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Anatomical and histological structure of ovary and salpinx in Red Foxes *(Vulpes vulpes) (Linnaeus, 1758)*

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Abstract: The Red fox is the largest of the true foxes and the most abundant wild member of the carnivora. This study aimed to determine the anatomical and histological structure of the ovary and salpinx of the Red foxes. The ovary and salpinx of four Red foxes of similar ages, which could not be rescued by the Center despite all interventions, were dissected. Measurements were taken from the right-left ovary and salpinx using digital callipers. The weights of each ovary and salpinx were measured using a precision scale (min: 0.0001 g, max: 220 g). The mean length of the ovary was 13.43 ± 2.38 mm, the width was 6.28 ± 1.99 mm, the thickness was 4.89 ± 0.18 mm, and weight was 0.93 ± 0.14 g. The mean length of the salpinx was 76.22 ± 3.02 mm, the width was 1.98 ± 0.07 mm, and the weight was 0.53 ± 0.31 g. Crossman's triple staining method was applied for histological examination of the ovary tissue. It was observed that the ovary was surrounded by germinative epithelium from the outside and consisted of the cortex with different follicles in the development stage, and the medulla layer with plenty of blood vessels and nerve plexuses inside. In conclusion, we believe that the findings of this study may be useful for further studies on foxes and surgical operations (ovariectomy, ovariohysterectomy). In addition, it is aimed to eliminate the insufficient information regarding the reproductive system of wild animals in this study.
Keywords: Anatomy, histology, ovary, Red fox, salpinx.

Kızıl tilkilerde (*Vulpes vulpes) (Linnaeus, 1758)* ovaryum ve salpinx'in anatomik ve histolojik yapısının incelenmesi

Özet: Kızıl tilki, tilkilerin en büyüğü ve vahşi yaşamın bir üyesi olan carnivorların en çok görülenidir. Bu çalışma ile kızıl tilki ovaryum ve salpinx'inin anatomik ve histolojik yapısını belirlemek amaçlandı. Tüm müdahalelere rağmen merkez tarafından kurtarılamayan benzer yaşlardaki 4 adet kızıl tilki ovaryum ve salpinx'i diseke edildi. Ölçümler digital kumpas yardımıyla sağ-sol ovaryum ve salpinx'ten alındı. Her bir ovaryum ve salpinx'in ağırlığı digital hassas terazide tartıldı (min: 0,0001 g, max: 220 g). Ortalama ovaryum uzunluğu 13,43 ± 2,38 mm, genişliği 6,28 ± 1,99 mm, kalınlığı 4,89 ± 0,18 mm ve ağırlığı 0,93 ± 0,14 g idi. Ortalama salpinx uzunluğu 76,22 ± 3,02 mm, genişliği 1,98 ± 0,07 mm ve ağırlığı 0,53 ± 0,31 g olarak belirlendi. Ovaryum dokusuna histolojik olarak incelenmesi amacıyla Crossman'ın üçlü boyama yöntemi uygulandı. Ovaryum'un dıştan germinatif epitelle çevrili olduğu, korteksinde farklı gelişim aşamasındaki foliküllerin bulunduğu ve içte bol miktarda kan damarı ve sinir pleksuslarının yer aldığı medulla tabakasından oluştuğu gözlendi. Sonuç olarak bu çalışmaya ait bulguların tilkiler üzerinde yapılacak olan cerrahi operasyonlarda (ovariektomi, ovaryohisterektomi) faydalı olacağı düşünülmektedir. Ayrıca, yapılan çalışma ile özellikle yaban hayvanlarının üreme sistemi ile ilgili bilgi yetersizliğinin giderilmesi amaçlanmaktadır.
Anahtar sözcükler: Anatomi, histoloji, Kızıl tilki, ovaryum, salpinx.

Introduction

The red fox (*Vulpes vulpes*) is a mammal of the Canidae family of carnivora, 70-90 cm tall and 7-10 kg of weight. This species, which can be seen in many regions of the world, is also available in Turkey. It is in the Canidae family. There are species living on the continent of Europe, Asia, North Africa, and America. It is a seasonally monogamous carnivores with big ears and a

long tail, famous for its intelligence and fraud (17). It has a 3-month gestation period and reaches sexual maturity at the age of 7-10 months (3).

The ovaries, which are the female reproductive organs, are a small, longitudinal, oval located in the caudal of the kidneys in the abdominal cavity (*cavum abdominis*) (28). In medium-sized adult bitches, the left ovary is located approximately 12 cm behind the 13th rib (*costa*) and 1-3 cm caudal of the kidney (*ren*), while the right ovary is located approximately 10 cm caudal of the last rib (*costa*) on the right (10). Its mean length is 20 mm in dogs and 10 mm in cats (28). It is a mean of 3-12 g in weight and 15 mm in diameter. Canine ovary length is 2 mm, while feline is 8-9 mm (4). The mean length of ovaries is 15 mm, width 7 mm, thickness 5 mm, weight 0.3 gr in dogs of 25 kg (10, 13). Each caudal end is associated with the kidneys (*renes)*. It is located at the level of the third and fourth lumbar vertebrae (*vertebrae lumbalis*) or between the last rib (*costa*) and iliac crest (*crista iliaca*) (28). The left ovary is usually larger than the right. The left ovary participates with the lateral of the spleen (*lien*) (28). The right ovary is located between the right part of the duodenum and the right abdominal wall. In bitches, each ovary is hidden inside the ovarian bursa (*bursa ovarica*), which is a completely peritoneal pouch (*peritoneum)*, while the cat is partially located in this pouch (28). Ovaries are connected by their mesovarium, which is the cranial part of the broad ligament of the uterus, (*ligamentum latum uteri*), which connects the reproductive organs to the abdominal wall and is a peritoneum wrap. Ovaries are found in the ovarian bursa (*bursa ovarica*), which formed by the mesosalpinx externally, the mesovarium with the round ligament of the ovary (*ligamentum ovarii proprium*) and the mesovarium internally. It has exocrine and endocrine functions. It is the exocrine function giving ovum from ovary to genital canal with oogenesis, endocrine function that produces its own hormones (23). It consists of two parts in the ovary: cortex ovarii (*zona parenchymatosa*) on the outside and medulla ovarii (*zona vasculosa*) on the inside (4). In all mammals except for mare, the cortex settles in the periphery. The structure of the cortex and medulla is very variable according to the stages of the sexual cycle, age, and type (23). Ovarian tissue is surrounded by surface epithelial cells that change from outer to cubic to prismatic or flattened, and this area is called stratum germinativum. The cells in this area are also called germinative epithelium (9). Germinative epithelial cells can be cubic-prismatic in developing females, prismatic in adults and flattening epithelium at later ages (12, 23). Under the stratum germinativum, tunica albuginea is found with connective tissue (5, 29). In most mammalian species, cortex and medulla can be distinguished. The cortex forms the follicles at different stages of development and the reticulum threads with collagen surrounding them (13). The medulla consists of blood vessels, lymph vessels, nerves, elastic, and reticular connective tissue fibers (5, 20).

Salpinx (*oviduct, tuba uterina*) is the closest part of the ovum paths to the ovary. It has an environment suitable for fertilization. Macroscopically, it has 3 parts the infundibulum, ampulla, and isthmus (23). There are many extensions called fimbria at the ends of the infundibulum, which is closest to the ovary. The infundibulum opens to the ampulla where fertilization takes place with ostium abdominale. After the ampulla, oocyte proceeds to isthmus, which is narrow and curved. Isthmus opens to cornu uteri with opening of the uterin tubae (*ostium uterinum tuba*) (15). In carnivores, salpinx (*oviduct, tuba uterina*) is small and mean length 50-80 mm (27, 28) or 60-100 mm and 1-3 mm in diameter (13). Salpinx (*oviduct, tuba uterina*) epithelium plays an important role in early embryonic development, in the opposite direction of ovum and spermatozoon, providing an appropriate environment for oocyte maturation and sperm capacitation (23).

There are studies on female genital system in various animal species (1, 6, 14, 26). However, in the literature searches, a study on the anatomy and histology of the female genital system in red foxes was not found. In this study, the red fox is a member of the wildlife (*Vulpes vulpes*) was conducted to examine the ovaries and salpinges of the anatomical and histological structure. Literature information reporting the morphological and histological features of the reproductive system of the red fox, which has an important place in the wildlife, especially in hunting, has not been obtained. We think that we will add new literature knowledge to the scientific world in order to overcome the mentioned deficiency with the presented study. In addition, the differences between the red foxes and other carnivores genital ducts were compared with the morphological and histological similarities. Considering these similarities and differences, it is thought that the findings obtained during the interventions (ovarian cysts or tumors) (18) may be a reference value or may help ovariectomy operations due to genital canal problems.

Material and Methods

These foxes (n=4) were brought to Kafkas University Wildlife Rescue and Rehabilitation Centre (Kars, Turkey) for various reasons such as traffic accidents and firearm injury, but could not be saved or needed to be euthanised according to the Wildlife Rescue and Rehabilitation Centre staff. This study was carried out after the approval from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (21264211-288.04-E.115365) and Ethical

Committee of Animal Experiments of Kafkas University, Kars, Turkey (KAÜ-HADYEK / 2019-018).

Anatomical examination: The study was carried out in Kafkas University Veterinary Faculty Anatomy Department and Histology and Embryology Department laboratory. The ovaries and salpinges (*oviduct, tuba uterina*) of red foxes of a similar age were dissected. Measurements were taken using a digital calliper (0.01, BTS, UK.). The ovaries lengths were determined from the extremitas uterina and extremitas tubaria. The ovaries width were measured between the margo mesovaricus and margo liber. Ovaries thickness were standard between the facies medialis and facies lateralis. Salpinges (*oviduct, tuba uterina*) length was measured from the between infundibulum and cornu uteri. The weights of the organs were measured using precision scales (min: 0.0001 g – max: 220 g, code: XB220A, Precisa®, Swiss). Nomina Anatomica Veterineria 2017 is used for scientific terms (22).

Histological examination: Ovarian tissue samples taken for histological examinations were determined in 10% formalin solution for 24 hours, then routine histological follow-up solutions and then blocked in paraffin. Crossman's triple staining (19) was applied to examine the histological structure of the 5 µm thick sections taken from paraffin blocks. The slides were examined under an Olympus EP50 microscope and their photos were taken.

Statistical analysis: The mean and standard error values of the morphometric measurements taken were determined in the SPSS® (20.0 version) package program. All morphometric parameters were expressed as mean ± standard error (SE). Lengths were expressed in millimeters and weights were stated in grams.

Results

Anatomical results: In anatomical examinations, it was observed that ovaries were oval shaped, completely in the ovarian bursa (*bursa ovarica*) between the 13th rib (*costae)* and the first lumbar vertebrae (*vertebrae lumbalis*). Ovary mean length is 13.43 ± 2.38 mm, width of 6.28 ± 1.99 mm (Figure 1), thickness 4.89 ± 0.18 mm, weight 0.93 ± 0.14 g, as determined (Table 1). The mean salpinx (*oviduct, tuba uterina*) length was 76.22 ± 3.02 mm, the width was 1.98 ± 0.07 mm, and the weight was 0.53 ± 0.31 g (Figure 2).

Histological results: The ovarian tissue of the foxes was surrounded by cubic germinative epithelial cells from the outside. In addition, a layer of connective tissue tunica albuginea was observed under the germinative epithelium. It was determined that the cortex layer was outside, and the medulla layer was inside (Figure 3). Follicles at different stages of development in the cortex layer (*primary, secondary, tertiary = Graafian follicles*) (Figure 4) and also the corpus luteum (Figure 5) were seen. While primordial follicles were distinguished in the periphery of the cortex, abundant blood vessels and nerve plexuses in the medulla attracted attention (Figures 3, 4, 5). It was observed that the intermediary tissue in the cortex and the medulla consisted of connective tissue.

Figure 1. Red fox ovary length (L) and width (W).

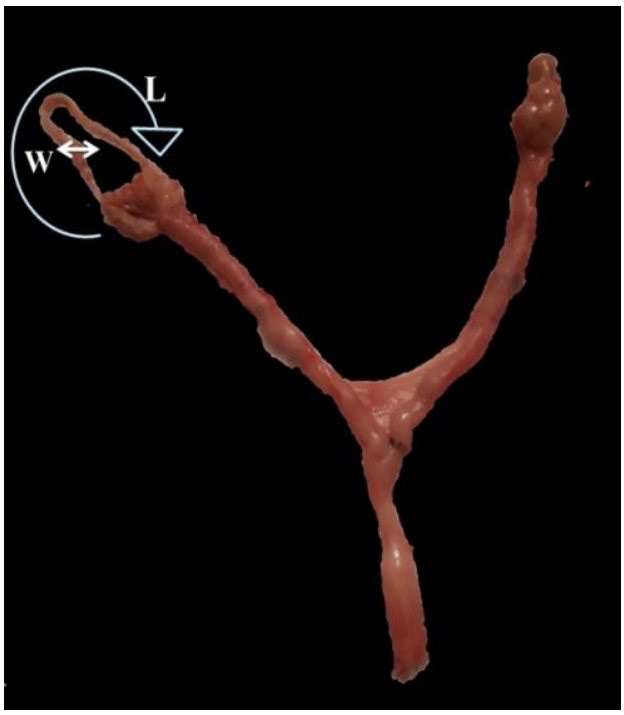

Figure 2. Red fox salpinx length (L) and width (W).

Table 1 Statistical results of some parameters of red fox ovary and salpinx.

Measurement	Mean ± SE		General mean ± SE
	Right	Left	
OV lengths (mm)	13.72 ± 0.79	13.14 ± 1.99	13.45 ± 0.96
OV width (mm)	6.84 ± 1.62	5.71 ± 0.79	6.28 ± 0.84
OV thickness (mm)	5.10 ± 0.53	4.69 ± 0.32	4.89 ± 0.29
OV weight (g)	1.08 ± 0.19	0.78 ± 0.04	0.93 ± 0.11
SAL lengths (mm)	74.96 ± 2.64	77.48 ± 1.05	76.22 ± 1.39
SAL width (mm)	1.93 ± 0.09	2.02 ± 0.02	1.98 ± 0.05
SAL thickness (mm)	0.68 ± 0.01	0.64 ± 0.02	0.66 ± 0.01
SAL weight (g)	0.50 ± 0.17	0.57 ± 0.19	0.53 ± 0.12

OV: Ovaryum, **SE:** Standard error, **SAL:** Salpinx.

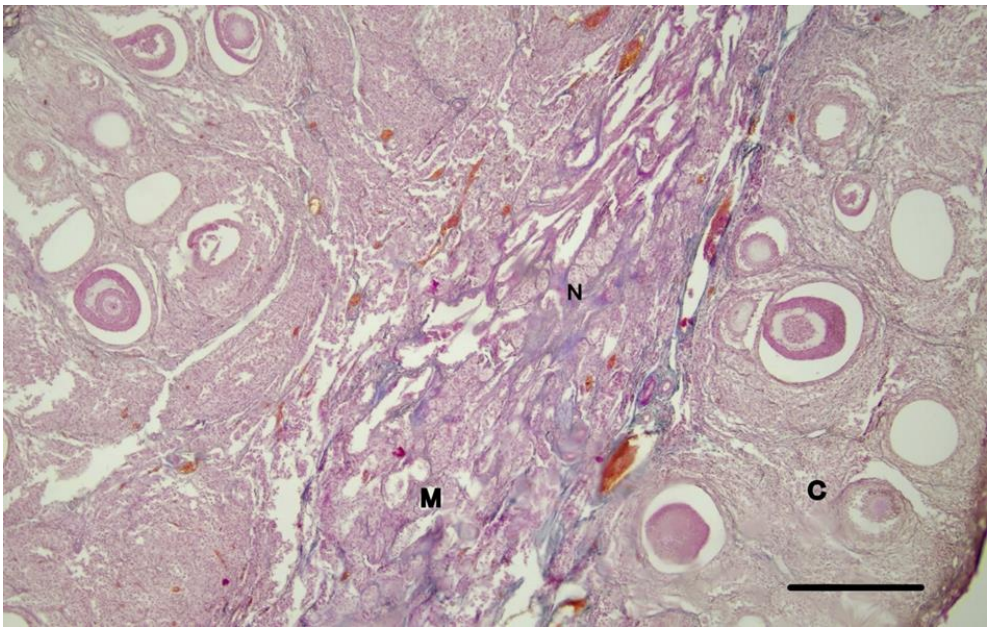

Figure 3. Red fox ovarian tissue. Medulla (M), cortex (C), nerve plexus (N), Triple Staining, Bar=500 µm.

Figure 4. Red fox ovarian tissue. Germinative epithelium (e), secondary follicle (S), Graafian follicle (G). Triple Staining, Bar=500 µm.

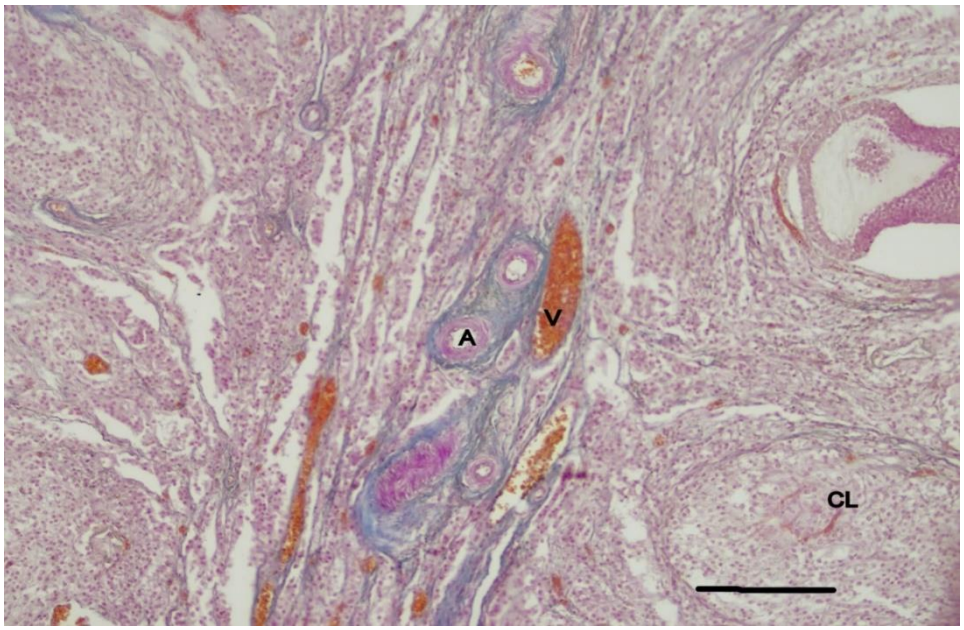

Figure 5. Red fox ovarian tissue. Arteriole (A), venule (V), corpus luteum (CL). Triple Staining, Bar=200 µm.

Discussion and Conclusion

Although there are about 270 species belonging to the Carnivora team, the Mustelidae, Felidae, Canidae and Ursidae families are mostly known. 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 economically important place in the fur industry. Artificial insemination in fox farming is one of the main methods applied in Scandinavian countries (2).

The shape of the ovaries was reported as kidney in horses and ellipsoids in other domestic mammals, while foxes were found to be oval (15). The mean ovary length is 10-20 mm in bitches (4) and 8-10 mm in cats (4, 15, 28). In another study in bitches, the length of the left ovary was 14.30 ± 2.20 mm and the right ovary was measured as 14.00 ± 2.00 mm (30). In foxes, the length of the ovaries was measured as 13.43 mm and the data obtained was between the mean length of the cat-dog ovaries. In bitches of 25 kg, the width of the ovary was 7 mm, the thickness was 5 mm (10, 13), while the foxes were 6.27 mm wide and 4.89 mm thick. In the bitches, the mean ovary weighs of 0.3-12 g (4, 10, 13). In our study, ovaries in foxes were determined as 1.12 g. It was determined that the mean weight of this ovaries obtained from the foxes was within the mean values determined for the bitches.

The mean length of salpinx (*oviduct, tuba uterina*) in carnivores is 50-80 mm (27, 28). However, there are sources reporting that the length of salpinx (*oviduct, tuba uterina*) varies between 60-100 mm in bitches (13). In foxes, the length of salpinx (*oviduct, tuba uterina*) was determined as 76.22 mm and was among the averages reported in the literature. While the width of salpinx (*oviduct, tuba uterina*) was 1-3 mm (13) in carnivores, this study was 1.97 mm in foxes. It is generally understood that the data obtained for salpinx (*oviduct, tuba uterina*) from foxes are similar to bitches.

The ovary is the organ where female germ cells (egg cells = ovum) are formed, at the same time, the genital cycle is regulated, and the hormones of the sex are released (8, 23). The ovary is covered with germinative epithelium ranging from prismatic to cubic to cubic to flattening. Under the germinative epithelium is found tunica albuginea which consisting of connective tissue. It has been reported that there are medulla inside and cortex outside in histological sections. It is stated that there are abundant blood vessels, lymph vessels, collagen, elastic fibers and nerve plexuses in the medulla. In cortex, it has been suggested that interstitial tissue rich in connective tissue where fibroblasts are abundant and follicles (primary, secondary, Graafian) at various stages of development are seen (5, 7, 29). In our study, findings similar to the information mentioned were observed. The corpus luteum, as well as the primary, secondary, Graafian follicles of the ovary, surrounded by cubic germinative epithelial cells from the outside, was seen in the cortex. In the medulla, abundant blood vessels and nerve plexuses attracted attention.

In conclusion with the presented study, information was given about the anatomical and histological structures of ovaries and salpinges from the female genital organs of the Red fox. Working with wild animals is very few in number due to difficulties in procuring materials. Therefore, it is difficult to compare the similarities and differences of other animals with regard to organs, systems and their functioning. It is noteworthy that the studies conducted are mostly based on observation. In addition, no study has been found to examine the

morphological and histological examinations of the genital systems of red foxes which are examples of wildlife. With this study, it is aimed to overcome the lack of literature in this regard. In addition, findings obtained during interventions (ovarian cysts or tumors) that can form in the genital canal (ovarian cysts or tumors), especially in red foxes, may be reference values. We believe that oocyte storage (25), cloning (11, 16), ovarian transplantation (21, 24) operations and ovariectomy operations that will be performed due to genital canal problems will be contributed.

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Ethical Statement

This study was carried out after the approval from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (21264211-288.04-E.115365) and Ethical Committee of Animal Experiments of Kafkas University, Kars, Turkey (KAÜ-HADYEK / 2019-018).

Conflict of Interest

The authors declared that there is no conflict of interest.

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