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RESEARCH ARTICLE

Evaluation of Testicular Arterial Doppler Ultrasonography and Spermatological Parameters in Pomeranian Dogs with Alopecia X

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ABSTRACT

The present study investigates the effects of vascular parameters in testicular vessels on sperm quality in Pomeranian dogs with alopecia X. In the study, Doppler parameters such as peak systolic velocity (PSV, cm/s), end-diastolic velocity (EDV, cm/s), resistive index (RI), and pulsatility index (PI) of testicular arteries were measured in the healthy control group and dogs in the alopecia X group, and motility, progressive motility, and abnormal morphology analyses were performed in semen collected from dogs. When the obtained results were examined, it was determined that RI and PI parameters were higher, and the examined spermatological parameters were significantly lower, in dogs in the alopecia X group compared to the healthy control group ($p < 0.05$). It is thought that increased RI and PI values may negatively affect spermatogenesis by causing insufficient blood flow in the testicles and that a decrease in sperm motility, progressive motility, and morphological parameters may be related to this situation. The results obtained provide important data on the negative effects of alopecia X on reproductive health and emphasize that this disease should be addressed in male dogs not only in terms of dermatological but also reproductive functions.
Keywords: Alopecia X, Doppler ultrasonography, Sperm evaluation, Testicular artery

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Alopecia X'li Pomeranian Köpeklerde Testiküler Arteriyel Doppler Ultrasonografi ve Spermatolojik Parametrelerin Değerlendirilmesi

ÖZ

Sunulan çalışma, alopecia X'li pomeranian ırkı köpeklerde testis damarlarındaki vasküler parametrelerin sperm kalitesi üzerindeki etkilerini araştırmayı amaçlamaktadır. Çalışmada sağlıklı kontrol grubu ile alopecia X grubunda bulunan köpeklerde testis arterlerinin pik sistolik hızı (PSV, cm/s), son diyastolik hız (EDV, cm/s), rezistif indeks (RI) ve pulzatilite indeksi (PI) gibi doppler parametreleri ile köpeklerden alınan spermadan motilite, progresif motilite ve anormal morfoloji analizleri yapılmıştır. Elde edilen sonuçlar incelendiğinde alopesi X grubunda bulunan köpeklerin sağlıklı kontrol grubuna kıyasla daha yüksek RI ve PI parametreleri, incelenen spermatolojik parametrelerde ise alopesi x grubunda bulunan köpeklerin sağlıklı kontrol grubuna kıyasla anlamlı derecede daha düşük olduğu belirlenmiştir ($p<0,05$). Artan RI ve PI değerlerinin testislerde yetersiz kan akışına yol açarak spermatogenezi olumsuz etkileyebileceği, sperma motilitesi, progresif motilite ve morfolojik parametrelerdeki düşüşün de bu durum ile ilişkili olabileceği düşünülmektedir. Elde edilen sonuçlar alopecia X'in üreme sağlığı üzerindeki olumsuz etkilerine dair önemli veriler sağlamaktadır ve bu hastalığın erkek köpeklerde yalnızca dermatolojik değil, aynı zamanda üreme fonksiyonları açısından da ele alınması gerektiğini vurgulamaktadır.
Anahtar Kelimeler: Alopesi X, Dopler ultrasonografi, Sperma analizi, Testiküler arter

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INTRODUCTION

The skin is the largest organ in the animal body and reflects the functionality and general health of the structures beneath it. Although the skin has many important functions, its primary role is to protects living beings from mechanical damage, chemicals, pathogens, ultraviolet radiation, and dehydration. Dermatological problems are among the most common health problems in pets. Since the skin is the organ most vulnerable to external factors and infections, such diseases can usually be easily noticed by pet owners (Weller et al. 2008).

Fertility can be affected by both hereditary and acquired skin diseases as well as their clinical management. The impact of skin diseases on fertility varies depending on the age of onset, severity, and chronicity of the disease. Although the prevalence of skin diseases negatively affecting fertility is relatively low, treatment strategies for the male reproductive system in these cases must be carefully evaluated (Abdel-Naser and Zouboulis 2016).

Alopecia refers to partial or complete hair loss. "Alopecia X" is a type of alopecia caused by a progressive hair cycle disorder without obvious systemic symptoms or triggering factors (Gökalp and Kırbaş 2021). This condition is most commonly seen in young adult dogs such as Pomeranian, Siberian Husky, Malamute, Samoyed, Keeshond, and Poodle, especially in unneutered males (Scott et al. 2001). Alopecia is commonly seen in young adult dogs, both males and females, regardless of their infertility status. Clinical symptoms are characterized by partial or complete alopecia on the neck, tail, caudo-dorsal region, perineum, base of tail, and eventually on the trunk, excluding the head and forelegs. In addition, skin hyperpigmentation may be observed in areas with alopecia (Frank et al. 2004; May et al. 2019). Although the etiology of alopecia X is not fully known, several theories have been proposed, including hyposomatotropism in mature dogs, gonadal sex hormone imbalance in intact males, and adrenal sex hormone disorders (Frank et al. 2003; Gökalp and Kırbaş 2021).

This study aimed to evaluate testicular arterial Doppler ultrasonography and spermatological parameters in male Pomeranian dogs diagnosed with alopecia X. The goal was to understand the effects of alopecia X on fertility and reproductive functions, to reveal vascular changes and spermatological parameters in these dogs and to gain new perspectives on the disease in terms of reproduction.

MATERIAL and METHODS

Animal Selection and Experimental Design

This study was approved by the Local Ethics Committee for Animal Experiments of Ondokuz Mayıs University (approval number: E-68489742-604.01-2400217735). Ten Pomeranian dogs were examined in the study. All dogs included in the study were owned dogs. The study design consisted of two groups: a healthy control group (n=5; Pomeranian breed, unsterilized dogs with no health problems) and an Alopecia X group (n=5; Pomeranian breed, unsterilized dogs diagnosed with Alopecia X). The mean age and weight in each experimental group were as follows: mean age in 2.40±1.14 years, kg 2.74±0.59 in the healthy group, mean age in 2.80±1.30 years, kg 2.66±0.61 in the alopecia x group. All dogs were regularly vaccinated, fed with commercial dry food, and had free access to water. Only dogs whose owners consented to participate the experiment were selected for ethical reasons. All animal owners signed a written consent form before participating in the study and were informed.

Within the scope of the study, male Pomeranian dogs of different age groups and body weights brought to Ondokuz Mayıs University Veterinary Faculty Animal Hospital with complaints of alopecia were evaluated. According to the anamnesis obtained from the owners brought to the internal medicine clinic, it was reported that hair loss was observed in the tail and back region for an average of 2 to 3 months. In the physical examinations, the areas with hair loss were examined with a Wood's lamp and deep skin scraping samples were taken for fungal and scabies diseases; however, no diagnosis was reached for these samples. In addition, routine hemogram examinations were performed on blood samples taken from the vena cephalica antebrachii of the dogs and collected in tubes containing ethylenediaminetetraacetic acid (EDTA), and it was determined that the obtained data were within the reference range. It was determined that cortisol, cholesterol, ALT, ALP, AST, and GGT values were within reference range. As a result of all these evaluations, the patients were diagnosed with alopecia X (Figure 1).

In this study, the dogs' testicles in both groups were examined in detail using arterial Doppler ultrasonography techniques. In addition, sperm samples were collected from each dog in the study and control groups and evaluated in terms of motility, progressive motility, and morphology.

Measurement of Testicular Arterial Hemodynamics by Doppler Ultrasonography Technique

Ultrasound examinations of testicular blood flow were performed in all dogs in the healthy control and alopecia X groups before sperm collection. To avoid the negative effects of anesthetic agents on TBF, the dogs were immobilized in a ventrodorsal position without sedation. An ultrasound device (Vetus 9, Mindray) equipped with a microconvex probe (6.5–7.5 MHz) was used for all evaluations. In this study, testicular blood flow was evaluated in the supratesticular (Fig. 2), intratesticular (Fig. 3), and marginal testicular (Fig. 4) arteries. A more detailed and comprehensive analysis of the distribution and waveform of blood flow was performed in the study; Doppler parameters such as peak systolic velocity (PSV, cm/s), end-diastolic velocity (EDV, cm/s), and resistive index (RI) and pulsatility index (PI) of the testicular arteries were recorded. The angle between the Doppler beam and the examined vessel was kept 45-60°, parallel to the blood flow direction. The Doppler probe was placed in a central region of the vessels, and at least three consecutive waves were monitored to ensure the automatic determination of spectral curves and vascular indices.

Figure 1. Dogs 1 and dogs 4 belong to the alopecia X group, respectively.

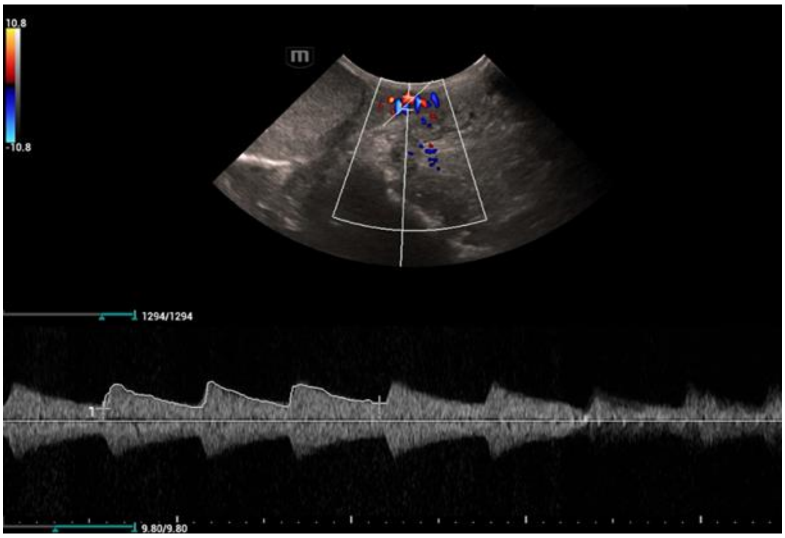

Figure 2. Hemodynamics of the left supra-testicular artery in dog number 1 belonging to alopecia X group

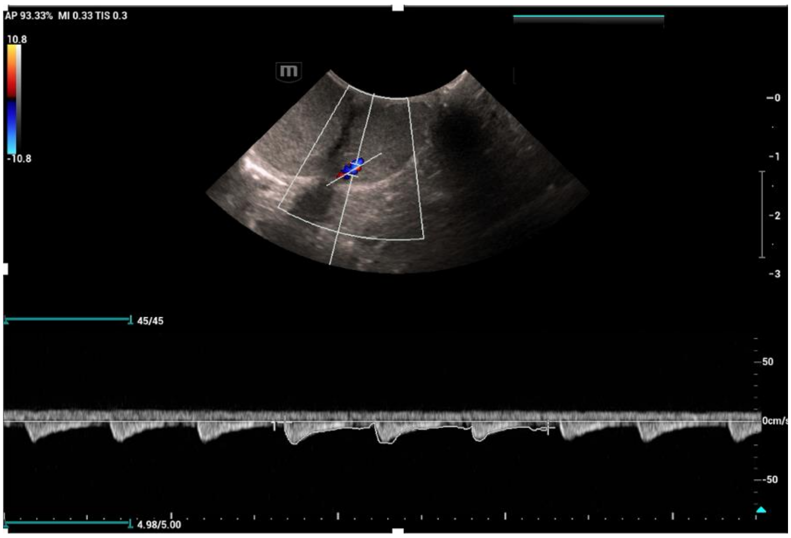

Figure 3. Hemodynamics of the right intratesticular artery in dog number 3 belonging to the alopecia X group

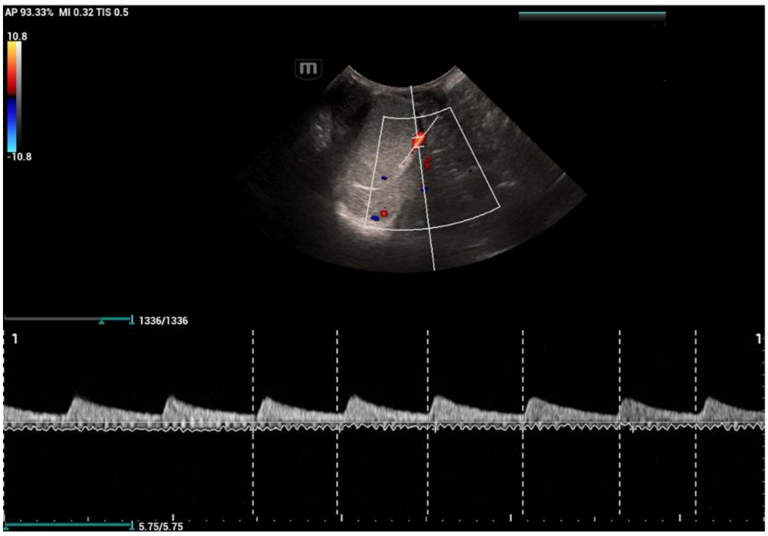

Figure 4. Haemodynamics of the right testicular marginal artery in dog number 5 belonging to the alopecia X group

Sperm Collection and Evaluation
Sperm samples were collected by digital manipulation from all dogs in healthy control and alopecia X group. The fresh spermatozoa obtained were diluted to a final concentration of 50-100x106/ml with a tris-based extender (3.025 g tris, 1.7 g citric acid, 1.25 g fructose, 5% v/v glycerol, and 20% v/v egg yolk with 100 mL distilled water) to ensure uniformity and then evaluated under the Computer Aided Sperm Analyzer (CASA) (SCA, Sperm Class Analyzer, Version 6.5.0.91; Microptic, Barcelona, Spain).

For the evaluation of spermatozoa motility, the obtained sperm were transferred to an Eppendorf tube. 10 µl of sperm was taken from each sample, placed on a slide, and covered with a coverslip. The prepared samples were examined using a phase-contrast microscope (Nikon, Eclipse, Tokyo, Japan), a heating stage at 37°C, 10x objective, and a camera recording at 60 frames/sec. As a result of the analyses, total motility (%) and progressive motility (%) parameters were obtained by measurements made in at least five different microscope fields after the dilution rate of the sperm sample was entered into the software. The results were recorded for statistical analyses.

For the morphological evaluation of spermatozoa, the morphology module of the CASA system and the SpermBlue® stain kit (Microptic, Spain) were used. 10 µl of the sperm sample was taken, and a smear was prepared on the slide and left to dry. After the drying process, the slides were dipped into a jar containing SpermBlue dye at room temperature and kept for 2 minutes. After the staining process, the slides were left to dry at an angle of 60-80 degrees. Once dried, at least 200 spermatozoa were evaluated morphologically using the CASA system phase-contrast microscope with a blue filter and a heating table. Spermatozoa were evaluated in terms of head, midpiece, tail, and total abnormal spermatozoon rate.

Statistical analysis

All analyses were performed using a commercially available software (SPSS®, IBM Inc.). All data are expressed as mean ± standard deviation (SD). Data normality checking was carried out using the Shapiro-Wilk test. Statistical analysis of normally distributed parameters between groups was evaluated using the Student's t-test for independent samples. Comparisons between measurements in the study group at different times were assessed with a paired sample t-test. P<0.05 value was considered significant.

RESULTS

The study groups' mean values of total motility (TM), progressive motility (PM), and morphological evaluations (morphological disorders of the head, midpiece, and tail and total abnormal spermatozoa) were analyzed. Comparative results of these values are presented in Table 1.

Table 1 Comparisons of spermatological parameters in healthy control and alopecia X groups

Parameters	Healthy control group				Alopecia X group				
	Mean	SD	Min	Max	Mean	SD	Min	Max	p value
TM (%)	94.75	2.11	92.25	97.23	84.78	4.48	80.21	91.88	**0.002**
PM (%)	58.17	6.26	51.69	68.53	39.86	9.81	23.88	48.62	**0.008**
Abnormal head	2.80	1.64	1.00	5.00	4.00	1.00	3.00	5.00	0.201
Abnormal midpiece	2.20	0.83	1.00	3.00	5.40	1.51	3.00	7.00	**0.003**
Abnormal tail	3.80	1.30	2.00	5.00	7.60	1.81	5.00	10.00	**0.005**
Total abnormality	8.80	1.92	6.00	11.00	17.00	3.93	11.00	21.00	**0.003**

Mean: Mean SD: Standart deviation Min: Minimum Max: Maximum p: Significance value

When these differences are analyzed, revealed that there is a significant decrease in the healthy control group TM (94.75±2.11%) and PM (58.17±6.26%) values of semen compared to TM (84.78±4.48%) and PM (39.86±9.81%) values of alopecia X group semen (p<0.05). In addition, there was a significant increase in the mean values of the head, midpiece, tail, and total abnormal sperm morphology values of the semen alopecia X group (p<0.05).

The mean values of PSV, EDV, RI, and PI for supratesticular, intratesticular, and marginal arteries in the study groups were evaluated by Doppler ultrasonography. The comparative results of these values are presented in Table 2, Table 3, and Table 4, respectively.

When Table 2 is examined, except for the statistical difference observed in the increase in the PI value of the alopecia X group (p<0.05), no statistical difference was found in other parameters. In Table 3, the increase in PSV, RI, and PI values, which are values other than EDV, was found to be statistically significant (p<0.05). In Table 4, which includes the measurement results of the marginal artery, the statistical difference is observed in the EDV and RI parameters (p<0.05).

Table 2. Comparisons of the Supratesticular artery hemodynamic parameters in healthy control and alopecia X groups

Supratesticular Artery	Healthy control group				Alopecia X group				
	Mean	SD	Min	Max	Mean	SD	Min	Max	p value
PSV (cm/s)	17.20	1.30	16.00	19.00	19.40	5.02	14.00	25.00	0.371
EDV (cm/s)	8.00	0.70	7.00	9.00	7.80	0.83	7.00	9.00	0.694
RI	0.52	0.05	0.43	0.57	0.57	0.10	0.42	0.68	0.368
PI	1.09	0.67	1.03	1.19	1.27	0.99	1.12	1.39	**0.009**

Mean: Mean SD: Standart deviation Min: Minimum Max: Maximum p: Significance value

Table 3. Comparisons of the Intratesticular artery hemodynamic parameters in healthy control and alopecia X groups.

Intratesticular Artery	Healthy control group				Alopecia X group				
	Mean	SD	Min	Max	Mean	SD	Min	Max	p value
PSV (cm/s)	6.20	1.30	5.00	8.00	13.40	6.26	6.00	21.00	**0.036**
EDV (cm/s)	4.00	0.70	3.00	5.00	4.20	0.83	3.00	5.00	0.694
RI	0.35	0.06	0.25	0.40	0.63	0.13	0.50	0.80	**0.002**
PI	0.70	0.07	0.60	0.81	1.30	0.18	1.10	1.53	**0.000**

Mean: Mean SD: Standart deviation Min: Minimum Max: Maximum p: Significance value

Table 4. Comparisons of the Marginal artery hemodynamic parameters in healthy control and Alopecia X groups.

Marginal Artery	Healthy control group				Alopecia X group				
	Mean	SD	Min	Max	Mean	SD	Min	Max	p value
PSV (cm/s)	12.20	1.30	11.00	14.00	16.60	4.44	12.00	22.00	0.067
EDV (cm/s)	7.80	0.83	7.00	9.00	6.00	1.00	5.00	7.00	**0.015**
RI	0.35	0.06	0.27	0.46	0.59	0.17	0.41	0.77	**0.020**
PI	0.82	0.08	0.70	0.91	1.01	0.21	0.80	1.30	0.107

Mean: Mean SD: Standart deviation Min: Minimum Max: Maximum p: Significance value

DISCUSSION

Alopecia X disease may be caused by many systemic diseases such as hypothyroidism, hyperadrenocorticism, and follicular dysplasia, and researchers have reported that sex hormone endocrinopathies such as testosterone and estrogen may also play a role in its etiology (Frank et al. 2004; Crawford et al. 2024). Dihydrotestosterone (DHT) is a more potent androgen than testosterone and is an end-organ effector that directs growth and differentiation in specific tissues. DHT is usually formed by the reduction of testosterone in target cells, and this peripheral conversion occurs primarily in tissues such as the prostate, male genitalia, and skin. Testosterone conversion in the skin supports the normal development of sexual characteristics at different stages of life. However, this process can occasionally increase abnormally, leading to undesirable effects (Price 1975).

Considering the existence of these mechanisms in dogs, similar excessive accumulation of DHT in the skin and other tissues may cause clinical signs such as hair loss. The role of DHT in alopecia X cases in dogs should not be ignored. This disease has been defined as a hair loss problem that is seen especially in certain breeds and develops due to abnormal hormonal activity in the skin. Considering the effects of DHT on the prostate, skin, and genital tissues, the effect of this type of alopecia seen in dogs on spermatogenesis, sperm quality, testicular functions, and fertility is not yet fully understood, but considering the role of testosterone and related hormones, it is thought that this condition may also affect spermatological parameters and testicular functions.

Our study shows that spermatological parameters examined in dogs with alopecia X are significantly lower than in the healthy control group ($p<0.05$). Systemic hormonal changes observed in dogs with alopecia X may explain the negative effects on sperm parameters. It is thought that these hormonal changes negatively affect spermatogenesis, and as a result, decreased sperm motility, decreased progressive motility, and increasing morphological disorders are observed (Safarinejad et al. 2011; Rosety et al. 2014). Alopecia X is a condition encountered in dogs and shares some clinical features with androgenic alopecia (AGA) in humans. AGA is a condition that leads to a hair loss process in men via dihydrotestosterone (DHT) and is characterized by a decrease in the anagen phase (Ntshingila et al. 2023). Güngör et al. (2016) found lower motility and higher morphologically disordered sperm rates in patients with androgenic alopecia when they examined spermatological parameters compared to the control group of healthy individuals. It is thought that oxidative stress-induced hair loss in the inguinal region where the testicles are located leads to decreased spermatogenesis and sperm damage (Omu 2013). The data we obtained in our study suggest that, as in humans, hormonal imbalance, oxidative stress, and systemic proinflammatory conditions may be associated with deterioration in sperm quality.

The vessels entering the testicles provide stable blood flow, necessary for metabolic processes and sperm production. Testicular blood flow is key in transporting nutrients, regulatory hormones, and secretory products to and from the testicles (Bergh 1993). Due to the low oxygen concentration in the seminiferous tubules, regulating blood flow in the testicles is critical (Setchell 1990). Inadequate blood flow can lead to disruptions at different stages of the spermatogenesis process. Doppler measurements are a crucial tool for assessing the effectiveness of this vascular regulation. In addition, detecting abnormal blood flow can be an important indicator in determining fertility problems (Samir et al. 2018). PSV and EDV values used in Doppler measurements indicate arterial blood flow in the standard cardiovascular cycle. In the Zelli et al. (2013) study, no correlation was found between spermatological parameters and PSV and EDV values. However, it has been stated that RI and PI values provide more robust data on the anatomical structure and blood flow velocity of the testicular vessels (Biagiotti et al. 2002). RI is a measure that reflects the resistance to blood flow caused by the microvascular bed distal to the measured area. PI measures the oscillations in the waveform of blood flow. It is assumed that RI and PI values are inversely proportional to blood flow perfusion and that the lower these indices are, the more efficient spermatogenesis can be qualitatively (Zelli et al. 2013). Higher RI and PI parameters in the three vessels examined in dogs with alopecia X indicate increased resistance and low blood perfusion in the testicular vessels. This situation may lead to insufficient blood flow in the testes and, thus, a decrease in oxygen and nutrients, which may negatively affect spermatogenesis. Increased vascular resistance disrupts the microenvironment required for spermatozoon production, which can be directly linked to the observed decreases in sperm motility, progressive motility, and morphological parameters. The high RI and PI values suggest that vascular abnormalities in the testes may be linked to adverse outcomes in sperm quality in dogs with alopecia X.

CONCLUSION

In conclusion, the findings of our study showed that alopecia X, which is characterized by hormonal changes, may adversely affect in testicular arterial hemodynamics and sperm motility, progressive motility, and morphological parameters. These findings demonstrate that there is a relationship between increased vascular resistance in testicular vessels (high RI and PI values) and decreases in sperm quality in dogs with Alopecia X. The obtained results reveal that alopecia X is not only a dermatological problem but also may negatively affect male reproductive health. These data are important in evaluating reproductive functions and optimizing treatment processes in dogs with alopecia X. Future studies may contribute to developing specific treatment approaches by examining the effects of this disease on reproductive health in more detail.

Conflict of Interest: The authors have no conflicts of interest to report.

Authors' Contributions: CK, BE, and ÇE contributed to the project idea, design, and execution of the study. CK, BE, and ÇE contributed to the acquisition of data. CK, BE, and ÇE analyzed the data. CK, BE, and ÇE drafted and wrote the manuscript. CK, BE, and ÇE reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical Approval: This study was carried out at Ondokuz Mayıs University Animal Hospital. This research was approved by the Animal Experiments Ethics Committee of the University Ondokuz Mayıs (approval number: E-68489742-604.01-2400217735).

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