Kocatepe Veterinary Journal

Kocatepe Vet J. (2021):14(3):293-302

DOI: 10.30607/kvj.908062

RESEARCH ARTICLE

Arterial Vascularization and the Macroanatomic and Histological Structures of the Testis, Penis, and Prostate Gland in Red Foxes (Vulpes vulpes)

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ABSTRACT

The aim of this study was to examine arterial vascularization and the macroanatomic and histological structures of the testis, penis, and prostate gland in the red fox. Five male red foxes were provided by the Wildlife Rescue and Rehabilitation Center of Kafkas University, Turkey. The arteries supplying the prostate, penis, and testes in the animals were exposed by dissection, the mean length, width, and weight of these organs were measured. After the anatomical features of the testis, penis, and prostate were assessed, tissue samples of each blocked in paraffin then handling standard histological procedures. The internal iliac artery was divided into two branches the caudal gluteal artery, which is the thicker branch and leads dorsally, and the internal pudendal artery, which is the thinner branch and leads ventrally. The testicular artery is asymmetrically separated from both sides of the abdominal aorta at the 5th lumbar vertebra, passes through the spermatic canal, and ends in the testes. It is thought that the findings of this study will contribute information to the literature on artificial insemination, castration, prostate, and urolithiasis surgeries on carnivores.
Keywords: Anatomy, Histology, Penis, Prostate gland, Red fox, Testis.

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Kızıl Tilkilerde (Vulpes vulpes) Testis, Penis ve Prostat'ın Arteriyel Vaskülarizasyonu, Makroanatomik ve Histolojik Yapısı

ÖZ

Bu çalışmanın amacı kızıl tilkilerde testis, penis ve prostat'ın arteriyel vaskularizasyonu, makroanatomik ve histolojik yapısını incelemektir. Kafkas Üniversitesi Yaban Hayatı Koruma ve Kurtarma Merkezi'nden 5 adet erkek kızıl tilki temin edildi. Testis, penis ve prostat'ı besleyen arterler diseke edildi. Bu organların ortalama uzunluğu, genişliği, ağırlığı ölçüldü. Testis, penis ve prostat'ın anatomik özellikleri değerlendirildikten doku örneklerine standart histolojik prosedür uygulanarak parafinde bloklandı. İnternal iliac arter, daha kalın dorsal'e yönelen caudal gluteal arter ve daha ince ventral'e yönelen internal pudendal arter olarak ikiye ayrılıyordu. A. testicularis'ler L5 hizasında abdominal aorta'nın iki tarafından asimetrik olarak ayrılıyordu. Spermatik kanal boyunca seyredip testislerde sonlanıyordu. Sunulan çalışmanın bulgularının kızıl tilkiler ve carnivorlarda yapılacak olan suni tohumlama, kastrasyon, prostat ve ürolithiasis operasyonlarına katkıda bulunacağına inanmaktayız.
Anahtar kelimeler: Anatomi, Histoloji, Penis, Prostat bezi, Kızıl tilki, Testis.

To cite this article: Kırbaş Doğan G. Dalga S. Akbulut Y. Aslan K. Asker H. Karadağ Sarı E. Arterial Vascularization and the Macroanatomic and Histological Structures of the Testis, Penis, and Prostate Gland in Red Foxes (Vulpes Vulpes). Kocatepe Vet J. (2021):14(3):293-302

Submission: 01.04.2021 **Accepted**: 07.06.2021 **Published Online**: 26.08.2021
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INTRODUCTION

The red fox (Vulpes vulpes) is a carnivorous mammal of the order Carnivora and family Canidae. Red fox is the biggest of the other fox species and the most plenty wild member of the order. It is listed as a species of least concern by the International Union for Conservation of Nature (Demirsoy 1992, Larivière and Pasitschniak-Arts 1996). As for all animals, the red fox's circulatory system, the main elements of which are the heart and vessels, is important for sustaining life.

Testes are two oval-shaped organs located in the scrotum along with the epididymis (Evans and Lahunta, 2013). The tunica vaginalis, which consists of the parietal lamina (lamina parietalis) and visceral lamina (lamina visceralis), surrounds the testes. The parietal lamina are firmly attached to the scrotum. Below the tunica vaginalis is the tunica albuginea, collagen strands, and large blood vessels. The testicular connective tissue consists of the tunica albuginea, septa of the testis (septula testis), and mediastinum testis. The tunica albuginea divides the lobule into the parenchyma through branches called the septa of the testis. The septa converge at the center to form the mediastinum testis (König and Liebich 2015). Although the septa in the cat are thin connective tissue, they form thicker-looking compartments in Canidae, which includes the red fox. Testicular parenchyma consists of the tubuli seminiferi contorti, tubuli seminiferi recti, rete testis, and ductuli efferentes testis (König and Liebich 2015). The testicular artery (arteria testicularis) is separated from the abdominal aorta (aorta abdominalis) (Dursun 2008, Rerkamnuaychoke et al. 1991). The testicular artery is of the same muscular type as that in bovine (Hees et al. 1984). Together, the testicular artery and testicular vein contribute to the formation of the plexus pampiniformis (Polguj et al. 2009, Polguj et al. 2011). It was indicated the existence of direct communication between the testicular artery and testicular vein in the spermatic cord of cattle, goats, dogs, and boars (Rerkamnuaychoke et al. 1990). On the testes during the reproductive periods of young and adult red foxes has reported that the velocity of the blood flow in the testicular arteries is closely related to testicular weight, except during prepubertal periods (Joffre 1977). In rodents, there is an association between testicular weight and baculum (os penis) length (Adebayo et al. 2011).

The penis consists of three parts the radix, corpus, and glans. The glans is divided into the bulbus glandis and pars longa glandis (Evans and Lahunta 2013, Miller 1964). The radix consists of two crus penises that emerge from either side of the ischiatic arch (arcus ischiadicus). The crus penises combine to form the corpus penis. The inner structure of the corpus penis comprises a septum made of connective tissue. Although the septum is a perforated sheet of tissue in the carnivore, it also contains slit holes in other types of animals (Bahadır and Yıldız 2014). There are two types of penis musculocavernous and fibroelastic. In carnivores, the erectile tissue in the musculocavernous type contains a large quantity of blood. In canids, the distal end of the corpus cavernosum has been modified for the os penis, in which the urethra in the corpus spongiosum is located. This location of the urethra within the groove of the os penis might prevent the passage of urethral stones and cause an obstruction (König and Liebich 2015). The penis is fed by the penis artery (arteria penis) and urethral artery (arteria urethralis) which separate from the internal pudendal artery (arteria pudenda interna) (Dursun 2008).

The prostate gland divides into two parts, corpus prostata and pars disseminate prostatae. The prostate gland is the largest in horses, cats, and dogs. In carnivores, the large, single-lobed prostate, comprises prostate artery (arteria prostatica) and middle rectal artery (arterial rectalis media), the two branches of the internal pudendal artery (Dursun 2008, König and Liebich 2015). The prostate is large in the dog and it surrounds the entire urethra, and it surrounds a large part of it in the cat (König and Liebich 2015). The secretory alveoli of the prostate gland, which are tubuloalveolar, are arranged radially to the lumen of the urethra and open to the lumen from short channels. The alveoli consist of a single layer of prismatic or cubic epithelium. The proximal part of the channels beginning from the alveoli is covered with transitional epithelium (Özer 2010).

Literature information reporting the morphological and histological features of the reproductive system of the red fox, which has an important place in the wild life, especially in hunting, has not been obtained. There are studies on the male genitalia of Carnivora species (e.g. dog, cat, badger and hoary fox) (Erdoğan 2011, Karan et al. 2010, Mehanna et al. 2018, Saadon 2016), but there are limited studies on the male genitalia of the wild red fox in the literature. Most assisted reproductive technology development studies are used as models of pet dogs and farm foxes from the Canidae family. Farm foxes also have an economic importance in the fur industry. Artificial insemination in fox farming is one of the main methods applied in Scandinavian countries (Amstislavsky et al. 2012). This study aimed to compare the genitalia of the male red fox with that of other carnivora species in hopes to contribute information to the literature on artificial insemination (Yatu et al. 2018), castration, and urolithiasis surgeries in the species. In accordance with this purpose arterial vascularization, macroanatomical and histological structure of testis, penis and prostate were examined.

MATERIAL and METHODS

This study was obtained permission from the General Directorate of Nature Protection and National Parks of the Ministry of Agriculture and Forestry (21264211-288.04/E.893702). Five male red foxes at similar ages (2-4 age) and 2-6 kg weight obtained from Kafkas University Wildlife Rescue and Rehabilitation Center and Kafkas University Veterinary Faculty Clinics were used in the study. These foxes were brought to the clinics because they were either shot or injured in traffic accidents and could not be saved despite all interventions. The study was conducted in the Department of Anatomy and Histology-Embryology of Kafkas University Faculty of Veterinary Medicine.

Anatomical Investigation

The abdominal aorta was ligated after fixation. Each red fox's arterial system was perfused with physiological saline and red latex (PolyTek, Midwest-Janesville, WI, USA) before fixing the aorta and refrigerated for 10 d to solidify the latex. After solidification, the arteries supplying the testis, penis, and prostate were dissected and photographed using the Kodak Easyshare M320 Digital Camera (Eastman Kodak Company, Rochester, NY, USA). Nomina Anatomica Veterinaria 2017 was used for identifications of the vessels. The testes and prostates were weighed using a sensitive balance (min 0.0001 g, max 220 g, precisa code XB220A). The testes, penis, prostate, and os penis were measured by using digital caliper (Company). All morphometric parameters were expressed as Mean ± Standard Deviation (SD) in the SPSS (version 20.0) packaged software.

Histological Investigation

Testis, penis, and prostate tissue samples were fixed in 10% formaldehyde solution for 24 h at room temperature then embedded in paraffin for histological examination. Serial sections were taken at 5 µm thickness, and then they were deparaffinized, rehydrated, and stained with Mallory's modified triple staining (Crossmon 1937) to observe the general structure of the testis, penis and prostate gland. The sections were examined using the Olympus BX51 light microscope (Tokyo, Japan).

RESULTS

Anatomical Results

The internal iliac artery (arteria iliaca interna) was separated into its two branches—the caudal gluteal artery (arteria glutea caudalis), which is the thicker branch and leads dorsally, and the internal pudendal artery (arteria pudenda interna), which is the thinner branch and leads ventrally (Fig. 1). The testicular artery separated asymmetrically from both sides of the abdominal aorta at the L5 (fifth lumbal) vertebra (5.50 mm between right and left), passed through the spermatic canal (canalis inguinalis), and terminated in the testes (Fig. 2). The right testicular artery (a. testicularis dextra) was separated from the abdominal aorta before the left testicular artery (a. testicularis sinistra). The mean length of the testis was 24.67 ± 4.92 mm, mean width was 14.10 ± 3.77 mm, and the mean weight was 2.99 ± 1.94 g (Table 1).

The penis artery was separated from the internal pudendal artery and divided into the artery of the bulb of the penis (arteria bulbi penis), deep artery of the penis (arteria profunda penis), and dorsal artery of the penis (arteria dorsalis penis) (Fig. 3). As shown in Fig. 4, the mean os penis length was 49.53 ± 5.35 mm (4.9 cm); the mean penis length was 96.56 ± 7 mm (Table 2). The os penis was thick at the proximal end and thinned toward the distal end, resulting in a cartilage tip. In one in five animals, the penis was found to exhibit sigmoid flexure (flexura sigmoidea penis) 40.90 mm after the ischiatic arch (Fig. 3).

The internal pudendal artery was observed to divide into two branches—the prostate artery and penis artery—while advancing to the ventral rectum. It was also observed that both sides of the prostate artery were divided into two branches before entering the prostate gland (Fig. 5). One branch terminated at the urinary bladder (vesica urinaria) without involving the prostate (middle rectal artery), while the other terminated in the prostate (a. ductus deferens). More branches terminated on the right side of the prostate than on the left side. The length and width of the prostate were measured (Fig. 6). As indicated in Table 3, the mean prostate length was 17.13 ± 1.03 mm, mean width was 13.04 ± 3.22 mm, and the mean weight was 1.74 ± 0.77 g.

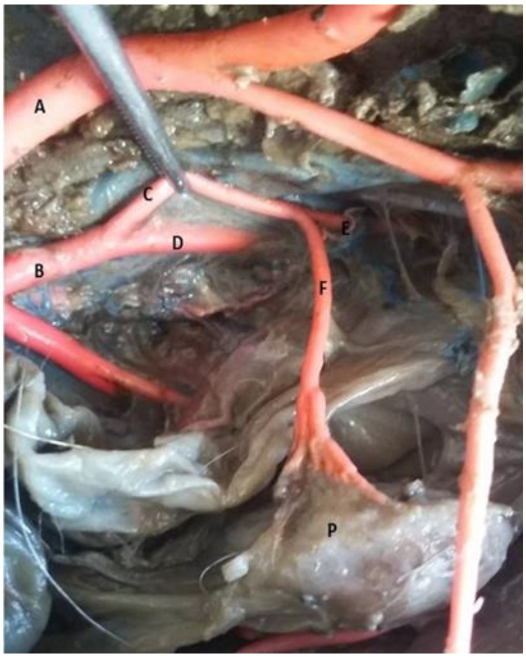

Figure 1: Arterial system feeding the prostate gland in red foxes
(**A**: a. iliaca externa, **B**: a. iliaca interna, **C**: a. pudenda interna, **D**: a. glutea caudalis, **E**: a. penis, **F**: a. prostatica, **P**: gl. prostatica)

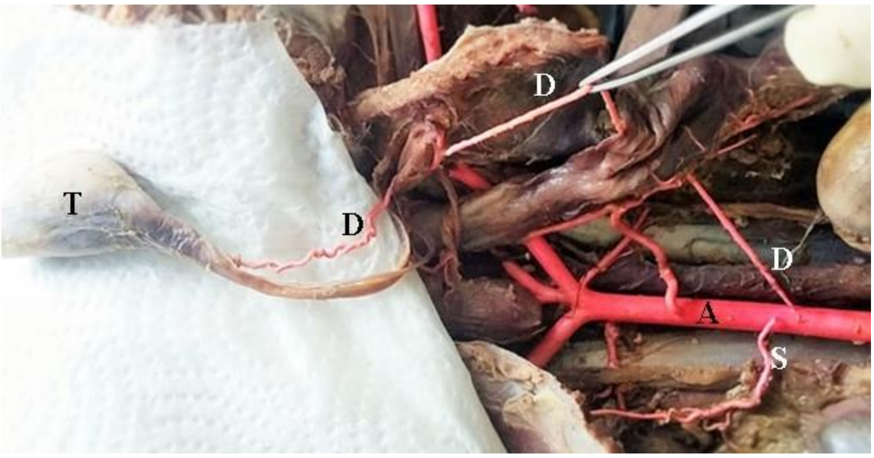

Figure 2: Arterial system feeding the testis in red foxes. **(A:** Aorta abdominalis, **D:** a. testicularis dextra**, S:** a. testicularis sinistra**, T:** Testis)

Table 1. Testis measurements in red foxes.

Measurement	RF1		RF2		RF3		RF4		RF5	
	right	left	right	left	right	left	right	left	right	left
Length of testis (mm)	19.45	18.14	28.3	31.05	30.40	29.44	23.65	20.97	25.78	19.56
Mean length of testis (mm)	18.80		29.68		29.92		22.31		22.67	
Width of testis (mm)	9.25	8.96	17.36	17.15	19.01	17.43	15.56	12.59	13.45	10.32
Mean width of testis (mm)	9.105		17.255		18.22		14.05		11.88	
Weight of testis (g)	0.967	0.884	5.280	4.99	4.718	4.871	3.035	2.564	1.53	1.026
Mean weight of testis (g)	0.9255		5.135		4.7945		2.7995		1.278	

RF: Red fox

Table 2. Penis measurements in red foxes.

Measurement	RF1	RF2	RF3	RF4	RF5	Mean ± SD
Length of penis (mm)	94.74	93.02	89.35	107.80	97.89	96.56 ± 7.00
Length of os penis (mm)	42.61	53.74	51.22	46.13	55.45	49.53 ± 5.35

RF: Red fox, **SD:** Standard deviation

Figure 3: A sigmoid flexure was observed in one in five red foxes and penil artery (**A**: a. profunda penis of penil artery).

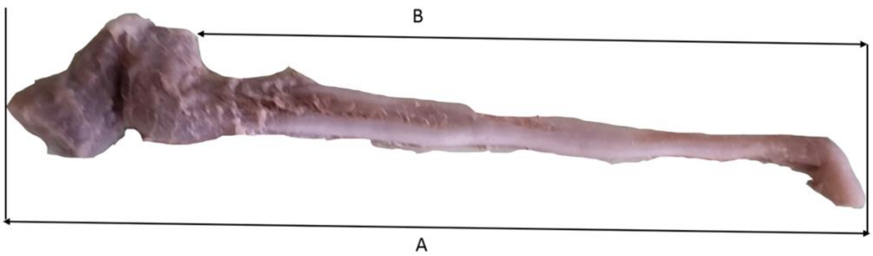

Figure 4: Penis and os penis measurements in red foxes (**A**: length of the penis **B**: length of os penis + cartilage end).

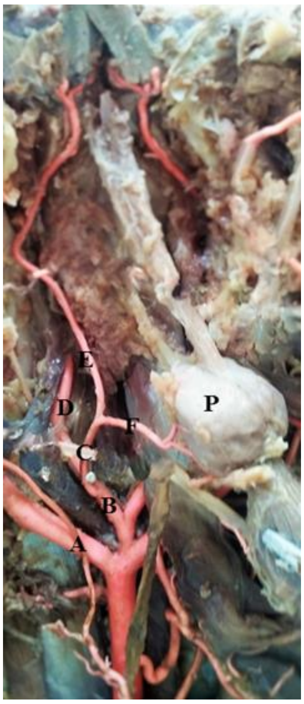

Figure 5: The arterial feed of penis and prostate gland in red foxes (**A**: a. iliaca externa, **B**: a. iliaca interna, **C**: a. pudenda interna, **D**: a. glutea caudalis, **E**: a. penis, **F**: a. prostatica, **P**: prostate gland).

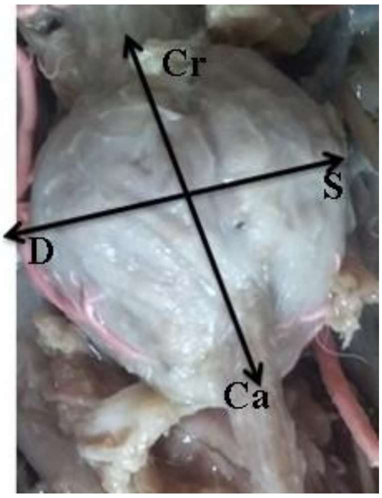

Figure 6: Prostate gland measurements in red foxes (**Cr**: cranial, **Ca**: caudal, **D**: dexter, **S**: sinister).

Table 3. Prostate measurements in red foxes.

Measurement	RF1	RF2	RF3	RF4	RF5	Mean ± SD
Length of prostate (mm)	15.60	17.74	17.10	18.34	16.89	17.13 ± 1.03
Width of prostate (mm)	13.56	15.78	16.40	9.03	10.45	13.04 ± 3.22
Weight of prostate (g)	1.545	2.95	1.732	0.8194	1.685	1.74 ± 0.77

RF: Red fox, **SD**: Standard deviation

Histological Results

The testis is surrounded by the external tunica albuginea and tunica vasculosa containing branches of blood vessels in the histological examination. The tunica albuginea is divided into lobes by the septum. Leydig cells were observed among the seminiferous tubules (Fig. 7).

The penis is surrounded by the tunica albuginea, and the penile urethral canal in the root is covered with pseudostratified Columnar epithelium. As it progresses toward the head of the penis, the epithelium becomes a single layer and columnar(Fig. 8).

The prostate gland is surrounded by a capsule of connective tissue containing smooth muscle cells. The prostate is divided into lobules by trabeculae, and serous tubuloalveolar secretory glands were observed within its lobules. These secretory glands are lined with simple columnar epithelium. Several smooth muscle cells were observed in the interlobular connective tissue (Fig. 9).

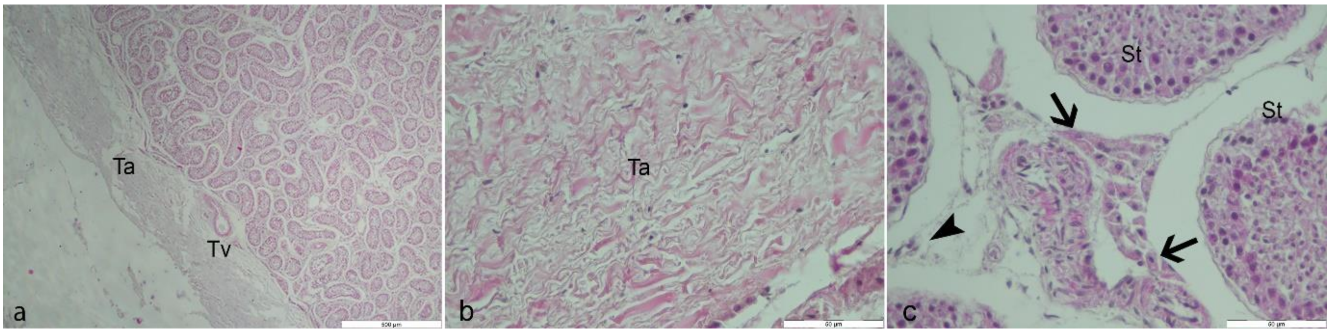

Figure 7: a (Bar 500 µm), **b** (Bar 50 µm), **c** (Bar 50 µm). Red fox testis. **Ta**: Tunica albuginea, **Tv**: Tunica vasculosa, **St**: Seminiferous tubule, **arrow head**: septum, **arrow**: Leydig cell. Mallory's modified triple staining.

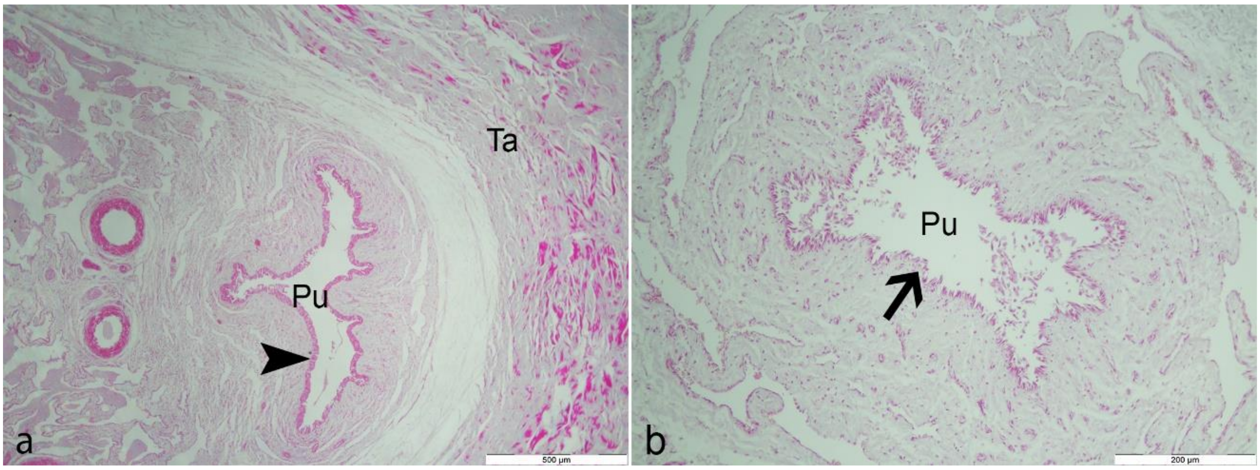

Figure 8: a (Bar 500 µm), **b** (Bar 200 µm). Red fox penis. **Pu**: Penis urethra, **Ta**: Tunica albuginea, **Arrow head**: Pseudostratified columnar epithelium, **Arrow**: columnar epithelium, **a**: penis root, **b**: head of penis. Mallory's modified triple staining.

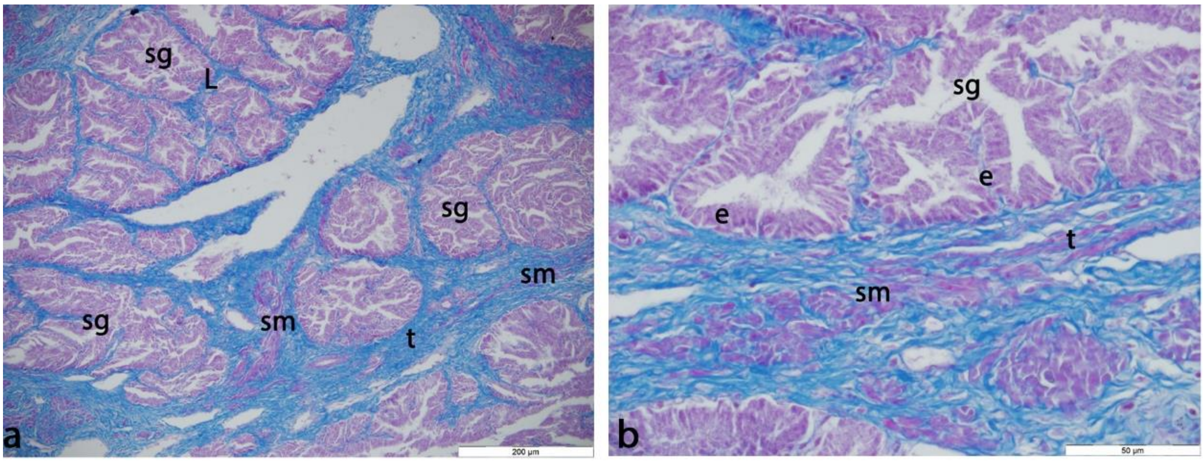

Figure 9: **a** (Bar 200 µm), **b** (Bar 50 µm). Red fox prostate gland. **L**: Lobules, **sg**: secretory glands, **sm**: smooth muscle cells, **t**: trabecula, **e**: epithelium. Mallory's modified triple staining.

DISCUSSION and CONCLUSION

The mean weight of the testes is 8 g in a 25-kg dog (Miller 1964), 1.25 g in rodents (Adebayo et al. 2011), and 2.99 ± 1.94 g in the red fox. The mean length of the testes in dogs is 30 mm (Evans and de Lahunta, 2013) and 24.67 ± 4.92 mm in the red fox, and the mean width is 20 mm in dogs (Miller 1964) and 14.10 ± 3.77 mm in red foxes. The difference between the values is believed to be the result of age, weight, and race differences. The testicular artery separates from the abdominal aorta at the L3–L4 vertebrae in dogs (Nickel et al. 1981), while it separates at the L5 vertebra in the red fox. As observed, these locations between the dog and red fox are very near to each other. Both the endocrine and exocrine glands of the testis of some animal species are covered with a vascular layer called the tunica vasculosa. In dogs and rams, the tunica albuginea is superficial, while in stallions and boars it is profound. In horses, the tunica albuginea may contain smooth muscle fibers (Banks 1993, Bacha and Bacha 2000). In the red fox, the inner surface of the tunica albuginea is covered with a vascular layer; no smooth muscle cells were found.

The mean penis length was 96.56 ± 7.00 mm in red foxes, 170 mm in Iraq dogs (Alaa 2016), 179 mm in all other dogs (Miller 1964), 50 mm in hedgehogs (Atalar and Ceribaşı 2004), 7.17 mm in the Western European hedgehog (Akbari et al. 2018), 54.6 mm in rodents (Adebayo et al. 2011), and 50–80 mm in the cat (König and Liebich 2015). The penis length in the red fox is between the minimum and maximum values of that in other animals within the same family. The length of the os penis is 130 mm (Alaa 2016), ≥100 mm (Nickel et al. 1981) in some dogs, 5 mm in cats (Bahadır and Yıldız 2014), and 12.6 mm in rodents (Adebayo et al. 2011). The mean length of the os penis in the red fox was 49.83 ± 5.35 mm. The penis and os penis in red foxes appear to be of average length when compared with that in other carnivores. As reported, the os penis thins from the proximal to the distal end and terminates in a cartilage tip (Gültiken et al. 2004). The penis artery is separate from the internal pudendal artery in male cats and rabbits (Takcı 1992). As reported, it was determined that the penis artery separates from the internal pudendal artery and divides into three branches—the bulb of the penis, the deep artery of the penis, and the dorsal artery of the penis (N.A.V. 2017). A pair of corpus cavernosum, separated by the septum, is observed in the dog penis, and each is reported to be surrounded by tunica albuginea. It has also been reported that the urethra is lined with keratinized stratified squamous epithelium as it progresses toward the head of the penis (Alaa 2016, Akbari 2018). In the red fox, it was determined that the urethral duct originated with pseudostratified columnar epithelium at the root of the penis, and the penile urethra changed into a simple columnar epithelium toward the head of the penis.

The prostate was the only male reproductive gland in red foxes to be reported in the literature (Halıgür and Özkadif 2016). The mean weight of the prostate in 25-kg dogs is 6.8 g (Evans and de Lahunta, 2013, Miller 1964), while it is 1.74 ± 0.77 g in red foxes; the mean length is 17 mm in dogs (Evans and de Lahunta 2013, Miller 1964) and 17.13 ± 1.03 mm in red foxes, and the mean width is 26 mm in dogs (Evans and de Lahunta 2013, Miller 1964) and 13.04 ± 3.22 mm in red foxes. Compared to that in the other carnivores studied, the prostate of the red fox is heavier and thinner but the length is similar. In this case, we suggest that the red fox prostate is the same length as that in the dog but weighs less. In the male rabbit, the prostate artery emerges from the caudal gluteal artery and divides into the middle rectal artery and urethral artery. In male cats, the prostate artery is separated from the internal pudendal artery. After dividing into the artery to the ductus deferens (arteria ductus deferentis) and middle rectal artery, it terminates at the lateral face of the prostate (Takcı 1992). In red foxes, the internal pudendal artery divides into two branches—the prostate and penis arteries—while advancing within the ventral part of the rectum. Both sides of the prostate artery are divided into two branches before entering the prostate gland. While one of these branches, the middle rectal artery, terminates on the urinary bladder without involving the prostate, the other branch, the artery to the ductus deferens, terminates on the prostate. It was found that the course of blood flow in the red fox prostate was the same as that in the cat. Although the prostate is a seromucous gland in most animals (Bacha and Bacha 2000), it was observed that it is a serous gland in red fox like dog. The tubuloalveolar glands are embedded in the fibrous collagenous connective tissue, which includes smooth muscle cells (Kuehnel 2003). The stroma of the canine prostate also contains elastic fibers (Marettová 2017). It has been reported that the epithelium of the secretory glands are simple columnar structures in dogs (Bartsch and Rohr 1980) but are cuboidal to tall columnar epithelial cells in Iraqi dogs (Hussin 2016). It has been demonstrated that the secretory glands are lined with simple columnar epithelium in the red fox prostate. The contents of gland secretions comprise citrate, lactate, and cholesterol (Smith 2008).

In conclusion the arteries suppling the prostate, penis, and testes in red foxes were observed through dissection, their mean measurements were taken, and histological structures were examined. It is believed that the findings of this study will contribute valuable information to the literature on artificial insemination, castration, prostate, and urolithiasis surgeries on red foxes and carnivores.

Conflict of Interest: The authors declared that there are no actual, potential, or perceived conflicts of interest for this article.

Ethical Permission: Permission was obtained for this study from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (21264211-288.04 / E.893702).

Financial Support: No support was received from any institution for this study.

Acknowledgement: We would like to thank Kafkas University Wildlife Conservation, Rescue and Rehabilitation Center for their support in this study.

Description: The summary of this study was presented orally at the 1st International Veterinary Anatomy Congress in Afyon/Sandıklı on 13-16 September 2017.

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