of spleen at (60-50) day of gestation. Capsule (C) mesothelium (M), parenchyma (P), blood vessels (B), and PALS (black circle). (H&E stain 10X)

The splenic parenchyma was completely enclosed by a connective tissue capsule has thickness (11.36±0.84 µm) (Table 2). It composed predominantly of collagen fibers, with sparse elastic and reticular fibers. Trabeculae were not evident at this stage of development (Figure 4). Haldar et al. (2021) reported in human fetuses, the splenic primordium has been appeared during the fifth week of gestation and according to Sonali et al. (2017) during the sixth week of gestation. In goats, Gupta et al. (2017) observed splenic primordia at 16 and 46 days of gestation.

Gupta et al. (2017) also reported the first appearance of reticular fibers in the fetal goat spleen at 60 days of gestation within the capsule and trabeculae. Whereas, collagen and elastic fibers were not observed at earlier stages. At the early prenatal stage (50-60 days), most histological structures were either absent or poorly developed. White pulp was not observed, and trabeculae were not distinguishable. While, capsule thickness showed a minimal value (11.36 ± 0.84 µm).

These findings indicate that the spleen at this stage is still in the early phase of organogenesis, with incomplete structural differentiation and absence of lymphoid tissue organization (Table 2). Scanning electron microscopy examination revealed that spleen in first trimester composed of thin capsule and parenchyma without appearance of trabecula. In this location, a primitive and poorly organized parenchyma mainly composed of undifferentiated mesenchymal cells and a delicate reticular framework and the visceral and parietal surface appear smooth relatively in textures (Figure 5).

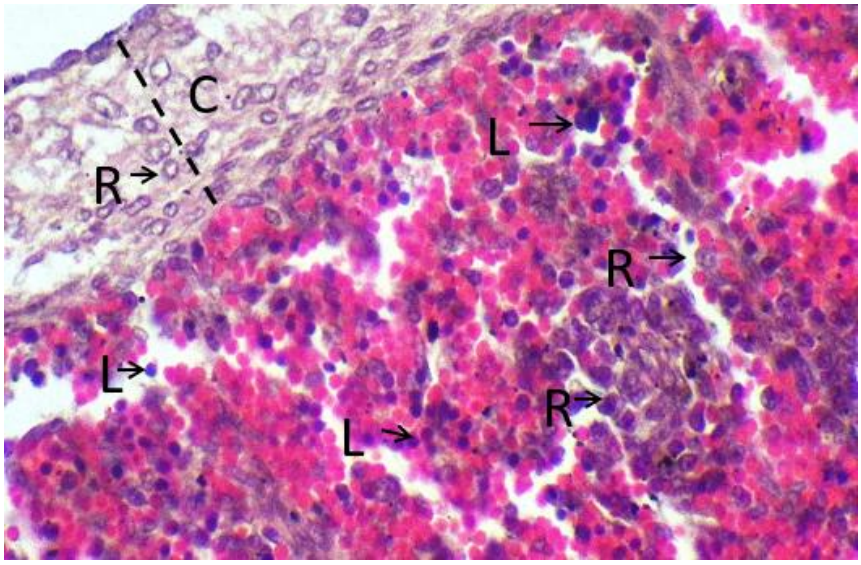

Figure 4. Cross histological section of spleen at (50-60) day of gestation. Capsule (C), lymphocyte (L), and reticular cell (R). (H&E stain 40X)

Table 2. Comparative Histomorphometric parameters of splenic white pulp during prenatal development in sheep (Mean ± SE)

Parameters Gestational age	Capsule thickness (µm)	Trabeculae thickness (µm)	Small W.P. diameter (µm)	Middle W.P. diameter (µm)	Large W.P. diameter (µm)	Number of W.P. (4×)
50-60 days pregnant	11.36 ± 0.84a	absent	absent	absent	absent	absent
90-100 days pregnant	28.8 ± 1.30b	11.5 ± 1.14b	32.76 ± 1.08b	41.2 ± 2.85 b	63.4 ± 1.14b	21.4 ± 1.12b
130-140 days pregnant	34.6 ± 1.14c	24.2 ± 0.86c	37.92 ± 0.84c	52.6 ± 1.56 c	76.0 ± 2.30c	9 ± 0.71c

* Values are expressed as Mean ± SE. Different capital letters (a, b, c) indicate significant differences ($P \leq 0.05$) among developmental stages. 'Absent' indicates lack of structural development at the early prenatal stage. W.P. = White Pulp

An immature and loosely organized splenic architecture predominantly composed of lymphocyte, erythrocyte and reticular cells without evidence of white and red pulp (Figure 6). Marwa-Babiker et al. (2023) mentioned that the scanning electron microscopy of spleen in camel

fetus lined with mesothelial cells and the reticular fibers appear in the cordal gaps, marginal zones, and parietal sheath were relatively small in the first trimester of gestation.

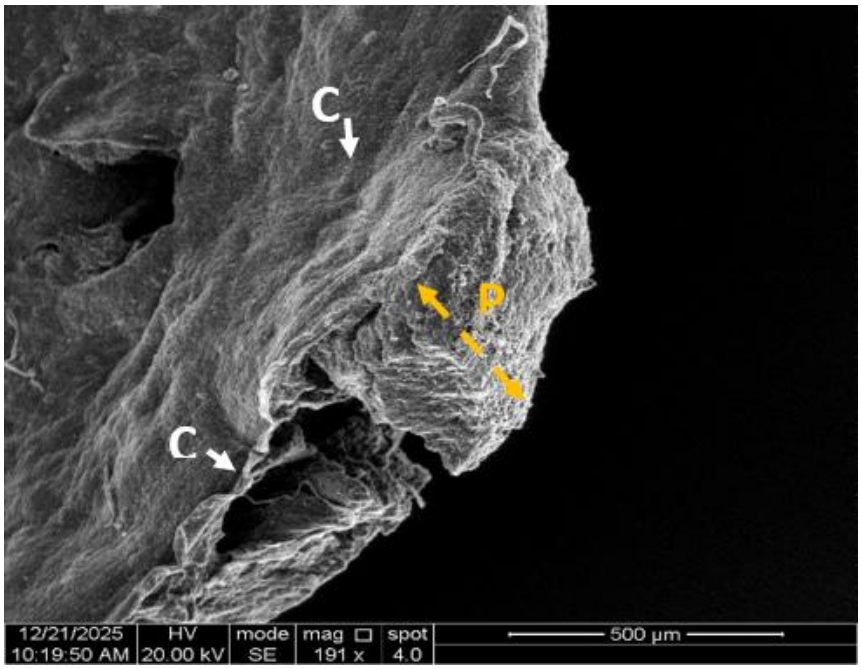

Figure 5. Scanning electron microscope. Thin capsule (C), and parenchyma (P) of spleen at the first trimester of gestation

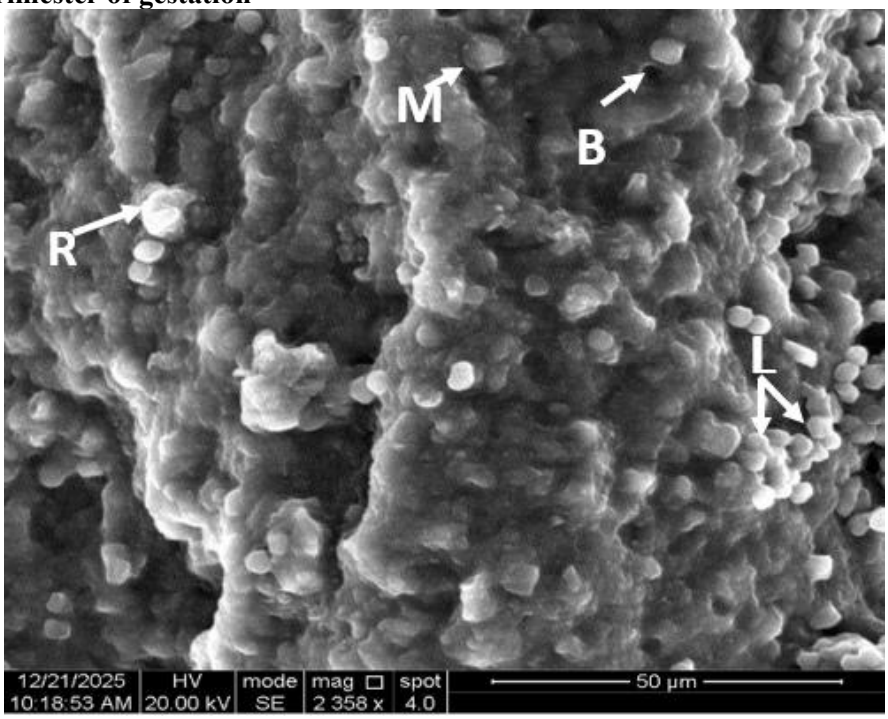

Figure 6. Scanning electron microscope. Cellular components of spleen at first trimester of gestation. Different size of lymphocyte (L), reticular cell (R), macrophage (M), and blood vessel (B)

Observation during second trimester (days 90-100): By mid-gestation, fetal body weight increased to approximately 1311± 60 gram, with crown rump length 259±6.67 mm. The spleen exhibited notable growth, biometric parameters of spleen in sheep fetuses as following. The

spleen weight 0.84±0.05 gram, volume 0.91±0.04 $cm^{3,}$ length 24.7±0.42 mm, width 15.7±0.78 mm, and thickness 8.44±0.98 mm (Table 1). The current study showed the color of spleen became dark bluish purple and has irregular triangular shape. Spleen progressively increases in size and weight with advancement of age and lies between the developing diaphragm and left dorsocranial surface of the rumen. It extended from 10^{th} to 12^{th} rib inside the upper thoracic part of abdominal cavity (Figure 7). Spleen have two extremities (cranial and caudal), two borders (lateral and medial), and two surfaces (parietal and visceral). They appear more concave and convex respectively than the first trimester where visceral surfaces was concave and less smoothness than parietal have hilus region contacted on the dorsal sac of the rumen. While, the parietal surface appears more convex and related to the diaphragm and ribs (Figure 8).

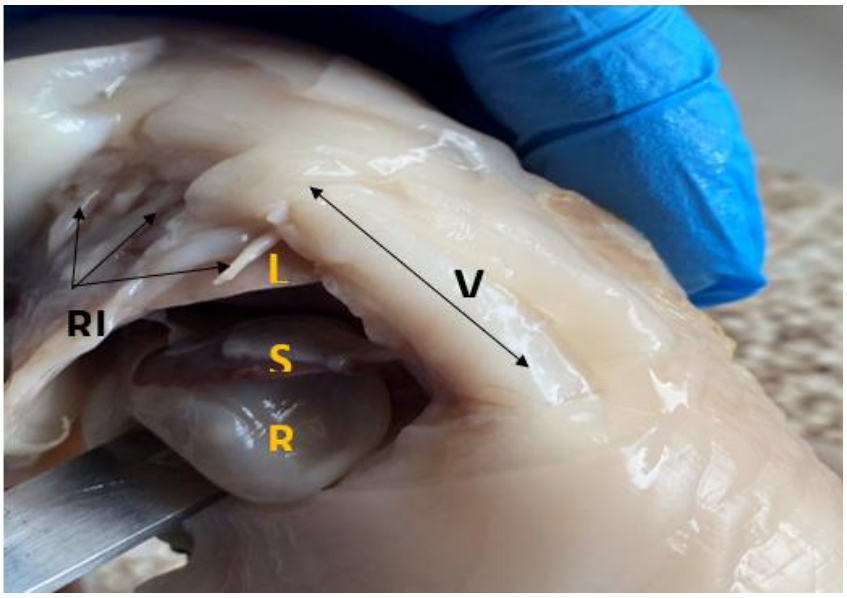

Figure 7. Photograph at 90-100 days of gestation. Spleen (S), lung (L), rumen (R), vertebral column (V), and ribs (RI)

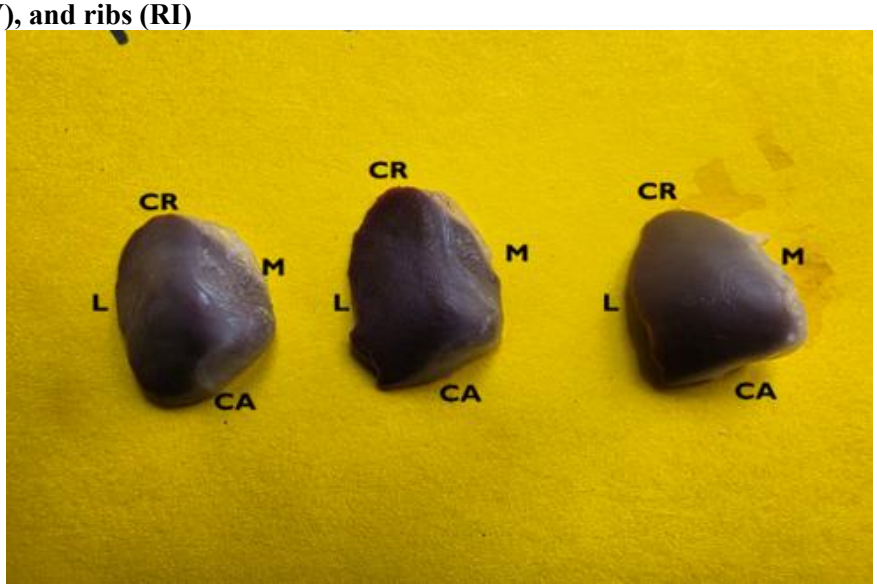

Figure 8. Photograph at 90-100 days of gestation. Parietal surface of spleen with cranial extremity (CR), caudal extremity (CA), lateral border (L), and medial border (M)

The current study consistent with Gupta et al. (1979) had observed that the parietal surface of spleen was convex and adherent to the diaphragm. Whereas, the visceral surface was concave and was related to the rumen in sheep. Mehta et al. (2016) noticed that the spleen of Chotanagpuri sheep was in the range of 10-13th ribs in all age groups. While, Kumar et al., (2011) observed remarkable increase of all gross dimensions of buffalo spleen after mid gestation. Microscopically by mid-gestation, the spleen exhibited advanced structural organization characterized by a fully trabeculated architecture surrounded by connective tissue capsule and well-differentiated cellular components. Ther was an increase in capsule thickness (11.36±0.84 µm) (Table 2). The splenic trabeculae consisted of dense connective tissue composed mainly of collagen and elastic fibers, fibroblasts, and smooth muscle cells. Subcapsular and peritrabecular sinuses were also evident (Figure 9). These findings are consistent with Jaji et al. (2019). They reported that trabeculae were formed and extended into the splenic parenchyma of camels during the second growth phase (5-7 months). However, they are not consistent with Gupta et al. (2017), who observed the appearance of trabeculae at 102 days of gestation days in goat fetuses.

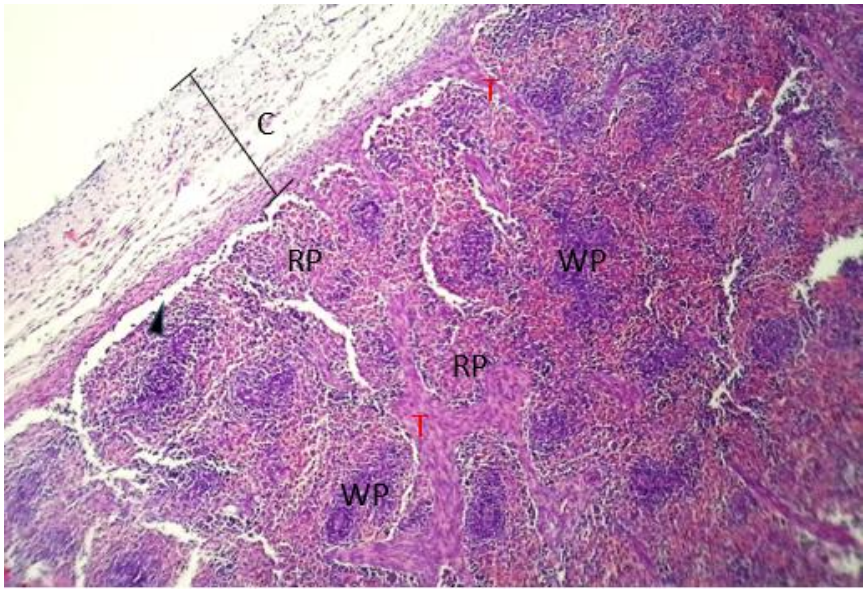

Figure 9. Cross histological section of spleen at 90-100 day of gestation. Capsule (C), supcapsular sinuses (black arrow), trabecula (T), white pulp (WP), and red pulp (RP). (H&E stain 4X)

At this developmental stage, a progressive accumulation of lymphocytes was observed in both the white and red pulp regions. The white pulp was characterized by a central arteriole surrounded by lymphocytes, macrophages, and reticular cells (Figure 10). While, the red pulp consisted of lymphocytes, reticular cells, plasma cells, and erythrocytes of varying sizes, along with the presence of some isolated lymphoid follicles lacking central arterioles. These follicles were enclosed by layers of collagen and reticular fibers (Figure 11). These findings are in agreement with Marwa-Babiker et al. (2023). They reported that in camels during the second trimester of gestation, the splenic capsule and trabeculae consisted of thick, dense, irregular connective tissue containing abundant collagen and smooth muscle fibers. Similarly, Malik et al. (2014) observed in goats that the splenic capsule became thickened, condensed, and fibrous with

the presence of smooth muscle cells, and that distinct trabeculae extended into the interior of the spleen.

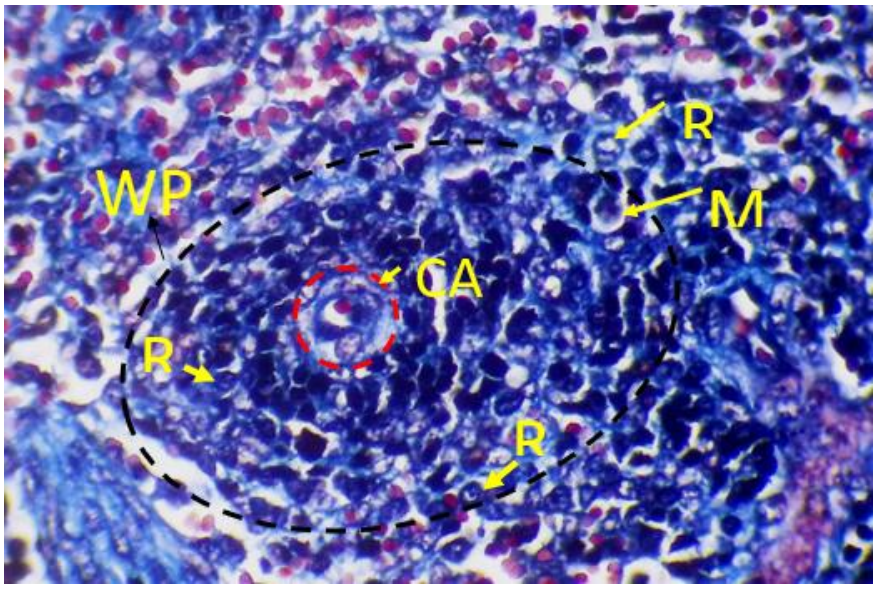

Figure 10. Histological cross section of spleen at (90-100) day of gestation. Central arteriole (CA), white pulp (WP), reticular cell (R), and macrophage (M). (Masson trichome stain 40X)

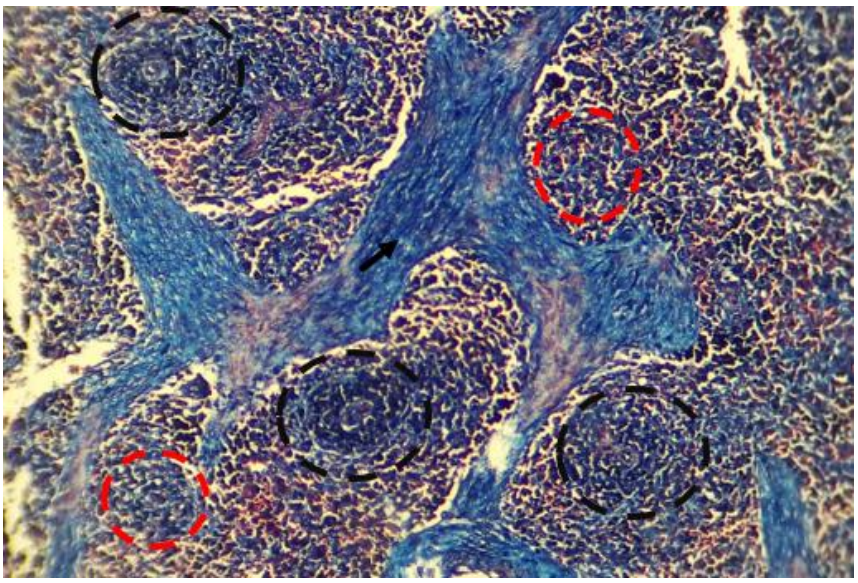

Figure 11. Cross histological section of spleen at 90-100 day of gestation. White pulp (black circle), pertrabecular sinuses (black arrow) and lymphoid follicle (red circle). (Masson trichome stain 10 X)

By mid-gestation (90-100 days), significant development of splenic architecture was evident. White pulp structures appeared clearly, with a relatively high number (21.4 ± 1.12), and their diameters and other measured values increased markedly (63.4 ± 1.14 µm). Trabeculae and capsule thickness also increased significantly compared to the earlier stage. This stage represents a critical period of structural differentiation, during which the spleen begins to establish its functional components, particularly those related to fetal hematopoiesis. Scanning electron

microscopy revealed that spleen exhibited a more organized and defined surface architecture compared with first trimester of gestation composed of capsule and parenchyma with cellular component. Where the capsule appeared as a thick dense fibrous layer with a relatively smooth wavy outer surface. This indicates the presence of elastic connective and collagen fibers beneath the surface and the primitive trabeculae originated gradually from capsule to extended into the parenchyma (Figure 12). In this stage the capsule progressively thickened with advancing gestation due to collagen deposition and differentiation of smooth muscle cells.

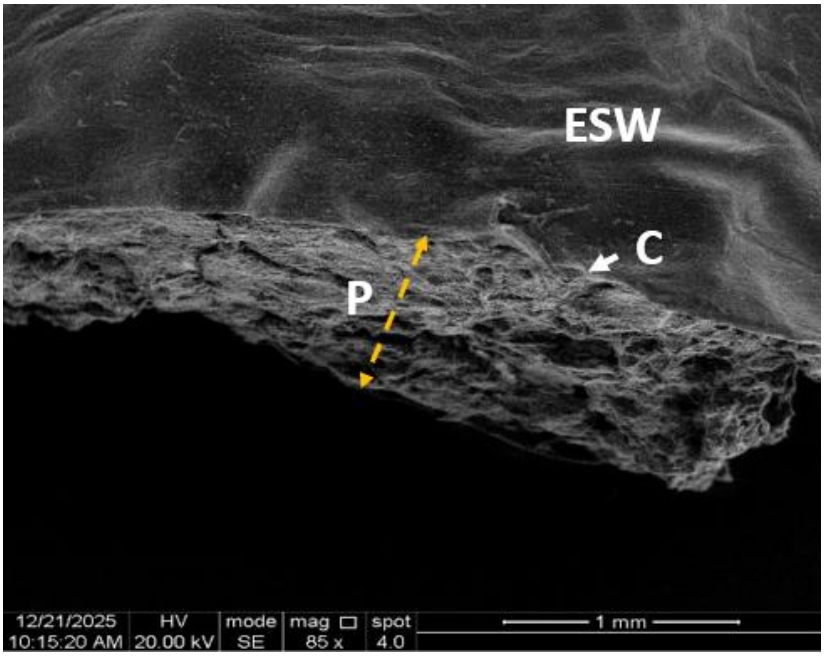

Figure 12. Scanning in electron microscope of spleen at second-trimester of gestation of local Awassa sheep foetuses. Thick dense fibrous capsule (C) and external smooth, wavy surface (ESW) and parenchyma (P)

The cellular elements appeared more uniformly distributed, indicating progressive differentiation of different types of cellular components in this stage of development to appear consist of different sizes of lymphocyte, macrophage, erythrocyte and reticular cells. Intercellular spaces became more prominent, indicating enhanced cellular migration and improved stromal arrangement within the tissue. These morphological changes signify the progressive establishment of the splenic microenvironment, which is essential for supporting lymphoid tissue development and maturation (Figure 13). In camel the second a well-developed network of reticular cells was established, housing dendritic macrophages, lymphoblasts, and varying populations of medium and small lymphocytes (Marwa-Babiker et al. (2023). By the late gestational stages, the reticular scaffold of the splenic white pulp was distinctly evident. It reflected advanced structural differentiation of the fetal camel spleen. While, in human, the splenic primordium was seen in the development stage of its primary vascular reticulum. It was mainly composed of mesenchymal tissue. The specific organization of the spleen was not yet identifiable. The area under the capsule was infiltrated by itinerant cells, chiefly erythrocytes, and their precursors. Whereas, the central areas were densely packed by argyrophilic reticular fibers.

Mesenchymal cells were with an irregularly shaped euchromatic nucleus and had a narrow rim of marginal heterochromatin according to Haldar et. al. (2021).

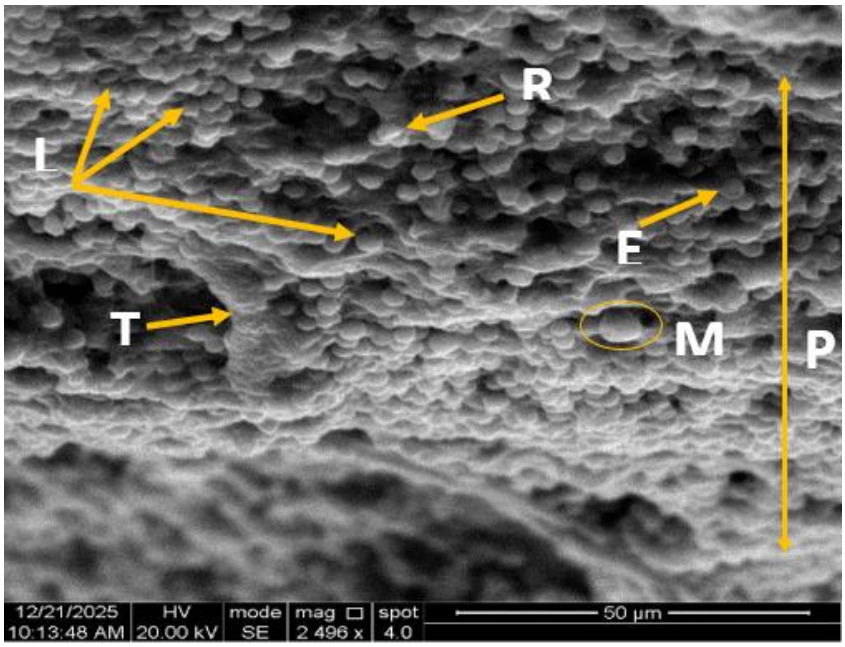

Figure 13. Scanning electron microscope of spleen at second-trimester of gestation. Cellular components are different size of lymphocyte (L) (small, middle and large) with macrophage (M), reticular cell (R), erythrocyte (E), and trabeculae (T)

Observation during third trimester (days 130-140): During the later stages of gestation, the average body weight of the sheep fetuses was 4870±121 gram, and crown rump length was 403±9.40 mm. The spleen grew significantly compared to earlier stages, the spleen weight (2.59±0.09 gram), volume (2.35±0.24 cm3), length (30.5±0.36 mm), width (20.9±0.80 mm) and thickness (9.97±1.1 mm) (Table 1). Morphologically, the spleen of the sheep fetuses was relatively larger and more development and growth with dark in color than that of the previous fetal group. However, its anatomical location remained nearly identical to that observed in the preceding group, attach to gastric tube. The spleen was fixed in position by two ligaments formed by peritoneal reflection, the phrenic splenic ligament, and gastro-splenic ligament (Figure 14). The triangular spleen exhibited three distinct angles: the cranioventral, caudodorsally, and caudoventrally angles. The cranioventrally angle was located in close proximity to the ruminoreticular groove. While, caudoventral angle highly relationship with hillus area and the caudodorsally related with vertebral column and the spleen was obliquely oriented irrespective of the long axis of the fetal body at third trimester of gestation (Figure 15). The findings are consistent with previous results (Chaurasia et al, 2019), which reported that the spleen in gout was present in close apposition to dorsal curvature of the rumen and report that the spleen in gout situation among growth diaphragm and the left dorsocranial surface of the rumen. At late gestation (130-140 days), the spleen parameters continued to increase significantly, reaching 2.59 g in weight, with dimensions of 30.5 mm in length, 20.9 mm in width, 9.97 mm in thickness, and a volume of 2.35 cm^3. Body weight also increased markedly to 4870 g. These findings indicate advanced maturation of spleen, with enhanced structural organization and functional readiness

prior to birth (Table 1). Histological these histological features indicate that the prenatal spleen is functionally competent at this stage.

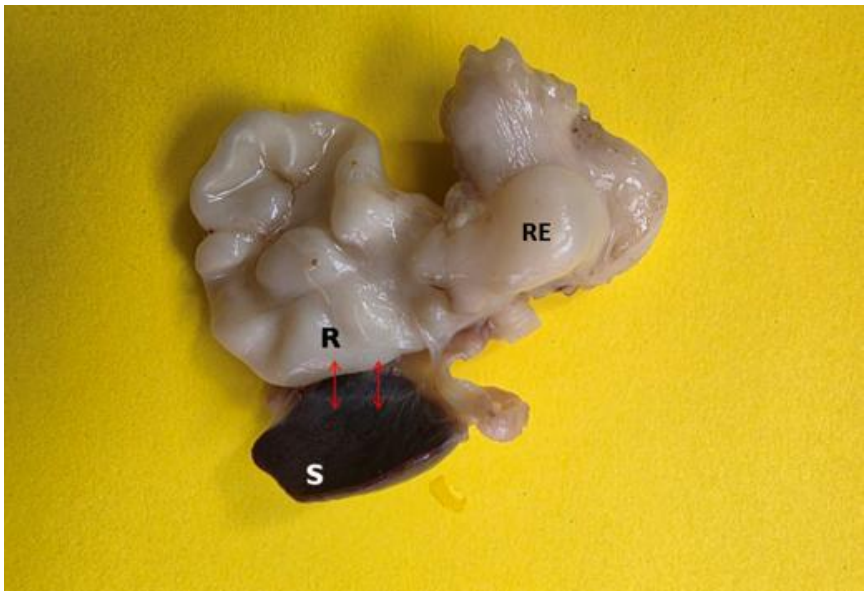

Figure 14. Photograph at 130-140 days of gestation. Rumen (R), reticulum (RE), and gastro-splenic ligament (red arrow) of spleen (S)

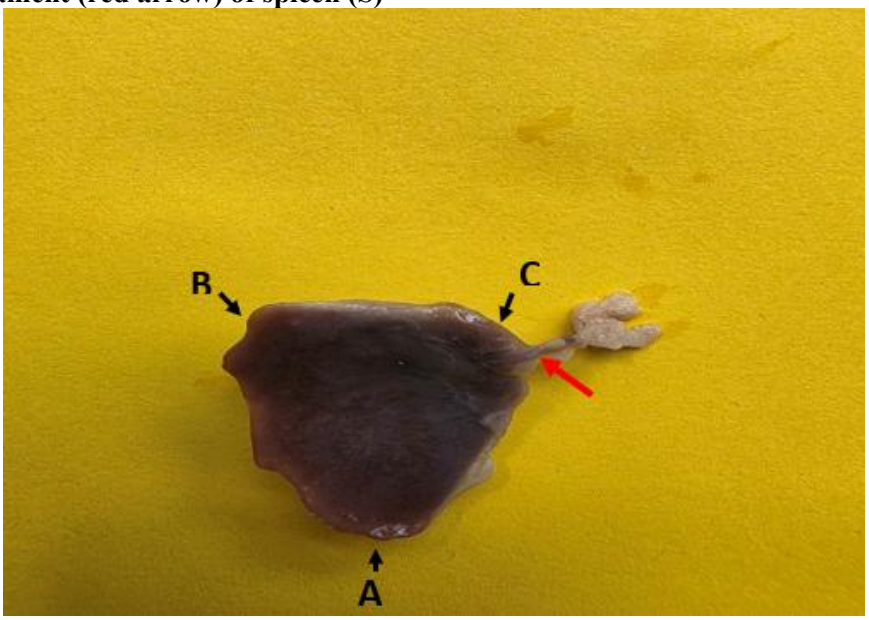

Figure 15. Photograph at 130-140 days of gestation. Three distinct angles. The cranioventral (A), caudodorsal (B), and caudoventral angles (C), and hillus area (red arrow)

Moreover, the spleen exhibits an advanced level of histological differentiation and organogenesis, characterized by an increased number of white pulp and red pulp, all surrounded by a relatively thick connective tissue capsule compared with earlier stages. With the progression of gestation and fetal growth, reticular fibers become more consolidated, appearing thick and continuous within both the stromal framework and the parenchyma. All the splenic structures appear more development with advancement of gestation and become relatively similar to that in postnatal periods and consist of the splenic capsule, trabeculae, red pulp, white pulp and with

present of cellular components and increased in trabecular network (The primary, secondary, and tertiary trabeculae were surrounded by peritrabecular blood sinuses). Muscular component to exhibiting a marked increase in the deposition of collagen, along with elastic and reticular fibers (Figure 16).

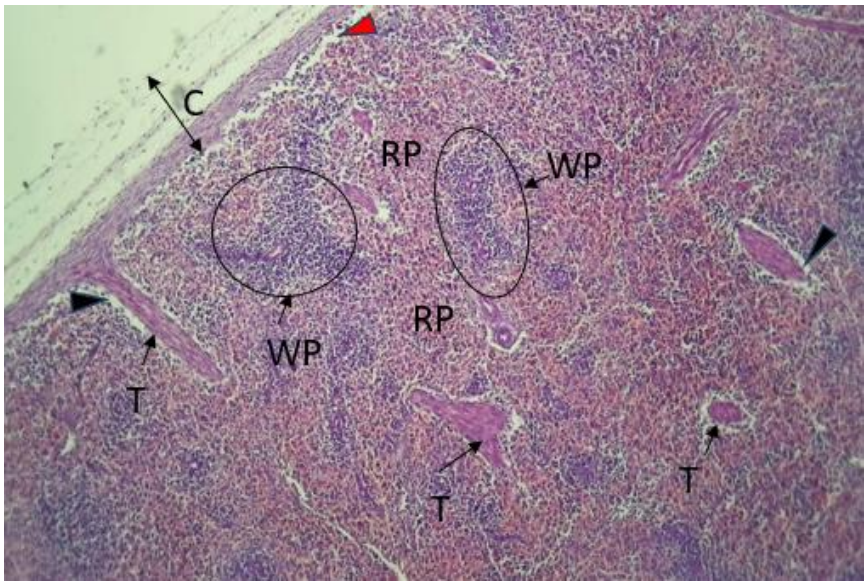

Figure 16. Cross histological section of spleen at 130-140 day of gestation. Capsule (C), type of trabecula primary (T1), secondary (T2), and tertiary trabeculae (T3), white pulp (WP) and red pulp (RP), subcapsular sinuses (red arrow) and peritrabecular sinuses (black arrow) (Masson trichome stain 4X). (H&E stain 4X)

This result is similar to the findings of Gupta et al. (2017) in goats, Kandil et al. (2025) in sheep, and Onu et al. (2016) in cattle, reported that the splenic capsule becomes increasingly fibrous with abundant collagen and reticular fibers. A clear differentiation between white and red pulp cells is observed by the third trimester of gestation. the pervious results agreement with Gupta et al. (2017) and Nishant et al. (2018) in gout who reported that during the third stage of gestation, the splenic trabeculae showed branching and anastomosing within the parenchyma, with variable thickness in different areas. They composed of fibroblasts, smooth muscle fibers, blood vessels, and nerves. The white pulp was characterized by one or two central arterioles surrounded by different sizes of lymphocytes along with the without presence of isolated lymphoid follicles (Figure 17). During the final prenatal growth phase, blood sinusoids were observed dividing the red pulp parenchyma into splenic cords. The parenchyma was further partitioned by secondary trabeculae that were irregularly distributed within the red pulp (Figure 18). These findings correspond with Nishant et al. (2018) in gout, Arey (1958) and Mc Geady et al. (2009) in mammals and bovines, respectively. They mentioned that principal structure of fetal spleen composed of capsule, trabeculae, white and red pulp and blood vessels. At late gestation (130-140 days), further significant increases were observed in all parameters. The diameter of white pulp increased, and trabeculae thickness reached higher values (24.2 ± 0.86 µm), indicating enhanced internal organization. Capsule thickness also increased significantly (34.6 ± 1.14 µm), reflecting improved structural support. These findings suggest that the spleen at this stage has

achieved a more advanced level of maturation, preparing for its postnatal functional role (Table 2).

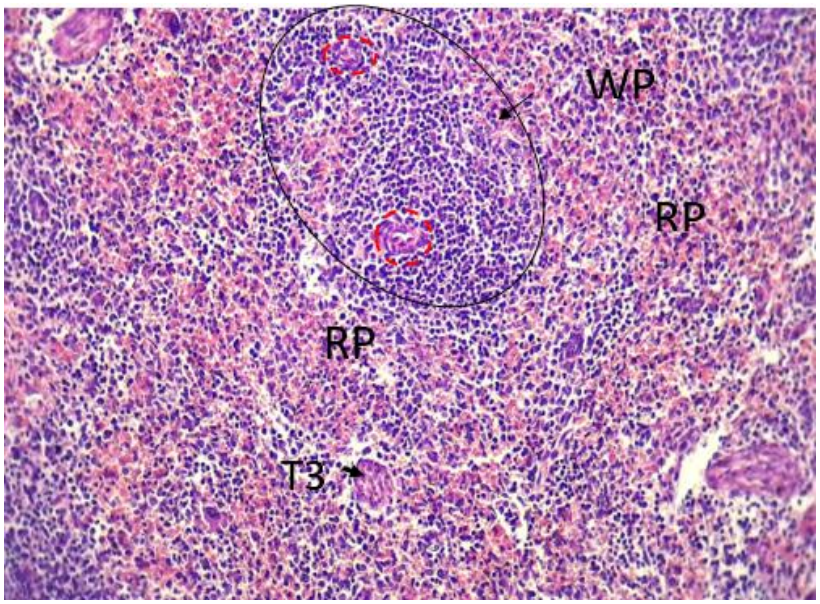

Figure 17. Cross histological section of spleen at 130-140 day of gestation. The white pulp (WP) with central artery (red circle), red pulp (RP), and tertiary trabecula (T3). (H&E stain 10X)

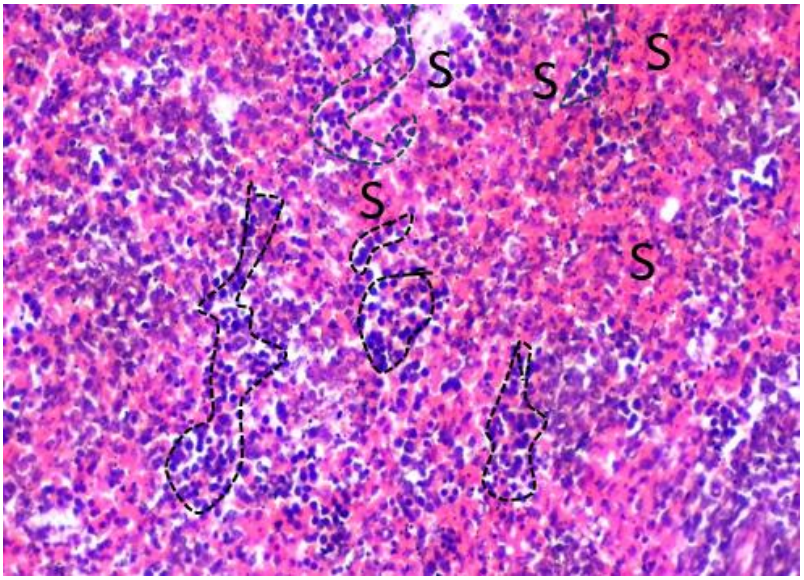

Figure 18. Cross histological section of spleen at 130-140 day of gestation. Red pulp with splenic cord (Dashed line) and splenic sinuses (S). (H&E stain 20X)

Scanning electron microscopy appear splenic structure exhibited a well-developed organs and cellular component. The organ was enclosed by a thick fibrous capsule relatively forming the outer boundaries that envelope of spleen at 130 -140 days of gestation. At fractured or cut edges of spleen, the capsule showed the trabeculae were occasionally observed extending from the capsule into the parenchyma where the trabeculae appeared as irregular fibrous bands projecting inward from the capsule, forming a supporting framework within the splenic tissue with subscapular sinus. These structures varied in thickness and exhibited a coarse surface due to

densely packed connective tissue fibers (Figure 19). The parenchyma appeared a clear distinction between red and white pulp in late stage of gestation composed of the red pulp composed of primitive vascular network with high erythrocytes. While, the white pulp appeared consist of lymphoid aggregations of lymphocytes, large macrophage and irregular reticular cells surrounding the central arteries (Figure 20).

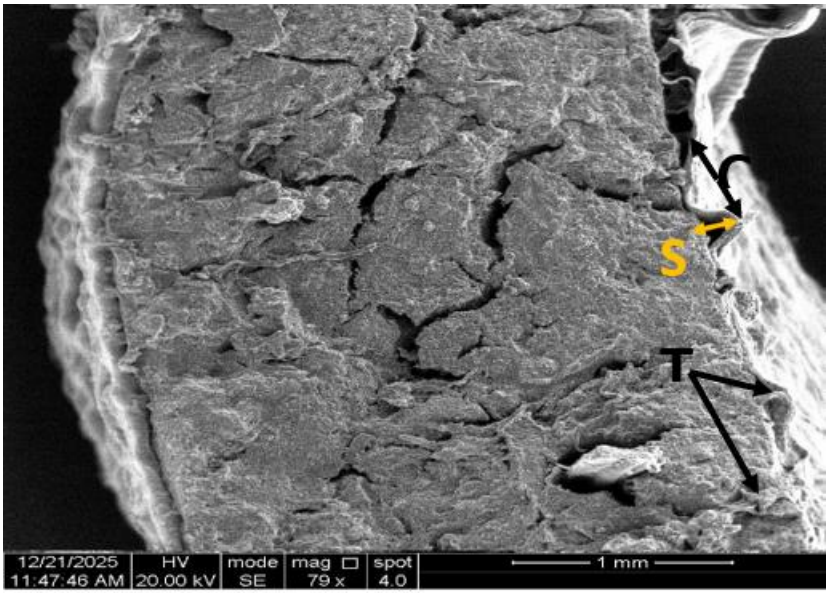

Figure 19. Scanning electron microscope at third-trimester of gestation of middle part of spleen of local Awassa sheep foetuses. Thick layer of capsule (C), subcapsular sinus (S) with trabeculae (T) as band extend from capsule

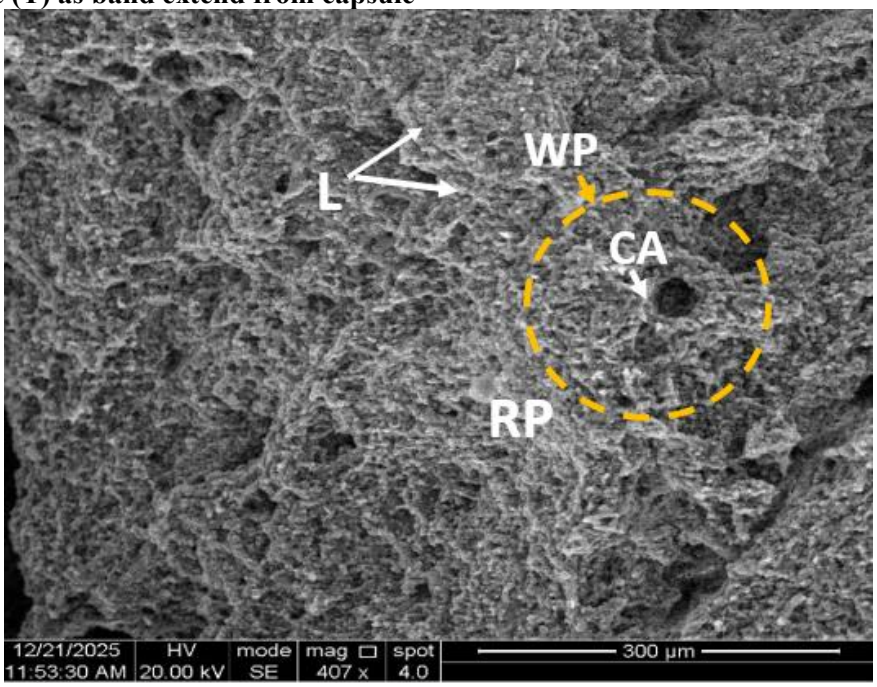

Figure 20. Scanning electron microscope at third-trimester of gestation of spleen of local Awassa sheep foetuses. Clear distinction between red pulp (RP) and white pulp (WP) with central artery (CA) and aggregation of different size of lymphocytes (L)

In camel, reticular cells formed a network where dendritic macrophages and lymphoblasts were present (Marwa-Babiker et al. (2023). In addition, medium-sized and tiny lymphocytes were seen. In humans, at third trimester, the spleen closely resembles the adult form, with a thick, well-defined capsule and trabeculae that partition the parenchyma into lobules, alongside clearly developed red and white pulp (Haldar et. al., 2021).

Novelty: Present report and finding can advanced several aspects of the spleen development and offered several markers to be considered during effective assess of animal health and production potential.

Conclusions: This study concluded that the spleen in sheep undergoes distinct age-dependent morphological and histological changes during prenatal development. Morphologically, the spleen exhibited a gradual increase in size and weight with advancing gestational age, accompanied by progressive maturation of the connective tissue capsule and trabeculae, with increasing deposition of collagen, elastic, and reticular fibers. By late gestation (130-140 days), the spleen exhibits advanced histological differentiation, with clearly defined red pulp, white pulp, and vascular structures. Scanning electron microscopy confirms the transition from a poorly organized primitive structure to a well-developed organ with distinct microarchitecture.

Authors contributions

Aisha Ayoub Essa and Jafar Ghazi Abbas confirm contribution to the paper equally.

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical considerations

The experimental procedures were approved by the Institutional Animal Ethics Committee and conducted at the Veterinary Medicine College, Al-Qassim Green University, Iraq. Ethical regulation of this work was assigned by the university research committee and adhered to the guidelines of the American Veterinary Medical.

Conflict of interest

The authors have no conflict to declare.

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پویایی‌های تکاملی مورفوژنز و هیستوژنز طحال در جنین‌های گوسفند آواسی بومی عراق: یافته‌هایی از میکروسکوپ نوری و الکترونی روبشی

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چکیده

هدف: طحال در دوران جنینی نقش مهمی در خون‌سازی ایفا می‌کند. در مرحله رشد کبدی، طحال همراه با کبد در تولید سلول‌های خونی مشارکت دارد و در مرحله میلوئیدی نیز همراه با کبد و مغز استخوان در خون‌سازی نقش دارد. همچنین تخریب گلبول‌های قرمز پیر، لنفوسیت‌ها و ترومبوسیت‌ها در طحال انجام می‌شود. هدف این مطالعه بررسی تکامل مورفولوژیک و بافت‌شناسی پیش از تولد طحال در گوسفندان آواسی بومی عراق با استفاده از میکروسکوپ نوری و الکترونی روبشی بود.

مواد و روش‌ها: تعداد ۳۰ نمونه طحال از میش‌های آبستن سالم نژاد آواسی در مراحل مختلف آبستنی جمع‌آوری شد. سن جنین‌ها با استفاده از معادله طول فرق سر تا دنبالچه (CRL) تعیین گردید و نمونه‌ها به سه گروه تقسیم شدند: گروه اول ۵۰ تا ۶۰ روز، گروه دوم ۹۰ تا ۱۰۰ روز و گروه سوم ۱۳۰ تا ۱۴۰ روز. برای بررسی با میکروسکوپ الکترونی روبشی (SEM)، قطعات کوچکی از طحال (حدود ۱ میلی‌متر مکعب) تثبیت شدند. از SEM برای مشاهده سطح طحال در بزرگنمایی‌های مختلف استفاده شد. تحلیل داده‌های مورفولوژیک و هیستولوژیک با استفاده از نرم‌افزار SPSS نسخه ۲۴ انجام گرفت.

نتایج: در گروه اول، طحال به صورت لکه خونی کوچک متمایل به قرمز مشاهده شد و سپس رنگ آن قرمزتر و منسجم‌تر گردید. بررسی‌های میکروسکوپ الکترونی روبشی و هیستولوژی نشان داد که سطح طحال نابالغ بوده و قشر و ترابکول‌ها هنوز به‌طور کامل تکامل نیافته‌اند که بیانگر مراحل اولیه تمایز طحال است. در گروه دوم، اندازه طحال افزایش یافت و ترابکول‌ها از کپسول به پارانشیم

امتداد پیدا کردند. همچنین تمایز واضح پالپ سفید و پالپ قرمز مشاهده شد که نشان‌دهنده بلوغ تدریجی ساختار طحال بود. در گروه سوم، طحال با پیشرفت دوره آبستنی تکامل بیشتری یافت و از نظر ساختاری تا حد زیادی مشابه طحال پس از تولد شد. این ساختار شامل کپسول طحال، ترابکول‌ها، پالپ قرمز و پالپ سفید همراه با اجزای سلولی مشخص بود و افزایش شبکه ترابکولی در بررسی‌های میکروسکوپ نوری و الکترونی مشاهده شد.

نتیجه‌گیری: یافته‌های این مطالعه بلوغ تدریجی طحال در دوران پیش از تولد را نشان داده و نقش حیاتی آن را در تکامل سیستم ایمنی برجسته می‌سازد. هرگونه انحراف از این الگوهای طبیعی ریزساختاری ممکن است نشان‌دهنده اختلالات پاتولوژیک یا ایمنی‌شناختی باشد.

کلمات کلیدی: تکامل پیش از تولد، جنین‌ها، طحال، گوسفند عواسی، SEM

نوع مقاله: پژوهشی

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