



Arterial Irrigation of the Spleen in Kangal sheep: A scanning Electron Microscopic study

Irrigación Arterial del bazo en ovejas Kangal: estudio mediante microscopia electrónica de barrido

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ABSTRACT

This study was performed to demonstrate the arterial irrigation of the spleen in Kangal Type Akkaraman sheep both under scanning electron microscopy and light microscopy. The spleens from 10 Kangal sheep were obtained from a slaughterhouse and used in this study. The necessary protocol for imaging under an electron microscope was applied to five of the spleens, and their images were obtained. After tissue tracing, the remaining five spleens were stained with Mallory's triple staining technique and examined under a light microscope. Macroscopically, it was seen that the spleen was leaf-shaped and that the splenic artery was divided into two main branches, the arteria dorsalis and arteria ventralis. It was found that many arteria trabecularis separated off from the main branches and that the arteria trabecularis divided into arteria centralis. These vessels continued with arteria penicillaris, the ends of which ended blindly in the form of bulbs. The findings obtained from the light microscope examination were parallel to those obtained from the scanning electron microscope analysis. The findings obtained from both microscope types are discussed, considering the available literature. The findings from the study offer guidance for the surgical intervention of the spleen. Furthermore, it provides researchers with the necessary information to compare studies that have been and will be performed in other species. This study addresses the deficiency in the literature on the subject.

Key words: Kangal sheep; scanning electron microscop; spleen

RESUMEN

Este estudio se realizó con el objeto de demostrar la irrigación arterial del bazo, en ovejas Kangal del tipo Akkaraman, mediante microscopía electrónica de barrido y microscopía óptica. Para ellos, se utilizaron los bazos de 10 ovejas Kangal, obtenidos de una sala de faenado local. El protocolo necesario para la obtención de imágenes a partir de un microscopio electrónico, se aplicó a cinco de los bazos. Después del estudio de los tejidos, los cinco bazos restantes se tiñeron con la técnica de triple tinción de Mallory y se examinaron bajo un microscopio óptico. Macroscópicamente, se vio que el bazo tenía forma de hoja y que la arteria esplénica se dividió en dos ramas principales, la arteria dorsal y la arteria ventral. Se encontró que muchas arterias trabeculares se separaron de las ramas principales y que estas se dividieron en las arterias centrales. Estos vasos continuaron con la arteria peniciladas, cuyos extremos terminaron ciegamente en forma de bulbos. Los hallazgos obtenidos del examen con microscopio óptico fueron paralelos a los obtenidos del análisis con microscopio electrónico de barrido. Se discutieron los hallazgos obtenidos con ambos tipos de microscopia, teniendo en cuenta la literatura disponible. Los hallazgos del estudio ofrecen una guía para la intervención quirúrgica del bazo. Además, proporciona a los investigadores la información necesaria para comparar estudios que se han realizado en otras especies. Este estudio aborda la deficiencia en la literatura sobre el tema.

Palabras clave: Bazo, microscopio electrónico de barrido, oveja Kangal

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INTRODUCTION

The spleen is an organ covered with a capsule consisting of fibrous connective tissue and smooth muscle [1], which has trabeculae extending into the organ interior. The parenchymal tissue of the spleen consists of red and white pulp. Approximately one-fifth of the volume consists of white pulp that includes diffuse and follicular lymphoid tissue, whereas the remaining volume is made up of red pulp consisting of venous sinuses covered with endothelium. As the vessel diameter of the splenic artery decreases, it branches and courses into the trabeculae. The vessels emerging from the trabeculae are surrounded by lymphoid tissue and form the central arteries within the white pulp. The central arteries enter the red pulp, where they branch into approximately 50 small, straight arterioles that open into capillary beds [2].

The functions of the spleen in the body include storing and concentrating erythrocytes, one of formed elements of blood of the blood. Furthermore, when necessary, it removes old erythrocytes, filters the blood, releases the iron contained in hemoglobin, and produces antibodies, lymphocytes, and monocytes as part of the immune system [1, 2, 3, 4].

The arterial vascularization of the spleen is provided by the splenic artery, a branch of the arteria celiaca [2, 5, 6, 7, 8]. The splenic artery also provides the rami pancreatici to the pancreas and the ramus epiploicus branch to the omentum majus. This also includes the arteria ruminalis sinistra branch, which runs in the opposite direction to the ramus epiploicus. Then, after the arteria ruminalis dextra branch, it ends by dividing into two branches at the hilus lienalis [2, 5, 6].

The spleen is differently shaped depending on the animal species [2, 6]. For example, it is sickle-shaped in horses (*Equus caballus*), boot-shaped in cats (*Felis catus*) and dogs (*Canis familiaris*), strap-shaped in cattle (*Bos taurus*), and leaf-shaped in goats (*Capra hircus*) [2]. It has been reported that in some carnivores, the splenic artery is a continuation of the arteria celiaca rather than being a third branch [6, 9]. In these animals, the arteria lienalis provides two branches along its course, one to the spleen and the other to the stomach [10]. It has been revealed that the arterial irrigation of the spleen in cattle is provided by branches that originate from the splenic artery and enters via the hilus [6]. In goats, the arterial vascularization of the spleen consists of branches arising from the splenic artery. In some cases, it has been shown to provide irrigation without branching, and its avascular region is parallel to its long axis [11].

It has been reported that the spleen in sheep (*Ovis aries*) resembles the shape of a leaf [2]. The arterial irrigation of the spleen occurs through branches that arise from the splenic artery, which is then bifurcated after entering via the hilum of the spleen. The dorsal branch is small, and the ventral branch is larger. Furthermore, in some cases, it has been found that it is divided into three branches; however, it has not been demonstrated to be divided into four branches. In other cases, it has been reported to branch irregularly after entering the spleen [11, 12].

The macroscopic and microscopic structure of the spleen has been examined by ultrasonography [13, 14], computed tomography, magnetic resonance, positron emission tomography [15], corrosion cast [14, 16, 17], latex [10, 17, 18], light microscopy [19, 20, 21], and scanning electron microscope

(SEM) [21, 22, 23, 24].

During any operative intervention within the abdominal region, the spleen, which is a sensitive organ in terms of arterial irrigation must be taken into consideration and protected. To avoid excessive bleeding during a biopsy of the spleen for diagnostic purposes, a location with less vascular density should be preferred [25]. In addition, it is necessary to know the vascularization of the spleen to decide whether to perform a partial or total splenectomy [21, 25, 26].

In this study, the arterial vascularization of the spleen in Kangal sheep was determined using the corrosion cast method and other histological methods. The cast were examined macroscopically and using SEM. And histological findings were evaluated under a light microscope. The findings obtained in the study will provide guidance for total or partial splenectomies, biopsies of the spleen, and operative interventions within the abdominal region in general. Moreover, the functional effects of spleen vascularization are discussed, considering the available literature.

MATERIALS AND METHODS

To demonstrate the arterial irrigation of the spleen in Kangal sheep, 10 fresh sheep spleens slaughtered at the Yarısoğlu Slaughterhouse in Sivas province were used. The corrosion cast method was applied to detect the arterial irrigation of the spleen. For this purpose, after the splenic artery was identified, a needle cannula number 16 (Grey) was placed in the vessel. To remove the blood in the splenic artery and its branches, 0.9% NaCl solution was perfused and a mixture of 20% monomethyl methacrylate and 80% liquid polymethyl-methacrylate prepared for corrosion cast was administered through a cannula placed in the splenic artery. The mixture was manually applied to the vessel under constant pressure, approximately 15 mL into each spleen. The spleens were kept at room temperature for 24 hour (h) to complete the polymerization of the substance administered into the vessel, and were kept in 40% potassium hydroxide (KOH) solution in an oven set at 40 °C for 48 h to melt the tissues outside the casts. After being removed from the oven, the lysed tissues of the spleen were removed with running water, and the remaining vascular casts were left to dry at room temperature for 24 h.

After the drying process was completed, splenic artery and its branches were photographed. In order to be viewed with a scanning electron microscope (JEOL JSM-5500LV, Japan), the dried cast were placed on aluminum plates and coated with gold. It was examined under a scanning electron microscope and the findings were photographed.

Spleen samples taken from animals were fixed in 10% buffered formal solution in tissue fixed for 24 h for histological preparation. The fixed samples were washed in running water for 24 h, passed through alcohol and xylol series and blocked in paraplast. Mallory's trichrome staining technique, modified by Crossmon [27], was applied to 5µm sections taken from the blocks for general histological examinations. The stained preparations were examined under a Carl Zeiss Primo Star (Germany) model research microscope and photographs were taken.

RESULTS AND DISCUSSION

When the corrosion casts that were prepared for electron microscopy of the Kangal sheep spleen were examined, it was observed that its shape resembled that of a leaf, which is consistent with the literature. It was observed that the splenic artery entered the spleen from hilum of the spleen, and after entering, it divided into two main branches: the arteria dorsalis and arteria ventralis (FIG. 1). It was determined that the arteria ventralis provided one or two side branches, which ran parallel to the arteria dorsalis as it coursed toward the tip of the spleen (FIG. 1). After providing these branches, it continued its course to the extreme point of the spleen. (FIG. 1). The arteries trabecular branches from each of the main branches according to the spleen functional units (FIG. 1). The vessels exhibited individual variations at the point where they separated from the splenic artery and during their course (FIG. 1).



FIGURE 1. Arterial Segmentation of the Spleen. (Corrosion Cast) 1: Arteria Lienalis, 2: Arteria Dorsalis, 3: Arteria Ventralis. Asterisks: Arteria Trabecularis.

In the SEM examination, it was found that after the main branches separated from the splenic artery, the arteria trabecular separated in accordance with the spleen's trabecular structure (FIG. 2). It was determined that the arteria trabecularis, whose diameter gradually narrowed toward the endpoint of the spleen, divided into many arteria centralis along its course (FIG. 2). A large number of arterioles with bulb-like endings (arteriola penicillated) branched from the arteria central (FIG. 2).

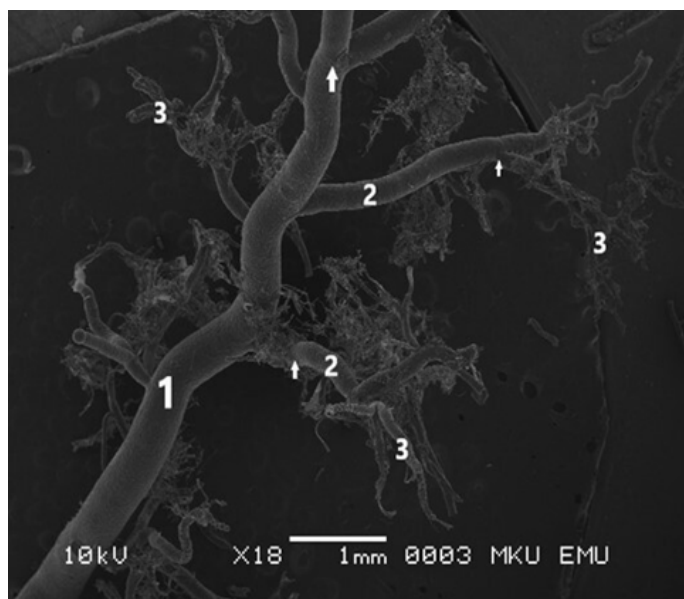


FIGURE 2. SEM image of splenic arteries. 1: arteria trabecularis, 2: arteria centralis, 3: arteriola penicillaris. Arrows: Narrowings of the arteria centralis as they separate from the arteria trabecularis. (The magnification coefficients are not repeated in the photographs as they are written under each photograph)

Furthermore, it was determined that in the regions where the arteria centralis separated from the arteria trabecularis, the vessels first showed a narrowing, but after they separated, they widened again (FIG. 1). The arteriola penicillated exhibited a bulb-like termination (FIG. 3), and obvious depressions in the arteriole walls were striking (FIG. 3). It was also observed that these arterioles came together in large numbers and exhibited a ball-like formation (FIG. 4).

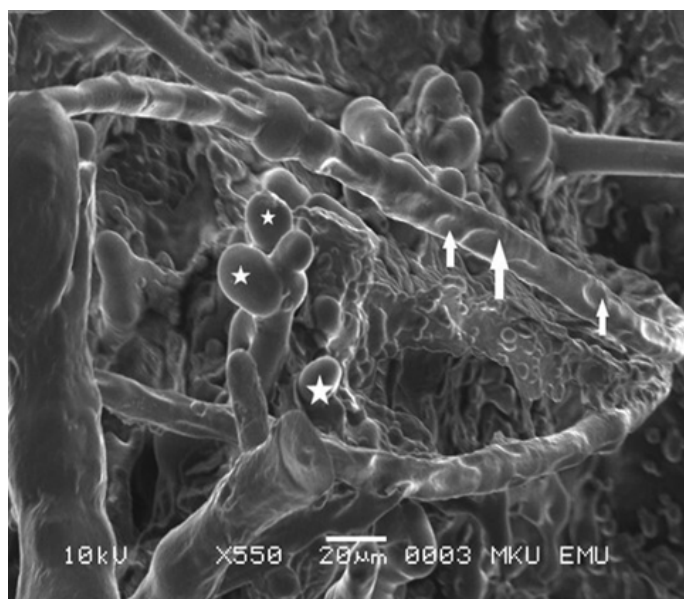


FIGURE 3. Sem Image of Arterioles. Stars: Bulb-Like Endings of the Arterioles, Arrows: Distinct depressions observed in the walls of the Arterioles

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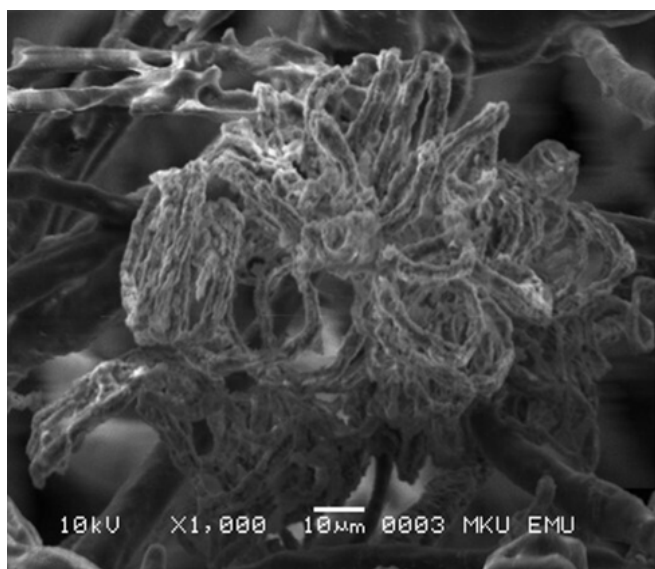


FIGURE 4. A Ball-Like Structure Formed By Arterioles (Arteriola Penicilaris) Coming Together

When the spleen sections were examined in terms of general histological appearance, the splenic venous sinuses, splenic cords, and trabeculae that entered the organ via the connective tissue capsule that surrounds the organ from the outside were observed. (FIG. 5). The trabecular arteries and veins carried by trabeculae that extended from the capsule were also seen (FIG. 6, 7). A periarteriolar lymphatic sheath (PALS) surrounded by T lymphocytes, which were formed by the trabecular artery that separated from the trabecula, was observed (FIG. 8). The white and red pulp areas that constitute the parenchyma of the organ were identified. (FIG. 6), and the central arteries were observed within the white pulp areas, which are considered the lymphoid parts of the spleen (FIG. 6, 8). Venous sinuses located within the red pulp areas between the lymph follicles were also noted (FIG. 8).

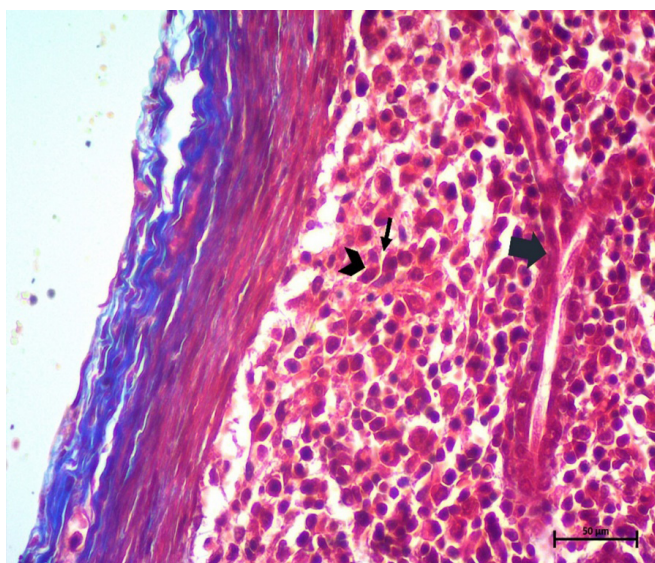


FIGURE 5. Spleen section in Kangal Sheep. Arrow: trabeculae, thin arrow: splenic venous sinuses, arrowhead: splenic cords. Mallory's trichrome. Bar: 50 µm

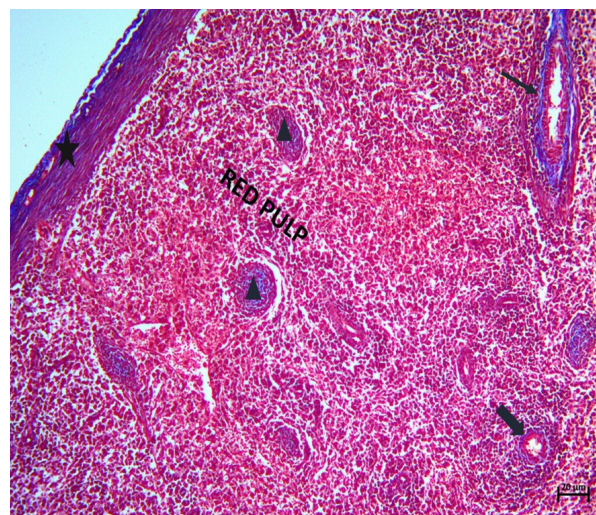


FIGURE 6. Spleen section in Kangal Sheep. Star: capsule, Triangle: white pulp, thick arrow: arteria centralis, thin arrow: vena trabecularis. Mallory's trichrome. Bar: 20 µm

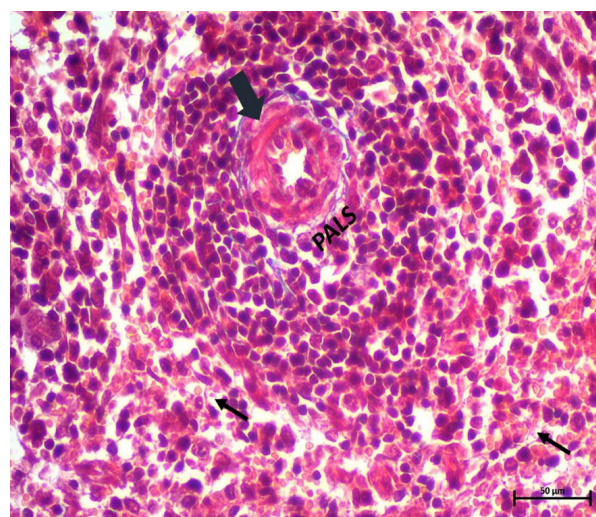


FIGURE 7. Spleen section in Kangal Sheep. Thick arrow: PALS located in arteria centralis and its surroundings, thin arrows: venous sinuses. Mallory's trichrome. Bar: 50 µm

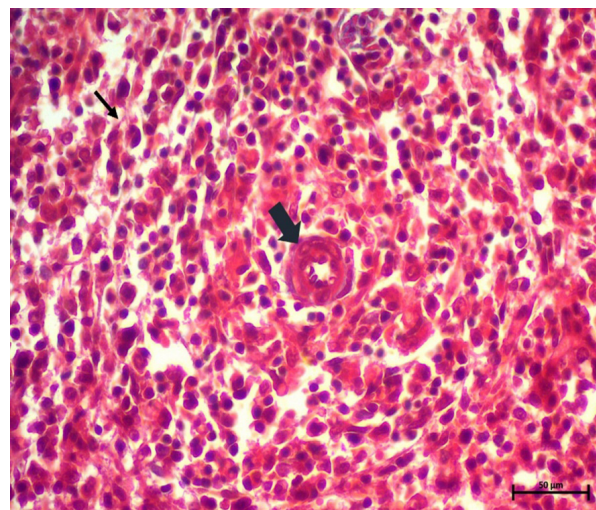


FIGURE 8. Spleen section in Kangal Sheep. Thick arrow: arteria trabecularis, thin arrow: venous sinuses. Mallory's trichrome. Bar: 50 µm

Many studies have been conducted on the arterial segmentation of the spleen [11, 12, 14, 25, 28, 29, 30, 31, 32]. In these studies, it was revealed how the splenic artery branches as it enters the spleen via the hilum of the spleen in humans as well as different animal species [32]. In a study performed in humans, it was reported that the splenic artery divided into two branches, the arteria superior, and arteria inferior, and did not exhibit any further branching [29]. In another study conducted with a large number of human samples ($n = 850$), it was determined that the majority exhibited a two-branched structure, but there were also vessels that were divided into single or three branches, although this number was low [31]. In another study, the splenic artery showed branching in the shape of the letter y when it entered the spleen [33].

There are also studies that consider the arterial irrigation of sheep spleens [12, 32]. In a study conducted on Merino sheep, it was shown that the splenic artery followed an inverted Y-shape course after it entered the spleen. In the same study, it was emphasized that the vein branches off into smaller venules before branching into the arteria dorsalis and arteria ventralis [32].

In this study, the spleen of Kangal sheep resembles that of a leaf, which is in agreement with the literature. The splenic artery courses on one side of the spleen, which is divided into two branches, and a branch (arteria trabecular) separates from these branches for each of the trabecula (FIG. 1). The course of the splenic artery in the extremitas dorsal and margo caudalis is important to be considered during a surgical approach to the spleen. In the study conducted on Merino sheep, it was observed that the two branches of the inverted Y-shape were almost equal, that is, the arteria dorsal and arteria ventral were equal in diameter [32]. In this study, it was seen that the diameter of the arteria dorsalis was thinner than that of the arteria ventralis (FIG. 1).

In studies on electron microscope images of spleen irrigation, the branches that separated from the arteria dorsalis and arteria ventralis for each spleen unit were called arteria trabecular [14, 32]. It has been determined that the arteria trabecular is also divided into the arteria centralis. The presence of arteriola penicillaris branching from the arteria centralis in the form of brushes has also been detected. In this study, the presence of the vessels mentioned above was observed (FIGS. 2, 3, and 4).

It has been reported that arteria penicillated terminates in the shape of a bulb [34] or a trumpet with a widened end [35]. In this study, the termination of these vessels was found to be in agreement with the literature (FIG. 3). In addition, it is thought that the obvious depressions seen in the arteria penicillated walls may be an inherent feature of the vessel wall structure (FIG. 3). The possibility that these depressions are artifacts should also be considered; however, other studies on the subject have reported that this arises from the cell structure of the vascular wall [36]. If, as stated in the studies, these structures are traces of cells on the vascular wall, further, more specific studies should be performed to investigate what these cells are and how they leave traces in the casts.

In a study conducted on the human spleen, it was reported that it is important to know the location, size and distribution of the vascular presence in the spleen for surgical operations. In the study, it was emphasized that knowing the segmentation that occurs in the spleen is of vital importance in operative

interventions such as splenectomy [29]. Another study conducted on the human spleen stated that dividing the spleen into lobes and segments will make the job of surgeons easier in partial splenectomy and allotransplantation applications [31]. The findings obtained in our study will also guide operative interventions to be performed on the Kangal sheep spleen.

It was observed that the findings obtained from the light microscope assessment of the spleens also support the findings obtained from the electron microscope evaluation. The findings obtained from the light microscope revealed the vascularization of the spleen as well as the relationship of the vascularization with the surrounding tissues. Similar to the histological results reported by the study conducted by Gnanadevi *et al.* [20] with sheep and goats collected from slaughterhouses in Chennai, India, this study found a capsule consisting of connective tissue from which many trabeculae containing major vascular branches extend. These trabeculae originated from the inner part of the capsule and extended to the parenchyma, and the white and red pulp [20]. It observed the formed parenchyma histologically. As reported by Suri *et al.* [19] in their study of sheep collected from slaughterhouses in Jammu, India, the red pulp areas in our study also consisted of spleen cords and sinusoids [19]. The PALS containing T lymphocytes around a central artery observed in the control group of the study conducted with rats in 2017 [37] was compatible with the PALS observed in this study using sheep. In the study conducted by Thanvi *et al.* [38] with sheep, the trabeculae, white and red pulp areas, splenic sinusoids, and splenic cords were revealed histologically, which were similar to our findings [38].

CONCLUSIONS

In the study, arterial vascularization in the spleen of kangal sheep was revealed by examining vascular corrosion patterns under electron microscopy and evaluating under light microscopy. It is thought that vascular corrosion cast findings will help to reveal segmentation in the spleen. Although electron microscopy findings are generally similar to the literature, it was concluded that the meaning of the depressions detected in the walls of penicillar arteries needs to be investigated. It was observed that light microscopy findings are similar to the literature. The findings obtained will guide surgeons in operative interventions such as splenectomy, partial splenectomy and resection to be performed on the spleen.

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Conflicts of interest

None of the authors have any financial or personal conflicts of interest that could inappropriately influence or bias the content of the article.

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