Special Issue:
Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Morphological and Histological Investigation of Prenatal Development of Spleen in Local Awassi Sheep Fetuses

Sabreen M. Al-Janabi[1], Jafar Ghazi Abbas Al-Jebori[1*], Ahlam JH Al-Khamas[1], Salim Salih Ali Al-Khakani[1], Ekhlas Abid Hamza Alalwany[1], Mohammed Salah Hasan Simawy[1], Osama Murtadha[1], Mustafa Fadhil[1], Dunia M. Al-Rubaie[1]

[1]Department of Veterinary Anatomy, Veterinary College, Qasim Green University, Qasim, Babylon, Iraq; [2]Department of medical physics, Hilla university college, Babylon, Iraq.

Abstract | This study aims to investigate morphological and histological changes in spleen of the fetuses of local Awassi sheep. Twelve samples were collected from healthy female's uterus during perinatal period. In order to record the spleen's developmental alterations, animals were grouped into Group I (50-55), Group II (70-75), and Group X (130-140) days of pregnancy based on age using the crown rump length formula (CRL). The spleen in group I was still developing as it was not fully formed, had a flat surface, and looked like a bloody batch. Histologically, it displayed two regions: the cortex and the medulla, which were covered in a capsule made of undifferentiated red and white pulp. The left side of group II is occupied by the spleen, which has an oval-elongated form and is more developed than in the preceding stage. the thoracic cavity with two surfaces and a border. Histologically, the cortex is clearly visible, with a layer of simple squamous epithelium mesothelial cells covering the splenic capsule, which contained the irregular connective tissue and parenchyma covering the organ. In comparison to the first stage, red pulp is separated from white pulp. Under a microscope, the spleen in group- II appears to be made up of trabeculae with sub-capsular and peri-trabecular sinuses lined by endothelium, and a visceral surface that was concave with the anterior half of it primarily attached to the dorsal curvature. The parietal surface was convex and attached to the diaphragm with only a small dorsal area. The spleen's whitish pulp was made up of he red pulp contains peri-arterial lymphatic sheaths (PALS) and splenic nodules. These histological changes in the developmental stage propose the healthy characteristics and any deviation from these changes may offer a clue of pathological alteration.

Keywords | Development, Fetuses, Spleen, Prenatal, Sheep

Received | August 22, 2025; **Accepted** | .October 22, 2025; **Published** | October 28, 2025
***Correspondence** | Jafar Ghazi Abbas Al-Jebori, Department of Veterinary Anatomy, Veterinary College, Qasim Green University, Qasim, Babylon, Iraq; **Email:** jafar@vet.uoqasim.edu.iq
Citation | Al-Janabi SM, Al-Jebori JGA, Al-Khamas AJH, Al-Khakani SSA, Alalwany EAH, Simawy MSH, Murtadha O, Fadhil M, Al-Rubaie DM (2025). Morphological and histological investigation of prenatal development of spleen in local Awassi sheep fetuses. J. Anim. Health Prod. 13(s1): 695-700.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.695.700
ISSN (Online) | 2308-2801

INTRODUCTION

The spleen is large a soft, highly vascularized secondary lymphoid organ involved in immune surveillance, hematopoiesis, and the filtration of blood. It is non-essential for life but plays a critical role in immune function, including antigen processing and the removal of senescent red blood cells (Guyton and Hall, 2021). Encased in connective tissue, the spleen is separated into two functional areas: The red pulp, which filters blood,

and the white pulp, which houses immune cells. With minor differences in size and location between species, it is situated in the upper left quadrant of the abdominal region and is connected to the abdomen, kidney, diaphragm, and colon in mammals (Moore *et al*., 2023). According to histology, the spleen is made up of the following: White pulp; lymphoid tissue arranged around central arterioles, which mostly contains B-cells in follicles and T-cells in the periarteriolar lymphoid sheaths (PALS); Finally, macrophages are abundant in the marginal zone, which is where the white and red pulp meet. and specialized for antigen trapping. The red pulp is thought of as a network of splenic cords and sinusoids filled by macrophages, reticular cells, and blood components (Mescher, 2021).

The purpose of this work was to describe the histomorphometric and developmental analysis of the spleen in prenatal local Awassi sheep fetuses (*Ovis aries*).

MATERIALS AND METHODS

Twelve sheep fetuses from pregnant ewes killed at the Najaf provinces' slaughterhouses and were used in the study. The fetuses' ages are calculated using the formula for crown rump length (CRL) below (Y=2.74X+30.15) where 'Y' is developmental age of fetus in days and 'X' is the crown rump length in cm according to (Niyf and Al-Jebori, 2024). After fixing in a 10% formalin solution, the spleen of sheep fetuses was dehydrated in an advanced sequence of alcohols, cleaned in xylene, and then embedded in paraffin wax. A rotary microtome was used to section the blocks at a slice thickness of 5–6 um. Hematoxylin and Eosin stain, trichrome mason's stain, and periodic acid schiff were used to stain histological sections (PAS) (Suvarna *et al*., 2018).

RESULTS AND DISCUSSION

Observation during first trimester of pregnancy (days 50–55)

The morphometric measurement during the 50–55 day first trimester of pregnancy, the body weight of sheep foetuses was about 38.4±1.03 grams and crown rump length was10.2±0.409 cm, the weight of spleen about was 0.015±0.005 mg; length (3.602± 0.467) and thickness was about 0.948± 0.155 (Table 1 and Figure 1A). The finding of current study didn't corresponding with Jaji *et al*. (2019) in camel who mentioned that the fetal spleen's first phase was measured to be 7.3 ± 2.4 cm long, 2.6 ± 0.6 cm wide, and 6.5 ± 4.0 g in weight.

In terms of morphology, the spleen is a tiny, bloody batch on the dorsal surface of the developing stomach in sheep fetuses. It is regarded as a lymphoid organ in the early stages of pregnancy and appears reddish in color. The spleen is located on the surface of the gut tube, extending to the partial surface of the first part from the small and large intestine in the upper left section of the liver and rib cage (Figure 1B). These findings concurred with those of both Nishant *et al*. (2018) and Gupta *et al*. (2017), but not with Chaurasia *et al*. (2019). The spleen in goats was creamish in color, and as development advanced from 58 days (9.2 cm CRL) onward, it turned reddish brown as a result of the development of hemopoietic areas in the splenic parenchyma. In camels, however, the spleen at this stage of pregnancy had a C-shape and was dark brown in pigmentation. These variations in size, shape, and morphometric measurements of the spleen because of variations in animal breed, species, and gestational periods.

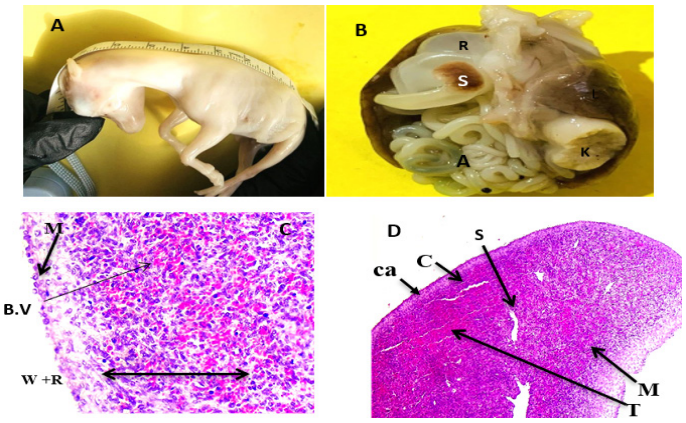

Figure 1: A show a picture demonstrating the crown measurement technique, B: Photograph at 50–55-day prenatal gestation showing spleen (S), rumen (R) and liver (L), small +large intestine(A), kidney (K), C: Histological section at 50-55-day gestation prenatal sheep showing blood vessels (B. V) white and red pulp (W.R). Mesothelial (M) H&E stain (X10) D- Histological section at 50-55-day gestation prenatal sheep showing: capsule (ca) cortex (C), sinus(S), trabecula (T) and medulla(M) H&E stain (X4).

Table 1: Crown rump length, weight of embryo and spleen, length and thickness spleen of sheep foetuses in different ages of embryonic life.

Parameters age	Grown rump length	Body weight foetuses	Spleen weight (gram)	Spleen length (mm)	Spleen thickness (mm)
(50-55) days gestation	10.2±0.409 A	38.4±1.03 A	0.015±0.005 A	3.602±0.46 A	0.948±0.155 A
(70-75) days gestation	18.2±0.483 B	246±2.90 B	0213±0.033 B	13.054±1.148 B	3.600±0.391 B
(130-140) days gestation	40.4±1.776 C	1360±3.53 C	1.500±0.334 C	26.792±1.153 C	6.308c±0.263 C

The values show the mean ±S.E. Significant differences (P ≤ 0.05) across various groups are indicated by distinct capital.

Histologically, the spleen appears as a mass of undifferentiated mesenchymal cells that separate into a capsule that is roughly 20±1.1) µm in thickness and a parenchyma that is 250±3.7) µm. The parenchyma region was undifferentiated into white and red pulp, and the capsule was made of irregular connective tissue (Figure 1C). Primitive trabecula appeared to stretch from the capsule to the parenchyma, and blood vessels with sinuses were found beneath the capsule region (Figure 1D). The current work contradicts Devi *et al*. (2016), who reported that the spleen was covered by a thick fibro-elastic muscular capsule that was invested by the serous peritoneal covering. The capsule was made up of smooth muscle fibers as well as collagenous, elastic, and reticular fibers. At the same time, branching trabeculae broke out of the capsule, invaded the splenic parenchyma, and split into smaller compartments.

Observation during the 70–75-day second trimester of pregnancy

The spleen weight was 18.2±0.483 grams, the body weight of sheep fetuses was around 246± 2.90 kilos, the crown rump length was 10.2±0.409 cm, and the morphometric measurements included the spleen weight, which was approximately 213±0.033 grams; the length was 13.054b±1.148 and the thickness was 6.308 ± 0.263 (Table 1 and Figure 2A). A picture demonstrating the crown measurement technique that the mean spleen weight, width, and length in goats at They were 13.08±0.74 mm, 8.95±0.32) mm, and 0.25±0.12 grammes at mid-gestation, respectively, and that the variations in parameters were caused by species differences and variations in the gestation stages.

Morphologically, the spleen is described as possessing two extremities (cranial and caudal), two borders (lateral and medial), and two surfaces (parietal and visceral) The partial surface of the spleen appears concave, while the visceral extremity is convex and the cranial extremity is located close to the rumen's cranial surface and appears semi-rounded. The caudal border of the spleen extended past the rib cage's edge and reached up to the level of the second lumbar vertebrae, with the caudal border shifting more towards the dorsal end (Figure 2B). According to Malik *et al*. (2001), in goats, the spleen's development was seen near the cranial sac of growing rumen, right below the upper end of the last left rib. In sheep fetuses, the spleen was moved slightly to the left of the cranial sac of forming rumen at 103 days of gestation.

The spleen was dark reddish brown and had distinct surfaces, resembling an oyster shell. edges. At 110 days of gestation, the cranial boundary was noticeably thicker than the other three borders, and all borders had sharp edges. The spleen's hue was significantly darker at 126 days of gestation than it had been at earlier ages. The cranial portion of the visceral surface was very concave at 143 days of gestation, while the caudal portion was almost flat. It was determined that the dorsal border extended caudally to a maximum extent beyond the 13th rib (Bhagyalakshmi and Balasundaram, 2021).

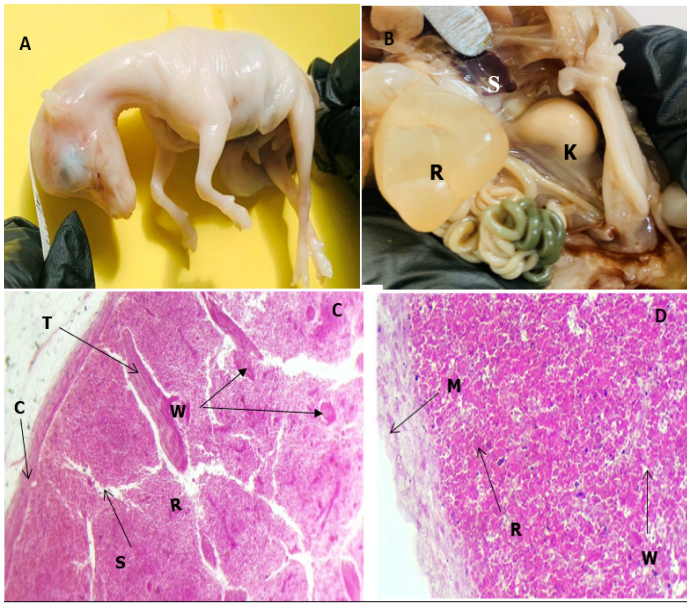

Figure 2: A: Photograph at 70-77 days prenatal gestation showing grown rump length at sheep foetuses, B: Photograph at 70-77day prenatal gestation showing spleen (s), rumen (R) and kidney (K), C: Histological section at 70-75 day gestation prenatal sheep showing sinuses (S), white pulp (W), Cortex (C), trabecula (T), red pulp (R) PAS stain (X20), D: Histological section at 70-75 day gestation prenatal sheep showing mesothelial (M).white pulp (W)red pulp .(R). Masson trichome stain (X10).

According to histological observations, the spleen appears to be covered with a clear capsule of about 20±0.34 µm made up of a layer of mesothelial cells of the splenic capsule's basic squamous epithelium. This layer contains the irregular connective tissue rise parenchyma that differentiates into white pulp and red pulp. Unlike the initial phase, where the red pulp appears near the capsule area and shows more development in this region trabecula than in the first trimester extended toward the center with the splenic cord, the sinuses are clearly visible with peritubular sinuses, blood vessels, and other cell types, and the white pulp appears in the center of the spleen in the medulla region (Figure 2C, D).

In the event that the spleen is injured, the mesothelial cells of the spleen in mammals may be a quick source of fibroblasts for wound healing. The trabeculae network that partially compartmentalizes the organ parenchyma originates from the capsule's connective tissue fibers, which also protect the splenic parenchyma (Corbin *et al*., 2008; Onkar and Govardhan, 2013).

Observation during third trimester of Gestation (130-140) days

According to morphometric measurements, sheep fetuses had an average body weight of 1360±3.53 grams, a crown rump length of 40.4±1.776 cm, and a spleen weight of around 1,500±0.334, a length of 26.792± 1.153, and a thickness of 6.308c ± 0.263 (Table 1 and Figure 3A). The spleen has four borders (anterior, posterior, dorsal, and ventral) and two surfaces: Visceral and parietal. With a tiny dorsal area, the parietal surface was convex and joined to the diaphragm. The visceral surface was concave, and the gastro-splenic ligament mostly connected the anterior part of this surface to the rumen's dorsal curvature. Dorsally, the anterior border was thick and straight.

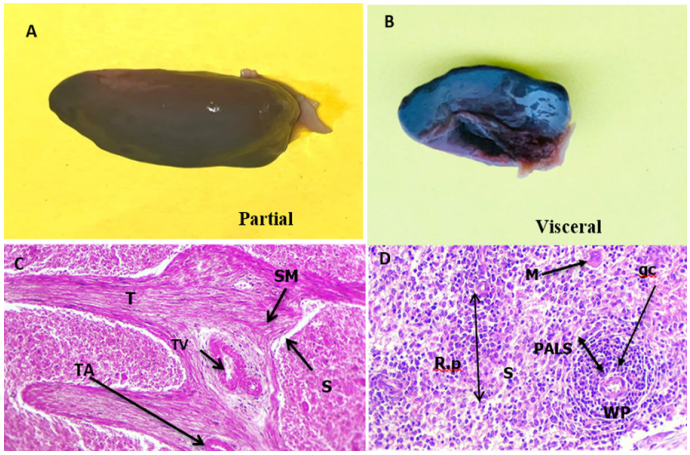

Figure 3: A: Photograph at (130-140) day prenatal gestation showing partial surface of spleen B-Photograph at (130-140) day prenatal gestation showing visceral surface of spleen C-histological section at (130-40) day gestation prenatal sheep showing sinuses (S), trabecula (T), Trabecular vein (TV) Trabecular artery, smooth muscle (SM)H&E stain (X10) D-histological section at (130-140) day gestation prenatal sheep showing sinuses (S), Small nodules (S.N), (R.P), red pulp (RP), white), pulp (WP), germinal center (gc), central artery (CA), and peri-arterial lymphatic sheath (PALS) macrophage (M) H&E stain (X20).

The dorsal boundary was thick in front and small in the back. The ventral border was somewhat rounded caudally and thinner than the dorsal border. The ventral and posterior boundaries were loose and slender. As gestation progressed, Hilus advanced toward the transverse groove of the rumen and was positioned on the visceral surface, near the anterior dorsal angle of the spleen, which was comparatively more rounded. in the goat's last stage of pregnancy (Figure 3B). These findings concurred with those made by Nishant *et al.* (2018) in goat fetuses demonstrating that the cranial of the visceral surface's part was very concave, whereas the caudal portion was almost flat. The spleen's dorsal border extended caudally as far as possible past the thirteenth rib. Histologically, Microscopic analysis showed that the spleen of lambs was made up of parenchyma and stroma. The capsule and trabeculae that penetrate the parenchyma are the primary components of stroma. This organ's capsule is made up of two layers: The inner sub-serosal layer is made up of irregular, dense connective tissue that is interfused with smooth and elastic muscle fibers, and the outer serosal layer is made up of peritoneal mesothelial cells and simple squamous epithelium tissue. The average thickness of the capsule was 31±0.51 µm (Figure 3C).

Collagen fiber bundles of elastic and smooth muscle fibers make up the trabeculae, and the presence of these fibers in the capsule and trabeculae may aid in adjusting the spleen's volume and expelling extra blood from the bloodstream. Arteries and veins can enter the spleen through the lanes of passageways provided by the big trabecula. The vascular sheath of the splenic hilus, which was loaded with erythrocytes and encircled by connective and adipose tissue, was where the splenic arteries and veins were most visible (Figure 3C, D).

The peri-arterial lymphatic sheath (PALS) and splenic nodule, which were dispersed throughout the red pulp, made up the parenchyma of the spleen's white pulp. The splenic lymphatic nodules had a nearly spherical shape, with a germinal center in the middle and a macrophage-filled marginal zone surrounding it (Figure 3C, D). To improve the interaction between the PALS cells and antigens in the material of the closely similar red pulp, a morphological change is the macrophage that surrounds the single central artery at the white pulp's periphery. Its ability to protect the body from harmful organisms in the splenic blood will improve as a result.

The areas between the trabeculae that comprised the crimson pulp and the white pulp, which is made up of cellular cords and splenic sinusoidal capillaries. The central artery, also known as the nodular arteriole, held the paracentral location in the nodule. One discontinuous epithelium, which rests on the basement membrane that encircles the sinusoids in a discontinuous manner, limits the splenic sinusoidal capillaries. Cellular cords occupy the spaces between the sinusoidal capillaries (Figure 3C, D).

According to Zidan *et al.* (2000) in camels and Devi *et al.* (2016) in goats, the central artery situated in the paracentral position in the nodule is in agreement with the presence of sub-capsular and peritrabecular sinuses lined by endothelium in pigs and goats (Usende *et al.*, 2014).

CONCLUSION

In summary, the study reveals important facets of the development of the spleen in the first, second, and third trimesters of pregnancy in sheep embryos. It explains

modifications. in morphology, such as the appearance of separate splenic sections and the differentiation of their form and placement during gestation. Histological observations highlight the differences between the cortex and medulla, as well as the appearance and extension of trabeculae to the organ's center. By pointing out similarities and differences with comparable species, these findings advance our knowledge of secondary organ development in sheep. This understanding is essential for veterinary and medical research because it shapes our understanding of the spleen's anatomy and opens the door to more studies of the spleen's embryonic development.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of Al-Qasim Green University and thank all colleagues and staff who contributed to the success of this study.

NOVELTY STATEMENT

Present report and finding can advanced several aspects of the uterus development and offer several markers to be considered during effective assess of animal health and production potential.

AUTHOR'S CONTRIBUTION

MHH and JGAA-J confirm contribution to the paper equally.

Funding

No funding was received.

Availability of data and materials

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

Ethics approval and consent to participate

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

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Bhagyalakshmi J, Balasundaram K (2021). Gross morphometrical studies on spleen (*Ovis aries*) at various age groups of prenatal sheep. https://www.thepharmajournal.com/special

Chaurasia S, Menaka R, Rao TKS, Tyagi KK (2019). Prenatal gross morphometrical studies on spleen with special reference to prediction of foetal age in Surti goat (*Capra hircus*). Indian J. Anim. Res., 53(12): 1619-1623.

Chaurasia S, Rao TKS, Menaka R, Tyagi KK (2019). Gross morphometrical investigations of the spleen during pregnancy, specifically in relation to predicting the age of the fetus in Surti goats (*Capra hircus*). Indian J. Anim. Res., 53(12): 1619–1623. https://doi.org/10.18805/ijar.B-3707

Corbin E, Vicente J, Martin-Hernando MP, Acevedo P, Pérez-Rodríguez L, Gortazar C (2008). Spleen mass as a measure of immune strength in mammals. Mamm. Rev., 38(1): 108–115. https://doi.org/10.1111/j.1365-2907.2007.00112.x

Devi H, Mathur R, Joshi S (2016). Histological studies on the spleen of Marwari goat (*Capra hircus*). Vet. Practitioner, 17(2): 190-191.

Gupta V, Farooqui MM, Prakash A, Pathak A, Kumar P (2017). Morphological and cytological differentiation of goat spleen. Indian J. Anim. Res., 51(6): 1027-1032. https://doi.org/10.18805/ijar.v0iOF.7820

Gupta V, Archana P, Prakash A, Farooqui MM (2017). Goat spleen (*Capra hircus*) morphological and cytological distinction. J. Anim. Res. India, 51(6): 1027-1032.

Guyton AC, Hall JE (2021). Textbook of medical physiology. Elsevier.

Jaji AZ, Girgiri IA, Tomar P, Mahre MB, Saidu AS, Yawulda MP, Faruk DU (2019). Morphology, morphometry and histogenesis of the prenatal dromedary (*Camelus dromedarius*) spleen. Macedon. Vet. Rev., 42(2): 141–149. https://doi.org/10.2478/macvetrev-2019-0018

Lakshmi MS, Ramayya PJ, Bharathi S, Veeresh PG (2022). Gross morphological alterations in sheep (Ovis aries) heart development during pregnancy. Pharma Innov. J., 11(9S): 2584–2588. Retrieved from https://www.thepharmajournal.com/specialissue?ArticleId=15815andissue=9Sandvol=11and-year=2022

Malik MR, Pillai PVA, Parmar ML Taluja JS (2001). Morphohistogenesis and mode of growth of spleen in goat (*Capra hircus*). Indian J. Anim. Sci., 71(3): 204-207. Indian J. Anim. Sci., 71(3): 2001.

Marwa, A.M. Alkhodair,K.M., Abdelhay,M.A. Elseory,A.M., zolain, H.B., AL-Ramaden, S.Y. Althnaian, T.A. (2023). Morphology of the Spleen in the Camel's Fetus (*Camelus dromedarius*): Gross anatomy, histology and scanning electron microscopic studies. J. Appl. Vet. Sci., 8(4): 54-61.

Mescher AL (2021). Junqueira's basic histology: Text and Atlas. McGraw-Hill. https://www.researchgate.net/publication/352693113_Junqueira's_Basic_Histology_16th_edition_2021

Moore KL, Dalley AF, Agur AMR (2023). Clinically oriented anatomy. Wolters Kluwer. shop.lww.com Moore's Clinically Oriented Anatomy

Nishant N, Ajay Prakash AP, Farooqui MM, Prabhakar KPK (2018). Topography and biometric studies of

prenatal goat spleen at different gestational age. https://integrityresjournals.org/journal/JASVM/article-full-text-pdf/273F402515?utm_source=chatgpt.com

Nishant PA, Gupta V, Farooqui MM, Kumar P (2020). Histological and histochemical studies on the spleen of goat foetus in early stages of gestation. https://www.researchgate.net/publication/343852028_Histological_and_histochemical_studies_on_the_spleen_of_goat_foetus_in_early_stages_of_gestation

Niyf AM, Al-Jebori JGA (2024). Ontogenesis of rectum in local awassi sheep fetuses (*Ovis aris*) during prenatal periods. J. Anim. Health Prod., 12(s1): 45-54. https://doi.org/10.17582/journal.jahp/2024/12.s1.45.54

Onkar DP, Govardhan SA (2013). Comparative histology of human and dog spleen. J. Morphol. Sci., 30(1): 16–20. Retrieved from https://www.researchgate.net/publication/287857829_Comparative_histology_of_human_and_dog_spleen

Suvarna KS, Layton C, Bancroft JD (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences.

Usende IL, Okafor CL, Aina OO, Onyiche TE, Durotoye TI, Omonuwa AO, Jarikre TA, Maina MM, Falohun OO (2014). Comparative studies and clinical significance of the spleens of Nigerian indigenous pig (*Sus scrofa*) and goat (*Capra hircus*). J. Vet. Adv., 4(7): 604-612. https://doi.org/10.5455/jva.20140723040030

Zidan M, Kassem A, Dougbag A, El-Ghazzawi E, El-Aziz M, Pabst R (2000). The spleen of the one humped camel (*Camelus dromedarius*) has a unique histological structure. J. Anat., 196(3): 425-432. https://doi.org/10.1046/j.1469-7580.2000.19630425.x