Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Kandil B, Erhan Bayçumendur F, İşbilir F (2025).** Investigation of Connective Tissue Fibers in the Spleen of Sheep Fetuses (Second and Third Trimester). *Van Vet J*, 36 (1), 41-46. DOI: https://doi.org/10.36483/vanvetj.1595092

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Investigation of Connective Tissue Fibers in the Spleen of Sheep Fetuses (Second and Third Trimester)

Banu KANDİL[1,*] Füsun ERHAN BAYÇUMENDUR[2] Fatma İŞBİLİR[3]

[1] *Siirt University, Faculty of Veterinary Medicine, Department of Histology and Embryology, 56100, Siirt, Türkiye*
[2] *Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Histology and Embryology, 58140, Sivas, Türkiye*
[3] *Siirt University, Faculty of Veterinary Medicine, Department of Anatomy, 56100, Siirt, Türkiye*

Received: 02.12.2024 Accepted: 11.02.2025

ABSTRACT The aim of this study was to reveal the development of connective tissue fibers in the spleen of sheep fetuses. For this purpose, two different study groups were formed, consisting of the second and third trimesters of pregnancy. In this study, a total of 12 sheep fetuses were used, with 6 fetuses in each group. The fetal spleen tissues were fixed in 10% formaldehyde. Subsequently, routine histological procedures were applied to the tissue samples. Tissue sections were stained using Gordon Sweet's silver staining method to identify reticular fibers, Masson's trichrome staining method to identify collagen fibers, and Verhoeff's Van Gieson staining method to identify elastic fibers. The collagen, elastic, and reticular fibers were observed in the capsules and trabeculae of the spleen of sheep fetuses in the second and third trimesters. The collagen fibers were long and wavy, while the elastic fibers were short and wavy in structure. The reticulum fibers formed networks by anastomosing. Additionally, the elastic fibers were interwoven with collagen fibers, and the collagen, reticular, and elastic fibers were denser in the third trimester compared to the second trimester. In addition, reticular fibers were dominant in the spleens of sheep fetuses in both trimesters. As a result, collagen, reticular, and elastic fibers were observed in the spleens of sheep fetuses during the second and third trimesters of pregnancy. It was determined that with fetal development, the connective tissue fibers developed.

Keywords: *Connective tissue fibers, Fetal development, Sheep, Spleen.*

ÖZ

Koyun Fetüslarının (İkinci ve Üçüncü Trimester) Dalağında Bağ Doku İpliklerinin Araştırılması

Bu çalışmanın amacı koyun fetüslarının dalağında bağ doku ipliklerinin gelişimini ortaya koymaktır. Bu amaçla gebeliğin ikinci ve üçüncü trimesteri olmak üzere iki farklı çalışma grubu oluşturuldu. Bu çalışmada her grupta 6 fetüs olmak üzere toplam 12 koyun fetüsü kullanıldı. Fetüsların dalak dokusu %10 formaldehitte tespit edildi. Daha sonra doku örneklerine rutin histolojik işlemler uygulandı. Retiküler iplikleri belirlemek için Gordon Sweet's gümüşleme boyama yöntemi, kollegen iplikleri belirlemek için Masson's trichrome boyama yöntemi ve elastik iplikleri belirlemek için Verhoeff' s Van Gieson boyama yöntemi ile doku kesitleri boyandı. İkinci ve üçüncü trimesterdeki koyun fetüslarının dalağının kapsül ve trabeküllerinde kollagen, elastik ve retiküler iplikler gözlendi. Kollagen iplikler uzun ve dalgalı, elastik iplikler ise kısa ve dalgalı yapıdaydı. Retikulum iplikler anastomozlaşarak ağlar oluşturmuştu. Ayrıca elastik iplikler kollagen iplikler ile örülü halde idi. Gebeliğin ikinci trimesterine kıyasla gebeliğin üçüncü trimesterinde kollagen, retiküler ve elastik ipliklerin daha yoğun olduğu gözlendi. Ayrıca her iki trimesterde koyun fetüslarının dalağında retiküler iplikler baskındı. Sonuç olarak gebeliğin ikinci ve üçüncü trimesterinde koyun fetüslarının dalağında kollagen, retiküler ve elastik iplikler gözlendi. Fötal gelişim ile birlikte bağ doku ipliklerinin geliştiği belirlendi.

Anahtar Kelimeler: *Bağ doku iplikleri, Dalak, Fötal gelişim, Koyun.*

✉ *Corresponding author: banukandil@siirt.edu.tr

INTRODUCTION

The spleen, the largest secondary immune system organ in the body, filters blood, not lymph, unlike the lymph nodes (Cesta 2006; Elhussieny et al. 2022). The spleen initiates immune responses against blood-borne antigens and cleanses the blood of foreign substances and old or damaged red blood cells (Cesta 2006). In addition, it plays various roles such as storing blood, producing blood cells, and participating in iron metabolism (Tanyolaç 1999).

The spleen is encased in a fibrous capsule composed of collagen and elastic fibers, as well as smooth muscle fibers. The capsule sends trabeculae into the organ, dividing it into compartments (Cesta 2006; Elhussieny et al. 2022). The spleen consists of two functionally and morphologically distinct areas: the red pulp and the white pulp (Alex et al. 2015; Thanvi et al. 2020). The structure and components of the spleen show distinct differences between various animal species and also undergo changes as the organism ages (Waghaye et al. 2017). Knowing about the structural components of the spleen is crucial for understanding its immunological role and for analyzing various diseases. Therefore, this organ requires continuous attention from anatomical, immunological, and clinical perspectives (Jadhav et al. 2019).

Connective tissue, originating from the mesoderm, holds cells and other tissues together, performing a supportive and shaping function that organizes systems. It also plays a role in defending the organism through cellular and fluid pathways. It also plays a role in the body's cellular and humoral defences. The connective tissue is composed of an intercellular substance (extracellular matrix) and various cells. The intercellular substance consists of fibers and an amorphous ground substance (Ergün 2014).

The fibers of connective tissue are composed of collagen, reticular, and elastic fibers (Junqueira and Charneiro 2005). Collagen fibers are made up of glycoproteins called collagen (Sağlam et al. 2008). Based on the combination of the chains they contain, at least 27 different types of collagen have been classified. Type I, II, and III collagen fibers are the most common (Erhan Bayçumendur and Ergün 2022). Collagen fibers are not elastic. They do not stretch under mechanical pressure and tension and show great resistance to such forces. However, they do have the ability to bend and flex. Elastic fibers, which are finer than collagen fibers, are primarily made of a protein called elastin (Sağlam et al. 2008). During their course, elastic fibers anastomose to form networks. Together with collagen fibers, elastic fibers give tissues flexibility but also limit their stretching (Rodrigues et al. 1999; Erhan Bayçumendur and Ergün 2022). Elastic fibers are the primary element responsible for reversing the stretching of connective tissue (Rodrigues et al. 1999). Reticular fibers are composed of type III collagen. These fibers are very thin and form narrow or wide meshed networks in tissues by branching and anastomosing. Reticular fibers have a certain degree of extensibility due to their thin structure and the loose bundling of their fibrils (Ergün 2014).

During fetal development, collagen, elastic, and reticular fibers work together to support the growth, shaping, and functional maturation of organs. Collagen fibers provide durability and support, elastic fibers confer flexibility and elasticity, and reticular fibers support cellular organization and tissue integrity. The proper functioning of these fibers plays a fundamental role in ensuring both the structural and functional development of organs. It is therefore important to identify changes in connective tissue fibers in organs that undergo structural changes during fetal development. A limited number of studies have examined connective tissue fibers in organs during fetal development. Therefore, the aim of this study was to determine the changes in connective tissue fibers in the spleen of sheep fetuses in the second and third trimesters of pregnancy.

MATERIAL AND METHODS

The present study was approved by Siirt University Local Ethics Committee for Animal Experiments (File no: 2024/43, Decision no: 2024/07/43).

Fetuses were collected from the uterus of pregnant healthy cross-bred Hamdani sheep (Ovis aries) slaughtered in the local slaughterhouse in Siirt province. In order to determine the age of the fetuses, the crown-anus length of the fetuses was measured. The formula X=2.1 (Y+17) was used to determine the days of gestation (X= gestation period in days, Y=crown-anus length) (Noakes et al. 2001; Işbilir and Güzel 2024). Thus, two separate study groups were formed: the second trimester (50-100 days) and the third trimester (101-150 days). A total of 12 sheep fetuses were used in the study, 6 in each group, regardless of sex.

Small tissue samples were taken from the spleen of sheep fetuses. Tissues were fixed in 10% formaldehyde (pH=6.9-7.1) for 24 hours at room temperature. After routine histological procedures, the tissues were embedded in paraffin. Tissue blocks were cut to 5 µm thickness. The tissue sections were stained using Gordon Sweet's silver staining method for reticular fibers, Verhoeff's Van Gieson staining method for elastic fibers, and Masson's trichrome staining method for collagen fibers (Erhan Baycumendur and Ergun, 2022). The stained tissue sections were examined under a light microscope (Zeiss Primo Star) and photographed.

RESULTS

Masson trichrome staining was used to demonstrate the presence and distribution of collagen fibers in the spleen of sheep fetuses. The collagen fibers were stained blue. The collagen fibers were prominently observed in the splenic capsule, trabeculae, and around the arteries. The collagen fibers had a long and wavy appearance. The collagen fibers were thicker and more abundant in the third trimester than in the second trimester (Figure 1, 2).

The elastic fibers in the splenic tissue were stained using Verhoeff's Van Gieson staining method. The elastic fibers were dyed pink-purple. The elastic fibers were observed in the splenic capsule and trabeculae. The elastic fibers extended in a short and wavy pattern and were interwoven with collagen fibers. The elastic fibers were denser in the third trimester compared with the second trimester (Figure 3, 4).

With Gordon Sweet's silver staining method, the reticular fibers in the spleen tissue were stained black. The reticular fibers were observed to form a network around blood vessels, in trabeculae and in the capsule. The reticular fibers were more stained in the third trimester compared to the second trimester. In both the second and the third trimesters of pregnancy, the spleen tissue was richer in terms of the reticular fiber network (Figure 5).

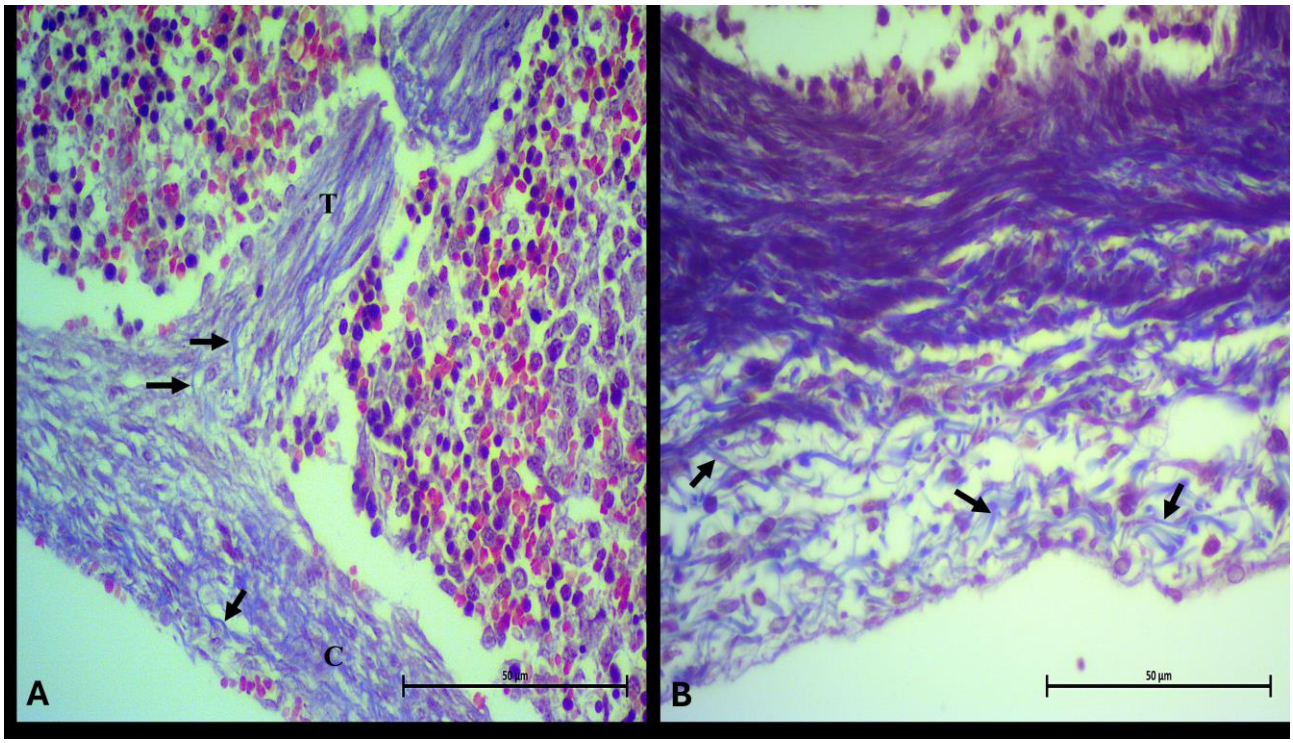

Figure 1: Second trimester (A) and third trimester (B) fetal sheep spleen sections. Arrows: Collagen fibers in the splenic capsule (C) and trabeculae (T). Masson trichrome staining, Bar: 50 µm.

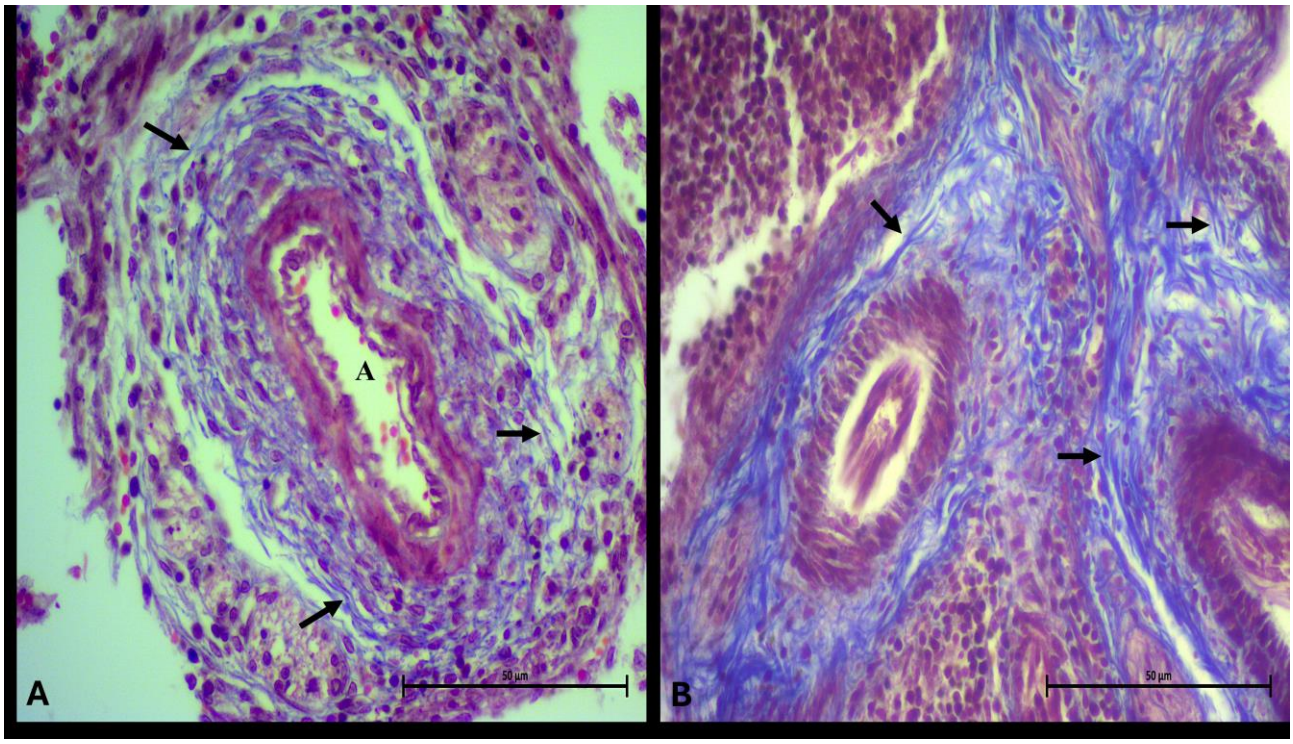

Figure 2: Second trimester (A) and third trimester (B) fetal sheep spleen sections. Arrows: collagen fibers around the arteries (A). Masson trichrome staining, Bar: 50 µm.

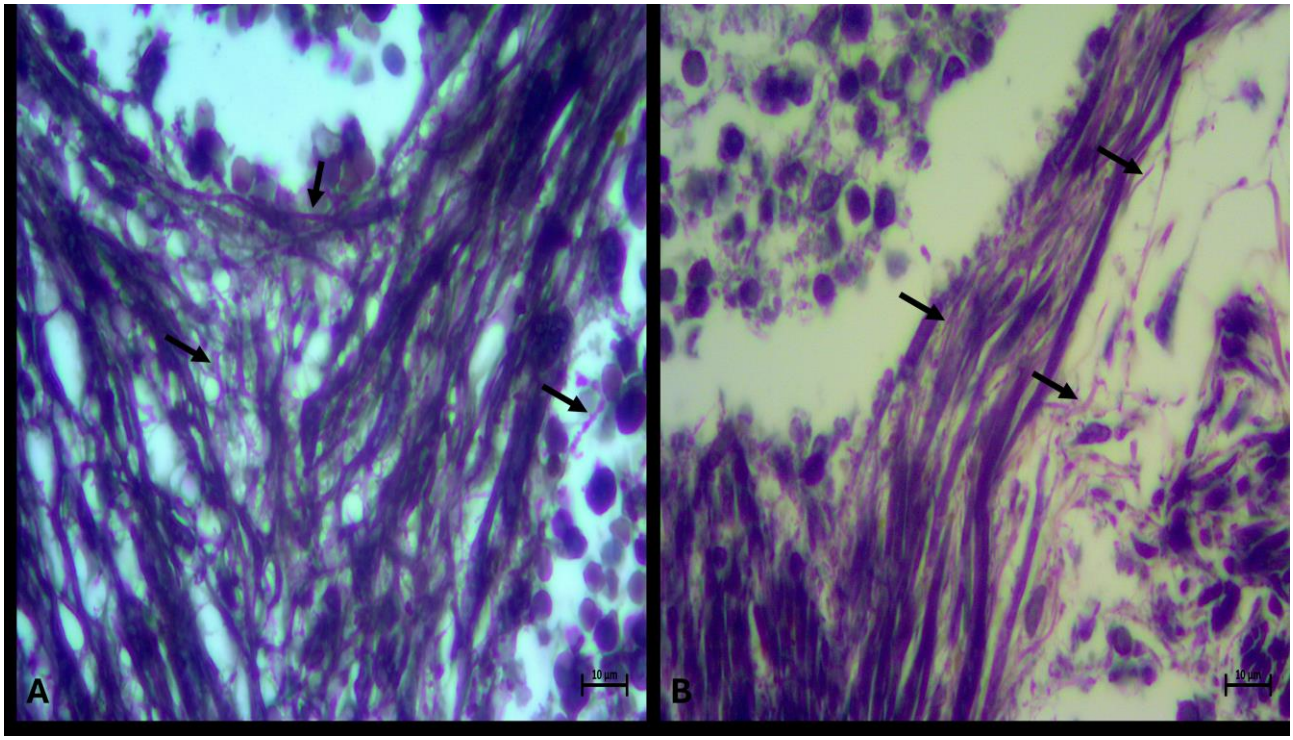

Figure 3: Second trimester (A) and third trimester (B) fetal sheep spleen sections. Arrows: elastic fibers in the trabeculae. Verhoeff' s Van Gieson staining, Bar: 10 µm.

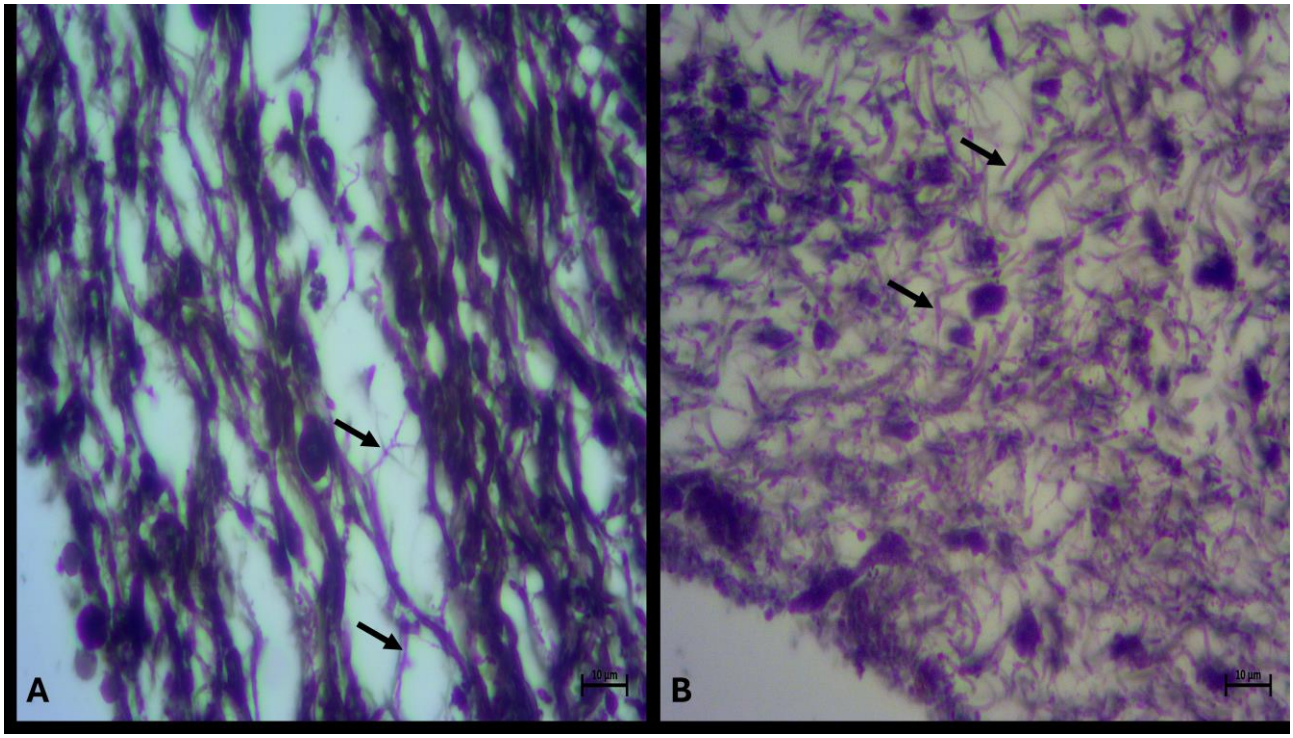

Figure 4: Second trimester (A) and third trimester (B) fetal sheep spleen sections. Arrows: elastic fibers in the splenic capsule. Verhoeff' s Van Gieson staining, Bar: 10 µm.

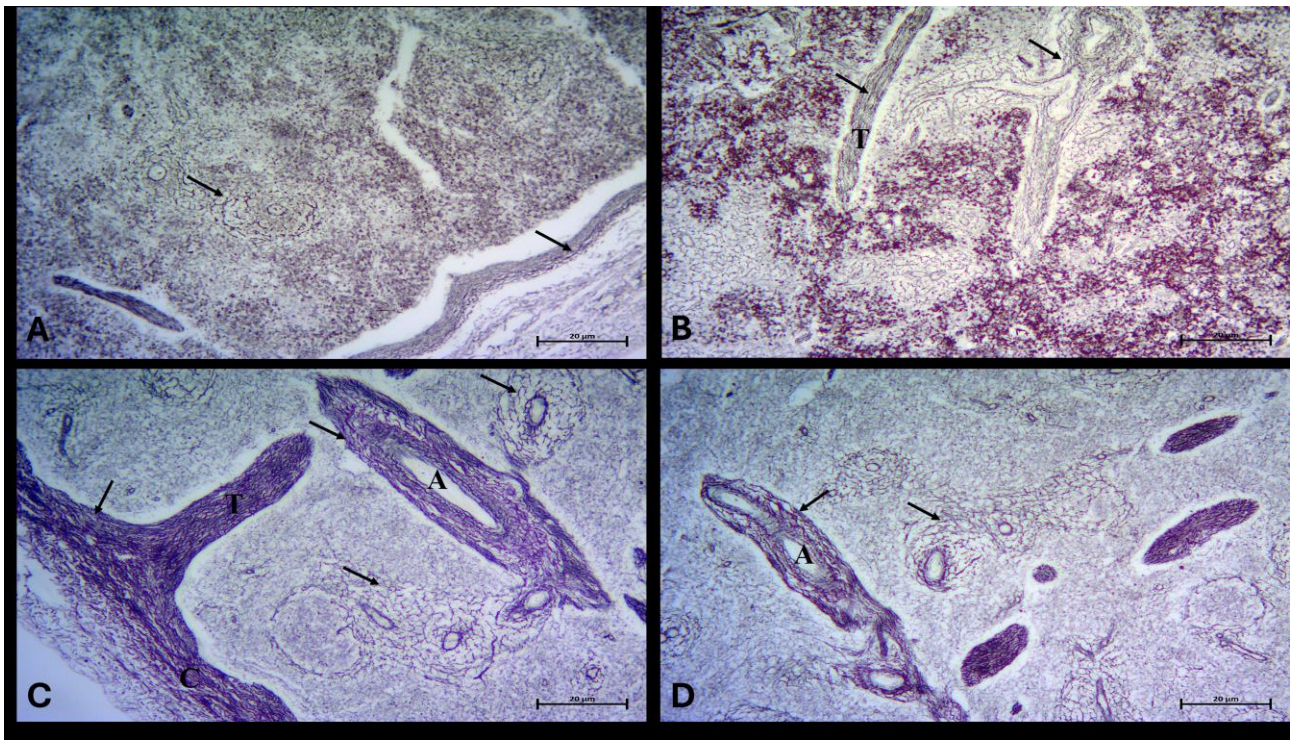

Figure 5: Second trimester (A, C) and third trimester (B, D) fetal sheep spleen sections. Arrows: reticular fibers. A: Artery, C: Capsule, T: Trabeculae. Gordon Sweet's silver staining, Bar: 20 µm.

DISCUSSION AND CONCLUSION

This study examined the development of collagen, elastic, and reticulum fibers in the spleen of sheep fetuses in the second and third trimesters of pregnancy.

In sheep, the stroma of the spleen consists of the capsule and trabeculae, with the trabeculae extending from the capsule and hilus into the parenchyma (Elhussieny et al. 2022). The components of the splenic stroma reflect the contractile role of the spleen in changing blood volume to expel circulating erythrocytes (Jadhav et al. 2019). The elastic fibers and smooth muscle fibers in the capsule and trabeculae assist in the change of the spleen's volume and help expel excess blood from circulation. These fibers play a critical role in the spleen's ability to contract, thereby facilitating the ejection of erythrocytes and maintaining proper blood flow dynamics (Devi et al. 2016)

In sheep and goats (Gnanadevi et al. 2019), rats (Saito et al. 1988), Marwari goats (Devi et al. 2016), white Yorkshire pigs (Shringi et al. 2018), and dogs (Das et al. 2005), reticular, elastic, and collagen fibers were reported in the trabeculae and capsule of the spleen. In adult Indian goats (Capra hircus), Raju et al. (2003) reported that reticular, collagen and, elastic fibers are evenly distributed in the splenic capsule. However, in pigs, Jadhav et al. (2019) reported that collagen, elastic, and reticular fibers were not evenly distributed in the splenic capsule. Researchers found that collagen fibers are more dominant than elastic and reticular fibers in the outer fibrous layer of the splenic capsule. Additionally, they noted that the elastic fibers in the splenic capsule are short, wavy, and interwoven with other connective tissue fibers, while the reticular fibers were sparse, wavy, and interwoven. In Barki sheep (Elhussieny et al. 2022), it was found that there were a few collagen fibers scattered among the smooth muscle fibers abundant in the splenic capsule and trabeculae. In the Iraqi camel (Alshamarry 2010), collagen and elastic fibers were observed intertwined with smooth muscle fibers in the splenic capsule. The structure and components of the spleen vary significantly across different animal species, and their composition serves as a reference for pathological conditions, aiding in the diagnosis of immunodeficiency diseases (Waghaye et al. 2017). Previous studies have shown that the connective tissue fibers in the capsule and trabeculae of the spleen have different characteristics in various animal species. The results of this study showed that reticular, collagen and elastic fibers were present in the capsule and trabeculae of the spleen of sheep fetuses in the second and third trimesters. While we found that collagen fibers were long and wavy, and elastic fibers were short and wavy, we also found that reticular fibers formed networks by anastomosing. Additionally, the elastic fibers were interwoven with the collagen fibers. The interwoven elastic and collagen fibers prevent distortion of the spleen capsule and the shape of the spleen (Rodrigues et al. 1999). Based on this information, it can be said that the interweaving of elastic and collagen fibers is crucial for the spleen to acquire its shape during fetal development.

The characteristics of the connective tissue fibers in the structure of the spleen not only vary between species but also change during the development of the spleen. In a study conducted on humans (Rodrigues et al. 1999), it was reported that in 7-month-old infants, elastic fibers were organized in a fine, straight, and uniform network between collagen bundles. However, in 32-year-old adults, elastic fibers were found to be thicker and shorter, with the elastic fiber network partially disrupted. In 76-year-old men, the elastic fiber network was completely disrupted and the elastic fibers were wrapped around thick collagen fiber bundles. Researchers have stated that with aging, the decrease in elastic fibers in the spleen capsule may contribute to the restriction of spleen expansion and the involution of the spleen as blood flow gradually decreases. In goats (Waghaye et al. 2017), collagen fibers in the splenic capsule were found to increase from prenatal to postnatal age groups. Researchers reported that reticular fibers predominated in the spleen capsule during the prenatal period and that the size and number of reticular fibers

decreased with age. They observed that with age, the size and quantity of elastic fibers increased, but in later ages, elastic fibers appeared shorter and fragmented. Researchers emphasized that the changes in the concentration of these fibers in the capsule with age are necessary to provide external firmness to the organs during the postnatal period and to maintain the elasticity of the organ during the prenatal and early postnatal periods. In camel fetuses, Marwa-Babiker et al. (2023) observed that in the first trimester of pregnancy, the spleen capsule consists of a thin mesenchymal connective tissue. In the second trimester of pregnancy, they identified collagen fibers in the capsule and trabeculae, and in the third trimester, they found that these fibers were thicker. In this study, consistent with the literature, it was determined that the collagen, reticulum, and elastic fibers in the spleen capsule and trabeculae were denser in the third trimester compared to the second trimester of pregnancy. During fetal development, the increase in elastic fibers may be to provide elasticity during the growth of the spleen, and the increase in collagen fibers may be to provide firmness to the spleen after the spleen has stopped growing.

During embryogenesis, most connective tissues contain abundant reticular fibers, which later transform into collagen fibers (Sağlam et al. 2008). The amount of reticular fibers indicates the maturity level of the tissues (Erhan Baycumendur and Ergun 2022). In a study conducted on chicken embryos (Fukuta and Mochizuki 1982), it was determined that on the 7th day of incubation, the splenic reticulum consisted only of reticular cells, without reticular fibers. The researchers reported that from day 9, the spaces between the extensions of the reticular cells began to fill with fine flocculent substances. It was observed that as the embryos grew, these materials were accumulated into felt-like bands that showed the characteristics of mature reticular fibers. In pigs, Jadhav et al. (2019) showed that reticular fibers are more dominant in splenic trabeculae compared with collagen and elastic fibers. In this study, reticulum fibers were observed in the spleens of sheep fetuses in the second and third trimesters. Additionally, in both trimesters, reticular fibers were dominant in the spleen compared to collagen and elastic fibers. Together with the reticulum cells, the reticulum fibers form the roof of the blood-forming organs (spleen, bone marrow, lymph nodes) (Sağlam et al. 2008). Therefore, the dominance of reticular fibers during fetal development is crucial for the development of the spleen.

Connective tissue fibers form the structural framework of the organ and provide the necessary support for the spleen to perform its functions. During fetal development, information about the presence and distribution of connective tissue fibers in the spleen can provide significant insights into the organ's developmental processes, structure, and functions. Additionally, understanding the development of connective tissue fibers in the spleen during the fetal period provides a basis for comparison to comprehend changes occurring in these fibers with aging. For these reasons, the results of this study are expected to make significant contributions to both basic science and clinical research.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

AUTHOR CONTRIBUTIONS

Idea / Concept: BK, FEB
Supervision / Consultancy: BK, FEB, Fİ
Data Collection and / or Processing: BK, FEB, Fİ
Analysis and / or Interpretation: BK, FEB
Writing the Article: BK
Critical Review: BK, FEB

REFERENCES

Alex L, Rajan ML, Xavier B et al. (2015). Microscopic study of human spleen in different age groups. *Int J Res Med Sci*, 3 (7), 1701-1706.

Alshamarry HA (2010). Histological and histometric study on the spleen of Iraqi camel (*Camelus dromedarius*). *Emir J Food Agric*, 22 (1), 65-70.

Cesta MF (2006). Normal structure, function, and histology of the spleen. *Toxicol Pathol*, 34 (5), 455-465.

Das SK, Alam MS, Islam MN, Awal MA (2005). Histology of the spleen of indigenous dog *(Canis familiaris)* of Bangladesh. *Bangl J Vet Med*, 3 (1), 59-61.

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

Elhussieny O, Abdelaziz M, Khalifa E (2022). New insights into the splenic stroma of the Barki sheep: a histological, immunohistochemical, ultrastructural and morphometrical study. *MJVM, 2* (1), 1-8.

Ergün L (2014). Bağ Dokusu. Özer A (Ed.). Temel Histoloji. II. Edition. Nobel Akademik Yayıncılık Eğitim Danışmanlık Tic. Ltd. Şti. Ankara

Erhan Bayçumendur F, Ergun L (2022). Histological examination of liver connective tissue fibers in broiler chickens and japanese quails. *Van Vet J,* 33 (3), 92-96.

Fukuta K, Mochizuki K (1982). Formation of reticular fibers in the developing spleen of the chick embryo. *Arch Histol Jap*, 45 (2), 181-189.

Gnanadevi R, Senthilkumar S, Ta K, Ramesh G (2019). Comparative histoarchitectural study of splenic components in sheep and goat. *Int J Curr Microbiol App Sci, 8* (5), 1387-1394.

Işbilir F, Güzel BC (2024). Developmental three-dimensional examination of the pelvic cavity of Hamdani crossbred sheep fetuses *(Ovis aries)* in the last two periods of gestation. *Vet Med Sci,* 10 (5), e1572.

Jadhav KA, Waghaye JY, Thakur PN et al. (2019). Comparative light microscopic study of splenic stroma in male and female local domestic pig (*Sus scrofa domesticus). J Entomol Zool Stud*, 7 (2), 898-903.

Junqueira LC, Charneiro J (2005). Basic Histology Text&Atlas. 11nd Edition. The McGraw-Hill Companies, New York.

Marwa-Babiker AM, ALkhodair KM, Abdelhay MA et al. (2023). Morphology of the spleen in the camel's foetus *(Camelus dromedarius):* gross anatomy, histology and scanning electron microscopic studies. *J Appl Vet Sci*, 8 (4), 54-61.

Noakes DE, Parkinson TJ, England GCW (2001). Arthur's Veterinary Reproduction and Obstetrics (pp.68). VIII Edition. Elsevier, China.

Saito H, Yokoi Y, Watanabe S et al. (1988). Reticular meshwork of the spleen in rats studied by electron microscopy. *Am J Anat*, 181 (3), 235-252.

Sağlam M, Aştı RN, Özer A (2008). Genel Histoloji. VI. Edition. Yorum Basın Yayın Sanayi Ltd. Şti. Ankara.

Shringi N, Mathur R, Kumar V et al. (2018). Histological studies on the spleen of large white Yorkshire pig *(Sus scrofa). J Entomol Zool Stud*, 6 (1), 1142-1144.

Raju NKB, Iyyengar MP, Gopinath S (2003). Histological study on the spleen of adult Indian goat *(Capra hircus). Indian J Vet Anat*, 15 (1/2), 44-47.

Rodrigues CJ, Sacchetti JCL, Rodrigues Jr AJ (1999). Age-related changes in the elastic fiber network of the human splenic capsule. *Lymphology*, 32 (2), 64-69.

Tanyolaç A (1999). Özel Histoloji. Yorum Basın Yayın Sanayi Ltd. Şti. Ankara.

Thanvi PK, Joshi S, Singh D (2020). Histomorphological studies on spleen of sheep *(Ovis aries). Vet Practitioner*, 21 (1), 48-53.

Waghaye JY, Banubakode SB, Charjan R et al. (2017). Age related histo-morphological changes of splenic connective tissue in goat *(Capra hircus). Indian J Vet Sci Biotech,* 13 (2), 1-6.