Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
xx (x): xx, 2025
DOI: 10.9775/kvfd.2025.34432

Research Article

Assessment of Testicular Artery Blood Flow Using Doppler Ultrasonography and Its Correlation with Spermatological Parameters in Kangal Shepherd Dogs

Burcu ESİN [1 (*)] Cumali KAYA [1] Çağatay ESİN [2]

[1] Department of Animal Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55200 Samsun - TÜRKİYE

[2] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55200 Samsun - TÜRKİYE

(*) **Corresponding author:**
Burcu ESİN
Phone: +90 362 312 1919
E-mail: burcuyalcin@omu.edu.tr

How to cite this article?

Esin B, Kaya C, Esin Ç: Assessment of testicular artery blood flow using doppler ultrasonography and its correlation with spermatological parameters in Kangal Shepherd dogs.*Kafkas Univ Vet Fak Derg*, 2025 (Article in Press).
DOI: 10.9775/kvfd.2025.34432

Article ID: KVFD-2025-34432
Received: 14.05.2025
Accepted: 11.08.2025
Published Online: 19.08.2025

Abstract

This study investigated the relationship between testicular artery hemodynamics and spermatological parameters in Kangal Shepherd dogs of different age groups using Doppler ultrasonography and computer-assisted sperm analysis. Fourteen clinically healthy, sexually mature Kangal Shepherd dogs were categorized into adult (3-5 years, n=7) and senior (7-9 years, n=7) groups. Each dog underwent three evaluations at two-week intervals, including a Doppler ultrasonographic assessment of supratesticular, intratesticular, and marginal arteries and comprehensive spermatological analysis. Testicular arterial blood flow was assessed by measuring peak systolic velocity, end-diastolic velocity, pulsatility index (PI), and resistive index (RI). Spermatological assessments included total motility, progressive motility, sperm concentration, and morphological analysis. The results revealed significant age-related alterations in testicular blood flow parameters, with senior dogs exhibiting increased PI and RI values. Moreover, strong negative correlations were identified between Doppler indices and spermatological parameters. Conversely, positive correlations were found between vascular resistance parameters and morphological sperm defects, particularly in the head, tail, and midpiece regions. The strongest correlations were observed in the left intratesticular artery, with RI showing a very strong negative correlation with motility (r=-0.91) and a very strong positive correlation with total morphological defects (r=0.92).

In conclusion, this study shows that testicular blood flow, assessed via Doppler ultrasonography, is closely linked to sperm parameters in Kangal Shepherd dogs, underscoring its value in clinical reproductive functions, especially for age-related changes.

Keywords: Doppler velocimetry, Kangal Shepherd dogs, Spermatological parameters, Testicular artery, Ultrasonography

Introduction

Fertility in male dogs is influenced by various intrinsic and extrinsic factors, including age, hormonal balance, testicular vascularization, and environmental conditions [1]. Among these, testicular blood flow (TBF) has gained particular attention in recent years, as Doppler ultrasonography (DU) parameters such as the pulsatility index (PI) and resistive index (RI)-have been proposed as potential indicators of sperm quality in dogs [2]. In clinical practice, DU is a valuable, non-invasive tool used to evaluate the hemodynamic characteristics of blood flow in various arteries and veins. It is widely employed for vascular assessment in multiple organs, including the liver, mammary glands, kidneys, placenta, and fetal structures. In recent years, research has increasingly focused on the Doppler parameters of the testicular artery (TA) in different animal species [3-6]. This focus is of critical importance, as impaired blood supply in the TA is considered a significant contributor to male infertility [7].

The testicular artery, which arises from the abdominal aorta, supplies blood to the testes. After passing through the inguinal ring to reach the spermatic cord, it travels along the posterior surface of the testis, emerging near the proximal pole. The artery then penetrates the tunica albuginea and follows a relatively straight course typically without branching-through the tunica vasculosa, a vascular layer beneath the capsule that extends along the epididymal margin of the testis.

In human medicine; Color Doppler (CD), Pulsed Wave Doppler (PW) and Power Doppler (PD) are routinely performed to evaluate the andrological status of the testes [8] as these modalities offer a simple yet accurate method of measuring blood flow by integrating anatomical and hemodynamic information [9]. In veterinary medicine, several studies have investigated the application of DU in dogs for evaluating TBF under both physiological and pathological conditions [10,11]. Although still considered a relatively novel technique in veterinary practice, testicular DU holds great potential for future development and clinical utility.

The testicular parenchyma is a high-metabolism tissue; any disruption in the transport of nutrients and oxygen via the blood can have adverse effects on the morphology and function of the testis [12]. Studies in dogs have shown a relationship between hemodynamic parameters of testicular artery blood flow and semen-related parameters [13,14]. Venianaki et al.[15] studied hemodynamic parameters of testicular artery blood flow in Beagles during prepubertal, pubertal and postpubertal ages. They obtained significant correlations between the hemodynamic parameters they obtained and semen evaluation parameters [15]. Souza et al.[16] reported possible changes in hemodynamic parameters of testicular artery blood flow during the peri-pubertal period; in general, these parameters can be used as indicators of future semen quality [17-19]. However, since the animals evaluated in the study were of different breeds, this did not support standardization of the results.

The testicular artery originates from the abdominal aorta and supplies blood to the testes via several branches. It first gives rise to the supratesticular artery, located along the spermatic cord, then enters the testis and continues as the marginal artery along the epididymal border, and finally branches into the intratesticular artery that penetrates the testicular parenchyma [13]. The intratesticular artery runs through the mediastinum testis and provides direct perfusion to the seminiferous tubules. Among these, the intratesticular artery is particularly important because it supplies the active spermatogenic tissue. Alterations in blood flow parameters (e.g., RI, PI) within this artery are closely linked to sperm motility, morphology, and concentration. Therefore, detailed Doppler assessment of these arteries can provide critical insights into testicular function and male dog fertility [15,16].

In addition to evaluating testicular artery blood flow, the objective and quantitative assessment of spermatological parameters is essential for determining male reproductive performance. A complete andrological examination -including physical evaluation of the testes and semen analysis- is fundamental for assessing testicular function. Computer-assisted sperm analysis (CASA) systems offer objective and reproducible measurements of sperm motility by tracking and analyzing the movement characteristics of individual sperm cells. Beyond motility, a comprehensive semen evaluation also includes sperm concentration and morphological assessment, both of which are critical for understanding the functional competence of spermatozoa [19].

Despite the clinical value of integrating vascular and spermatological data, there remains a need for more studies investigating the relationship between testicular hemodynamics and detailed semen quality parameters in dogs [2]. In particular, studies are needed on local or regional breeds such as the Kangal Shepherd dog, which may exhibit different reproductive physiology influenced by genetic background and environmental conditions [20].

This study investigated age-related changes in testicular artery hemodynamics and their potential association with semen quality in Kangal Shepherd dogs. Adult (3-5 years) and senior (7-9 years) dogs were compared regarding Doppler ultrasonographic parameters and spermatological findings. In addition to examining the effects of aging on testicular blood flow, the study also sought to determine whether alterations in vascular dynamics are correlated with differences in semen quality between the two age groups.

Material and Methods

Ethical Statement

This study was conducted at the Animal Hospital of the Faculty of Veterinary Medicine, Ondokuz Mayis University, and was approved by the Animal Ethics Committee of Ondokuz Mayis University (Approval No: 2024/41).

Experimental Design

A total of 14 healthy, sexually mature male Kangal Shepherd dogs were enrolled in the study. The dogs were categorized into two age groups: adult (n=7; 3.5±1.5 years; 48.98±1.02 kg) and senior (n=7; 7±1.5 years; 50±6.94 kg). All animals were presented to the university hospital for routine procedures such as vaccination or artificial insemination. Before participation, informed consent was obtained from all dog owners.

Each animal underwent a complete physical examination and a detailed reproductive tract evaluation. Only clinically healthy dogs without any observable physical or reproductive disorders, and with no known history of reproductive diseases, were included in the study. Testicular Doppler ultrasonography and spermatological evaluations were performed three times on each dog, at two-week intervals.

All dogs were maintained on a standardized diet throughout the study to minimize variability and ensure uniform metabolic conditions. Dogs diagnosed with orchitis, testicular morphological abnormalities, systemic illness, or those that failed to provide semen samples were excluded from the study.

Testicular Doppler Ultrasonography

Testicular blood flow was assessed in all dogs before semen collection. All ultrasonographic examinations were conducted by the same experienced ultrasonographer involved in the study. The dogs were positioned supine without sedation to minimize potential interference from anesthetic agents on TBF measurements. Vetus 9 (Mindray®) equipment equipped with a microconvex probe operating at 6.5-7.5 MHz was used for the ultrasound. A thin layer of ultrasound gel was applied to the skin, and the transducer was placed on the testes of the animals, which were positioned in dorsal recumbency [21].

Color Doppler ultrasonography was used to assess the testicular artery (TA) and visualize blood flow in both the right and left testes. TBF was evaluated in the supratesticular, intratesticular, and marginal testicular arteries *(Fig. 1)*. During each measurement, at least three consecutive Doppler waveforms were recorded and averaged to enhance measurement accuracy *(Fig. 2)*. For each region of both testicles, Hemodynamic PSV, EDV, PI and RI measurements were repeated three times and averaged. Doppler parameters were automatically calculated and recorded by the ultrasound system.

Collection of Semen Samples and Spermatological Analysis

Semen was collected from each dog three times, at two-week intervals using digital stimulation, to ensure that the animals met the minimum reproductive requirements [22]. During each collection, the ejaculate was divided into three distinct fractions: pre-sperm, sperm-rich, and post-sperm fractions. For spermatological assessments, only the sperm-rich fraction was used, as it contains the highest concentration of spermatozoa and provides the most reliable parameters for assessing semen quality. Parameters assessed included total motility (%), progressive motility (%), sperm concentration (mL), and sperm morphology (%). During evaluation, semen was maintained at a constant temperature of 37°C.

To ensure homogeneity, the fresh spermatozoa were diluted with a tris-based extender to achieve a final concentration of 50-100 x 10^6 spermatozoa/mL [23]. Prior to computer-assisted analysis, semen samples were subjected to macroscopic evaluation, including assessment of ejaculate volume, color, and consistency. Subsequently, a CASA system (Sperm Class Analyser, Version 6.5.0.91, Microptic, Barcelona, Spain) was utilized to assess sperm concentration, motility (%), progressive motility (%), and morphology.

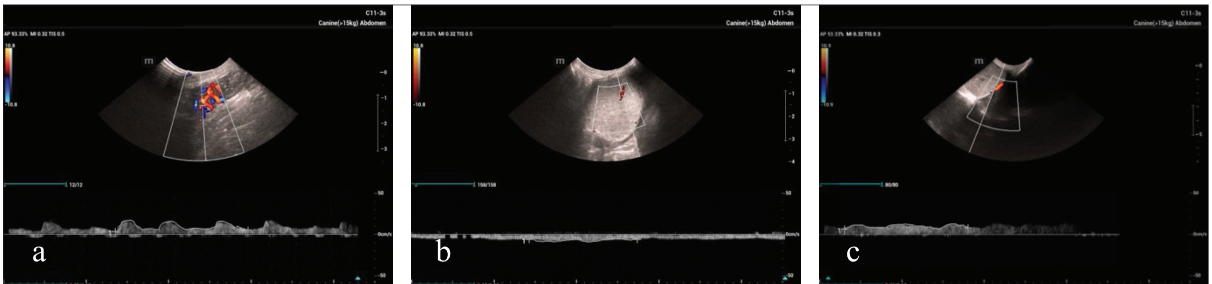

Fig 1. Doppler waveform of the testicular artery in dogs: supratesticular (a), intratesticular (b), and marginal (c)

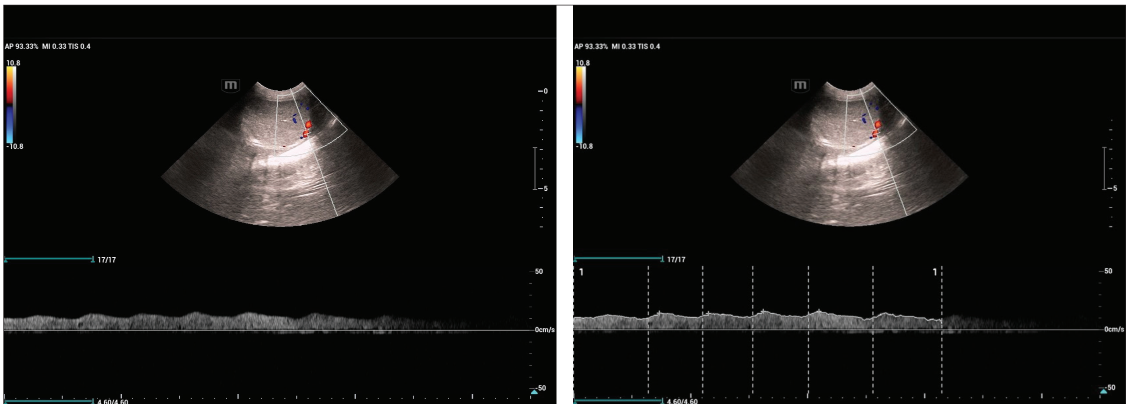

Fig 2. Spectral Doppler measurement of the testicular marginal artery

Sperm morphology was evaluated using the "Sperm Blue® kit" (Microptic®), and the results were also analyzed via the CASA system [24]. The Sperm Blue kit stains various parts of the sperm (head, midpiece, and tail) in different shades of azure, allowing for detailed morphological assessment. Sperm smears were prepared for each dog, allowed to air dry at room temperature, and stained following the manufacturer's instructions. At least 200 spermatozoa were assessed per animal, and the percentage of abnormal spermatozoa was determined.

Dogs were randomly selected for inclusion in the study, regardless of their initial sperm parameters as assessed by the CASA system, as the objective was to evaluate the natural variability of testicular hemodynamics and its association with spermatological parameters across different age groups.

Statistical Analysis

IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., NY, USA), was used for all statistical analyses. For each dog, repeated measurements taken at two-week intervals were averaged, and these mean values were used for statistical analysis to avoid pseudo-replication. Before hypothesis testing, the normality of the data distribution was assessed using the Shapiro-Wilk test, complemented by visual inspection of histograms and Q-Q plots. Additionally, skewness and kurtosis values were examined to further confirm the distribution characteristics of the variables. For normally distributed data, differences between the adult and senior groups were evaluated using the Independent Samples t-test. Homogeneity of variances was assessed with Levene's test, and the appropriate t-test results (assuming equal or unequal variances) were reported accordingly.

Doppler ultrasonographic measurements, including PSV, EDV, PI, and RI of the supratesticular, marginal, and intratesticular arteries in both the right and left testes, were normally distributed and thus analyzed using parametric tests (Independent Samples t-test). In contrast, spermatological parameters such as motility, progressive motility, sperm concentration, and morphological defects (head, midpiece, tail, and total morphological defects) did not follow a normal distribution. Although these variables exhibited homogeneity of variances, they were analyzed using the non-parametric Mann-Whitney U test due to their deviation from normality.

Spearman's rank correlation coefficient (ρ) was used to assess the relationships between Doppler parameters and spermatological variables. The strength of correlations was interpreted based on the absolute value of ρ. A P-value of less than 0.05 was considered statistically significant for all tests.

Results

Doppler ultrasonographic evaluation revealed significant age-related alterations in testicular arterial blood flow (*Table 1*). In the right testis, the supratesticular artery exhibited significantly higher PSV values in the senior group compared to the adult group ($P<0.01$). In contrast, EDV and RI were significantly lower in adults ($P<0.01$). Intratesticular arterial flow in the right testis also demonstrated significantly elevated PSV and PI values in the adult group ($P<0.01$), whereas RI was lower ($P<0.05$). No significant differences were observed in EDV values between the groups. In contrast, marginal artery measurements in the right testis revealed no significant differences in PSV, EDV, or RI values ($P>0.05$), although PI was significantly higher in adult dogs ($P<0.05$).

Similar trends were observed in the left testis. In the supratesticular artery, senior group exhibited significantly higher PSV and PI values ($P<0.05$ and $P<0.01$, respectively) and lower EDV and RI values compared to adult group ($P<0.05$ and $P<0.01$, respectively). The intratesticular artery on the left side also significantly reduced RI and PI values in the adult group ($P<0.01$ and $P<0.05$, respectively). However, the marginal artery showed no significant differences between groups in most parameters ($P>0.05$). These findings suggest that testicular arterial hemodynamics, particularly within the supratesticular and intratesticular arteries, are significantly influenced by age in large-breed dogs.

Significant differences between the adult and senior groups were observed in all spermatological parameters (*Table 2*). Total motility and progressive motility were significantly higher in adult dogs ($P<0.01$ and $P<0.001$, respectively), while senior dogs exhibited markedly reduced motility levels. Similarly, sperm concentration was significantly higher in the adult group compared to the senior group ($P<0.01$). In contrast, morphological abnormalities were significantly more prevalent in the senior group. The mean percentages of head, midpiece, and tail defects were significantly higher in older dogs ($P<0.01$ or $P<0.001$), resulting in a significantly increased total defect percentage in the senior group ($P<0.001$). These findings suggest a clear age-related decline in semen quality, characterized by reduced motility and increased morphological abnormalities in senior dogs.

Significant correlations between Doppler ultrasonographic vascular hemodynamics and spermatological parameters in the study dogs are presented in *Fig. 3*.

The correlation analysis between Doppler ultrasonographic indices and spermatological parameters is detailed in *Table 3*. A strong negative correlation was observed between right supratesticular PSV and total motility (r=-

Table 1. Comparison of doppler ultrasonographic parameters of the testicular arteries between adult and senior dogs

Doppler Ultrasonography			Groups of Animals (Mean±SD)		P
			Adult Group	Senior Group	
Right Testis Doppler Parameters	Supratesticular artery	PSV (cm/s)	18.15±0.93	22.14±2.11	**<0.01****
		EDV (cm/s)	5.73±1.45	3.67±0.33	**<0.01****
		RI	0.67±0.08	0.83±0.14	**<0.01****
		PI	1.27±0.28	1.93±0.05	**<0.01****
	Intratesticular artery	PSV (cm/s)	4.44±0.5	6.15±1.49	**<0.01****
		EDV (cm/s)	3.21±0.47	3.48±0.47	>0.05
		RI	0.27±0.04	0.41±0.10	**<0.05***
		PI	0.36±0.05	0.57±0.14	**<0.01****
	Marginal artery	PSV (cm/s)	13.71±2.04	14.42±2.05	>0.05
		EDV (cm/s)	6.97±1.57	6.23±0.49	>0.05
		RI	0.48±0.11	0.55±0.05	>0.05
		PI	0.77±0.18	1.07±0.08	**<0.05***
Left Testis Doppler Parameters	Supratesticular artery	PSV (cm/s)	18.15±1.47	20.74±1.68	**<0.05***
		EDV (cm/s)	5.73±1.45	3.84±0.34	**<0.05***
		RI	0.68±0.1	0.81±0.1	**<0.01****
		PI	1.76±1.08	1.93±0.3	**<0.01****
	Intratesticular artery	PSV (cm/s)	9.72±1.42	5.73±1.27	>0.05
		EDV (cm/s)	3.85±0.61	3.87±0.61	>0.05
		RI	0.29±0.01	0.52±0.1	**<0.01****
		PI	0.43±0.03	0.54±0.08	**<0.05***
	Marginal artery	PSV (cm/s)	14.20±1.96	14.69±1.38	>0.05
		EDV (cm/s)	6.81±1.83	6.32±0.63	>0.05
		RI	0.48±0.06	0.53±0.04	>0.05
		PI	0.65±0.1	0.72±0.17	>0.05

**P<0.05, **P<0.01, SD: standard deviation*

0.81, P<0.01), progressive motility (r=-0.84, P<0.01), and sperm concentration (r=-0.87, P<0.01). Conversely, right supratesticular PSV showed strong positive correlations with head defects (r=0.85), tail defects (r=0.77), and total morphological defects (r=0.76).

Similarly, the right supratesticular RI was negatively correlated with motility (r=-0.82), progressive motility (r=-0.84), and concentration (r=-0.71) while exhibiting positive associations with head (r=0.75), tail (r=0.73), and total defects (r=0.75). The PI in the same artery also demonstrated strong negative correlations with motility (r=-0.88), progressive motility (r=-0.90), and concentration (r=-0.76), alongside strong positive correlations with head (r=0.81), tail (r=0.82), and total defects (r=0.84).

In the right intratesticular artery, RI showed strong negative correlations with motility (r=-0.78) and progressive motility (r=-0.75) and strong positive correlations with head (r=0.89), tail (r=0.70), and total morphological defects (r=0.77). PI values followed a

Table 2. *Comparison of spermatological parameters between adult and senior groups*

Spermatological Parameters	Adult group			Senior group			P
	Mean±SD	Median	Interquartile Range	Mean±SD	Median	Interquartile Range	
Motility	78.71±6.08	80.13	8.38	33.71±6.27	31.23	8.38	**<0.01****
Progressive Motility	68.95±5.38	72.23	10.98	13.62±4.29	13.34	6.94	**<0.001*****
Concentration	368.19±33.19	380	41.98	184.27±37.85	178.67	74.84	**<0.01****
Head Defects	1.42±0.78	1	1	6.14±1.57	6	2	**<0.001*****
Midpiece Defects	4.42±0.53	4	1	9±2.08	9	4	**<0.01****
Tail Defects	4±0.81	4	2	12.42±1.61	12	3	**<0.001*****
Total Defects	9.85±0.89	10	3	27.57	3.64	8	**<0.001*****

**P<0.05, **P<0.01, ***P<0.001, SD: standard deviation*

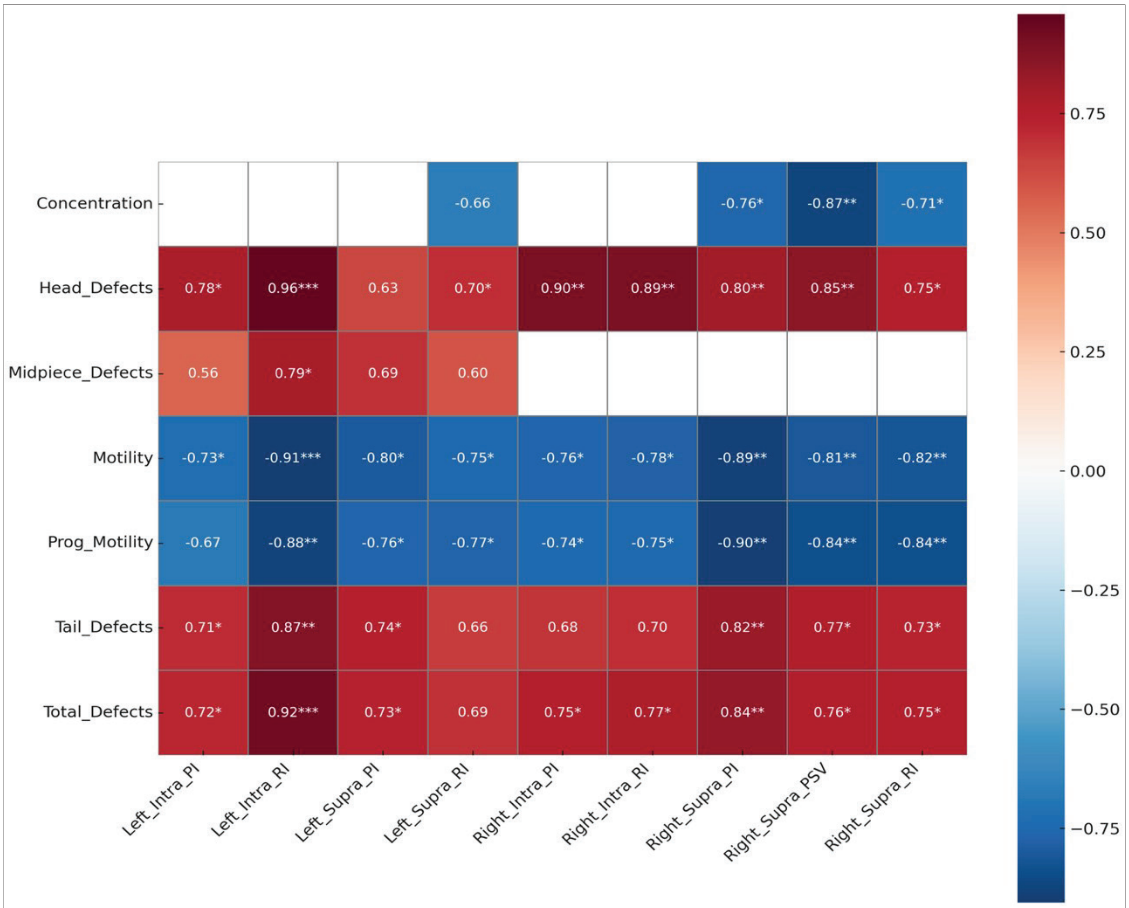

Fig 3. Significant correlations between doppler ultrasonographic vascular hemodynamics and spermatological parameters in study dogs

similar trend, being negatively correlated with motility (r=-0.76) and progressive motility (r=-0.74) and positively correlated with head (r=0.90), tail (r=0.68), and total defects (r=0.74).

For the left testis, left supratesticular RI showed strong negative correlations with motility (r=-0.75) and progressive motility (r=-0.77) and a moderate negative correlation with sperm concentration (r=-0.66). Positive correlations were observed with head (r=0.70), midpiece (r=0.60), tail (r=0.66), and total defects (r=0.69). Left supratesticular PI was also negatively associated with motility (r=-0.80) and progressive motility (r=-0.76) and positively associated with head (r=0.63), midpiece (r=0.69), tail (r=0.74), and total defects (r=0.73).

The strongest associations were found in the left intratesticular artery. RI exhibited very strong negative correlations with motility (r=-0.91) and progressive motility (r=-0.88) and very strong positive correlations with head (r=0.96), midpiece (r=0.79), tail (r=0.87), and total morphological defects (r=0.92). Lastly, left intratesticular PI showed a strong negative correlation with motility (r=-0.72), a moderate negative correlation with progressive motility (r=-0.67), and positive associations with head (r=0.78), midpiece (r=0.56), tail (r=0.71), and total defects (r=0.72).

Discussion

This study evaluated the relationship between testicular artery hemodynamics and spermatological parameters in Kangal Shepherd dogs across different age groups. The hemodynamic data revealed that PSV, PI, and RI values measured in the supratesticular and intratesticular arteries were significantly elevated in senior dogs, whereas end-diastolic velocity (EDV) values were significantly lower. No significant differences were observed between the left and right testicles in any hemodynamic parameters, and the measurements remained consistent across different examination days.

Regarding the spermatological findings, adult dogs exhibited significantly higher total motility, progressive motility, and sperm concentration than their senior group. Conversely, the incidence of morphological defects -specifically in the head, midpiece, and tail regions- was significantly higher in the senior group. Correlation analysis demonstrated a strong negative association between increased arterial resistance and pulsatility and sperm motility and concentration. In contrast, a positive correlation was identified with morphological abnormalities.

Age-related changes in testicular blood flow have been extensively documented in human medicine. Doppler ultrasonography studies have consistently shown that advancing age is associated with increased PSV, decreased EDV and elevated PI and, RI values in the testicular arteries [25]. These hemodynamic alterations reflect diminished vascular elasticity and impaired microcirculation within aging testicular tissue. The consequent reduction in testicular perfusion contributes to the decline in spermatogenic function commonly observed in older men. Clinically, such age-related Doppler changes are increasingly utilized as non-invasive indicators of subclinical testicular dysfunction and as part of the broader assessment of male reproductive health. Accordingly, testicular Doppler ultrasonography has become an important diagnostic modality in the evaluation of age-associated testicular decline and male infertility [26].

In veterinary medicine, assessing testicular blood flow using Doppler ultrasonography has proven to be a valuable diagnostic tool [27,28]. Numerous studies have demonstrated a positive relationship between testicular arterial blood flow and spermatological parameters, including overall sperm quality [13,17,22]. In Doppler evaluations conducted across various animal species -including dogs [17], rams [6] and stallions [5]- hemodynamic parameters such as PI and RI have been extensively studied. These indices, in particular, have been widely accepted as reliable indicators of testicular perfusion status and potential markers of sperm quality [15].

Considering the relevant literature, Souza et al.[22] reported the PI index values in the range of 0.7-1.15 and the RI index values in the range of 0.4-0.7 in the study conducted to examine the Doppler velocimetry parameters of the testicular artery in dogs. In 2015, conducted another study including the measurement and evaluation of the velocimetry parameters of the marginal testicular artery. In this study, the PI index results ranged from 0.4 to 0.7 and the RI index results ranged from 0.3 to 0.6. In addition, PSV and EDV values were obtained significantly lower in infertile dogs. RI and PI values did not differ between fertile and infertile dogs [29]. Venianaki et al.[15] studied the doppler examination of the testicular artery in dogs from birth to adolescence and reported the PI index values in the range of 0.1-0.5 and the RI index values in the range of 0.1-0.4. Based on these results, it can be said that the doppler velocytometric values of the testicular artery measured by us are consistent with other studies.

In the context of infertility and aging, increases in PI and RI observed in testicular arteries are thought to be associated with impaired testicular microcirculation and reduced parenchymal perfusion. Elevated PI and RI values reflect increased resistance to arterial blood flow and diminished diastolic velocity, which in turn hampers the delivery of oxygen and nutrients essential for spermatogenesis [30]. In vascular pathologies such as varicocele, increased RI and PI values have been reported, indicating compromised testicular microvascular function [31]. Moreover, aging-related changes including the disruption of elastin-collagen balance, the development of endothelial dysfunction, and structural thickening of arterial walls contribute to diminished vascular compliance and heightened arterial resistance. This leads to a more pulsatile pattern of blood flow to the testes, resulting in elevated PI and RI parameters, which may ultimately contribute to germinal epithelial damage and a decline in spermatogenic activity.

Several studies have attempted to establish reference values for testicular blood flow in dogs and to elucidate their association with spermatological parameters [14,16,32,33]. In a study conducted by de Souza et al.[29] to define regional differences in testicular arterial blood flow in clinically post-pubertal and pre-pubertal dogs, they found that PSV, EDV, RI and PI values were significantly lower in pre-pubertal dogs compared to post-pubertal dogs in testicular hemodynamic values. Zelli et al.[33] reported a negative correlation between PI and RI with total progressive motility, while PSV was also negatively associated with the live of sperm. This result is inconsistent with that obtained by England et al.[32] who could not prove a relationship between RI and PI with total sperm output or percentage of live of sperm. Trautwein et al.[19] investigated the effect of testicular arterial blood flow on sperm motility and spermatozoa morphology in dogs and reported a correlation between Doppler velocimetry parameters and motility.

In the present study, PI and RI values, which have been previously proposed as potential indicators of sperm quality in dogs [33], were consistent with those reported in earlier studies. A strong negative correlation was identified between testicular arterial RI and PI values and key spermatological parameters such as sperm motility and concentration, which tended to decline with age. Conversely, RI and PI values were positively correlated with the incidence of morphological abnormalities, which increased with age. These findings suggest that age-related impairment in testicular blood flow, as reflected by elevated vascular resistance parameters, may negatively influence spermatogenesis. Consequently, the increase in vascular resistance with age may play a critical role in the deterioration of male fertility.

Trautwein et al.[19] investigated the effect of testicular artery blood flow on epididymal sperm motility and spermatozoa morphology in dogs and reported a positive correlation between Doppler velocimetry parameters PSV, PI and RI and motility parameters. In 2020, Lemos et al.[14] reported a positive correlation between sperm concentration and PSV and EDV in their study comparing normozoospermic and non-normozoospermic groups. In our study, a negative correlation was obtained between sperm concentration and PSV.

In our study, PSV values were higher in senior dogs than in the adult group; however, a significant decrease in sperm concentration was observed. This apparent paradox may indicate a compensatory vascular response to age-related microcirculatory impairment. Elevated PSV in the presence of increased PI and RI suggests that while systolic pressure rises to maintain perfusion, overall flow efficiency declines due to elevated resistance. This high-resistance, low-efficiency state hampers effective oxygen and nutrient delivery to the testicular tissue, thereby compromising spermatogenesis [33,34].

Additionally, a study examining the Doppler examination of testicles in dogs of different sizes reported that the velocitometric index values varied depending on the size and weight of the dog [16]. Larger and heavier dog breeds show different velocitometric parameter values compared to smaller dog breeds. In our study, correlations between velocitometric values and spermatological parameters were applied to Kangal Shepherd dogs. The differences in our findings with the study conducted by Lemos et al.[14]. In the study, evaluations were made using more than one breed. Therefore, this difference may have been observed.

In conclusion, Doppler velocimetric evaluation plays an important role in the evaluation of reproduction in male dogs. This study shows that spermatological parameters can be associated with testicular arterial blood flow in male Kangal Shepherd dogs of different age groups. It also forms the basis for establishing basic reference values that can be used for clinical diagnosis. Due to the differences in these parameters depending on the location of measurement, season, age, breed, laterality and operator, further research is needed to determine reference physiological parameters according to species and breed and to establish reference values for species of certain sizes.

Declarations

Availability of Data and Materials: The datasets and analysed during the current study available from the corresponding author (BE) on reasonable request.

Ethical Statement: This study was conducted at the Animal Hospital of the Faculty of Veterinary Medicine, Ondokuz Mayis University, and was approved by the Animal Ethics Committee of Ondokuz Mayis University (Approval No: 2024/41).

Funding Support: There is no funding source.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: BE: Writing–original draft, Project administration, Methodology, Data curation, Conceptualization. BE, CK and ÇE: Methodology, Conceptualization, Writing – review & editing.

References

1. Arlt SP, Reichler IM, Herbel J, Schäfer-Somi S, Riege L, Leber J, Frehner B: Diagnostic tests in canine andrology - What do they really tell us about fertility? *Theriogenology,* 196, 150-156, 2023. DOI: 10.1016/j.theriogenology.2022.11.008

2. Stefanizzi E, Valenčáková A, Schmiesterová K, Figurová M, Horňáková Ľ: The use of Doppler ultrasonography in the examination of testicles in dogs. *Acta Vet Brno,* 72 (4): 453-467, 2022. DOI: 10.2478/acve-2022-0037

3. Bigliardi E, Denti L, De Cesaris V, Bertocchi M, Di Ianni F, Parmigiani E, Bresciani C, Cantoni AM: Colour Doppler ultrasound imaging of blood flows variations in neoplastic and non-neoplastic testicular lesions in dogs. *Reprod Domest Anim,* 54 (1): 63-71, 2019. DOI: 10.1111/rda.13310

4. Trautwein LGC, Souza AK, Martins MIM: Can testicular artery Doppler velocimetry values change according to the measured region in dogs? *Reprod Domest Anim,* 54 (4): 687-695, 2019. DOI: 10.1111/rda.13410

5. Ortiz-Rodriguez JM, Anel-Lopez L, Martín-Muñoz P, Álvarez M, Gaitskell-Phillips G, Anel L, Rodríguez-Medina P, Peña FJ, Ortega Ferrusola C: Pulse Doppler ultrasound as a tool for the diagnosis of chronic testicular dysfunction in stallions. *PLoS One,* 12 (5):e0175878, 2017. DOI: 10.1371/journal.pone.0175878

6. Batissaco L, Celeghini ECC, Pinaffi FLV, de Oliveira BMM, de Andrade AFC, Recalde ECS, Fernandes CB: Correlations between testicular hemodynamic and sperm characteristics in rams. *Braz J Vet Res Anim Sci,* 50 (5): 384-395, 2013. DOI: 10.11606/issn.2318-3659.v50i5p384-395

7. Konovalenko S, Kritsak M, Stechyshyn I, Pavliuk B: Male infertility as a consequence of endogenous and exogenous factors. *Pharmacologyonline,* 3, 265-274, 2021.

8. Dubinsky TJ, Chen P, Maklad N: Color-flow and power Doppler imaging of the testes. *World J Urol,* 16, 35-40, 1998. DOI: 10.1007/s003450050023

9. Atilla MK, Sargin H, Yilmaz Y, Odabas O, Keskin A, Aydin S: Undescended testes in adults: clinical significance of resistive index values of the testicular artery measured by Doppler ultrasound as a predictor of testicular histology. *J Urol,* 158 (3): 841-843, 1997. DOI: 10.1016/S0022-5347(01)64332-5

10. Samir H, Radwan F, Watanabe G: Advances in applications of color Doppler ultrasonography in the andrological assessment of domestic animals: A review. *Theriogenology,* 161, 252-261, 2021. DOI: 10.1016/j.theriogenology.2020.12.002

11. Bracco C, Gloria A, Contri A: Ultrasound-based technologies for the evaluation of testicles in the dog: Keystones and breakthroughs. *Vet Sci,* 10 (12):683, 2023. DOI: 10.3390/vetsci10120683

12. Colli LG, Belardin LB, Echem C, Akamine EH, Antoniassi MP, Andretta RR, Mathias LS, Rodrigues SF de P, Bertolla RP, de Carvalho MHC: Systemic arterial hypertension leads to decreased semen quality and alterations in the testicular microcirculation in rats. *Sci Rep,* 9 (1):11047, 2019. DOI: 10.1038/s41598-019-47157-w

13. Moxon R, Bright L, Pritchard B, Bowen IM, de Souza MB, da Silva LDM, England GCW: Digital image analysis of testicular and prostatic ultrasonographic echogencity and heterogeneity in dogs and the relation to semen quality. *Anim Reprod Sci,* 160, 112-119, 2015. DOI: 10.1016/j.anireprosci.2015.07.012

14. Lemos H, Dorado J, Hidalgo M, Gaivão I, Martins-Bessa A: Assessment of dog testis perfusion by colour and pulsed-doppler ultrasonography and correlation with sperm oxidative DNA damage. *Top Companion Anim Med,* 41:100452, 2020. DOI: 10.1016/j.tcam.2020.100452

15. Venianaki AP, Barbagianni MS, Fthenakis GC, Galatos AD, Gouletsou PG: Doppler examination of the testicular artery of Beagle-breed dogs from birth to puberty. *Tomography,* 9 (4): 1408-1422, 2023. DOI: 10.3390/tomography9040112

16. de Souza MB, Barbosa CC, England GCW, Mota Filho AC, Sousa CV, de Carvalho GG, Silva HV, Pinto JN, Linhares JCS, Silva LDM: Regional differences of testicular artery blood flow in post pubertal and pre-pubertal dogs. *BMC Vet Res,* 11 (47): 1-6, 2015. DOI: 10.1186/s12917-015-0363-3

17. Zelli R, Troisi A, Ngonput AE, Cardinali L, Polisca A: Evaluation of testicular artery blood flow by Doppler ultrasonography as a predictor of spermatogenesis in the dog. *Res Vet Sci,* 95 (2): 632-637, 2013. DOI: 10.1016/j.rvsc.2013.04.023

18. Carrillo JD, Soler M, Lucas X, Agut A: Colour and pulsed Doppler ultrasonographic study of the canine testis. *Reprod Domest Anim,* 47 (4): 655-659, 2012. DOI: 10.1111/j.1439-0531.2011.01937.x

19. Trautwein LGC, Souza AK, Cardoso GS, da Costa Flaiban KKM, de Oliveira Dearo AC, Martins MIM: Correlation of testicular artery Doppler velocimetry with kinetics and morphologic characteristics of epididymal sperm in dogs. *Reprod Domest Anim,* 55 (6): 720-725, 2020. DOI: 10.1111/rda.13672

20. Akyazi I, Ograk YZ, Eraslan E, Arslan M, Matur E: Livestock guarding behaviour of Kangal dogs in their native habitat. *Appl Anim Behav Sci,* 201, 61-66, 2018. DOI: 10.1016/j.applanim.2017.12.013

21. Yayla S, Ozturk S, Aksoy O, Kilic E, Yildiz S: Normal ultrasonographic anatomy of the prostate in Kars Shepherd dogs. *Kafkas Univ Vet Fak Derg,* 18 (1): 27-30, 2012. DOI: 10.9775/kvfd.2011.4528

22. de Souza MB, da Cunha Barbosa C, Pereira BS, Monteiro CLB, Pinto JN, Linhares JCS, da Silva LDM: Doppler velocimetric parameters of the testicular artery in healthy dogs. *Res Vet Sci,* 96 (3): 533-536, 2014. DOI: 10.1016/j.rvsc.2014.03.008

23. Nazeri E, Niasari-Naslaji A, Ghasemzadeh-Nava H, Panahi F: The effect of varying concentrations of chicken plasma egg yolk and glycerol on the viability of canine sperm following short and long-term preservation. *Iran J Vet Res,* 24 (1): 6-13, 2023. DOI: 10.22099/IJVR.2022.43403.6339

24. Tekin K, Kurtdede E, Salmanoglu B, Uysal O, Stelletta C: Osteopontin concentration in prostates fractions: A novel marker of sperm quality in dogs. *Vet Sci,* 10 (11):646, 2023. DOI: 10.3390/vetsci10110646

25. Yang H, Chryssikos T, Houseni M, Alzeair S, Sansovini M, Iruvuri S, Torigian DA, Zhuang H, Dadparvar S, Basu S: The effects of aging on testicular volume and glucose metabolism: An investigation with ultrasonography and FDG-PET. *Mol Imaging Biol,* 13, 391-398, 2011. DOI: 10.1007/s11307-010-0341-x

26. Wang JJ, Wang SX, Tehmina, Feng Y, Zhang RF, Li XY, Sun Q, Ding J: Age-related decline of male fertility: mitochondrial dysfunction and the antioxidant interventions. *Pharmaceuticals,* 15 (5): 519, 2022. DOI: 10.3390/ph15050519

27. Semiz I, Tokgöz Ö, Tokgoz H, Voyvoda N, Serifoglu I, Erdem Z: The investigation of correlation between semen analysis parameters and intraparenchymal testicular spectral Doppler indices in patients with clinical varicocele. *Ultrasound Q,* 30 (1): 33-40, 2014. DOI: 10.1097/RUQ.0000000000000055

28. Esin C: Effect of systemic hypertension on prostatic arterial hemodynamics in dogs with benign prostate hyperplasia. *Kafkas Univ Vet Fak Derg,* 18 (1): 27-32, 2025. DOI: 10.9775/kvfd.2024.32734

29. de Souza MB, England GCW, Mota Filho AC, Ackermann CL, Sousa CVS, de Carvalho GG, Silva HVR, Pinto JN, Linhares JCS, Oba E: Semen quality, testicular B-mode and Doppler ultrasound, and serum testosterone concentrations in dogs with established infertility. *Theriogenology,* 84 (5): 805-810, 2015. DOI: 10.1016/j.theriogenology.2015.05.015

30. Ok F, Durmus E, Ayaz M: The role of the resistive index in predicting testicular atrophy after orchiopexy in unilateral undescended testis. *Pediatr Surg Int,* 39 (1): 38, 2022. DOI: 10.1007/s00383-022-05336-3

31. Dalili AR, Madani AH, Joni SS: The comparison of resistance Index of testicular artery using color Doppler ultrasound in infertile men undergoing varicocelectomy. *J Reprod Infertil,* 22 (2): 110, 2021. DOI: 10.18502/jri.v22i2.5796

32. England GCW, Bright L, Pritchard B, Bowen IM, de Souza MB, Silva LDM, Moxon R: Canine reproductive ultrasound examination for predicting future sperm quality. *Reprod Domest Anim,* 52 (52): 202-207, 2017. DOI: 10.1111/rda.12825

33. Zelli R, Orlandi R, Troisi A, Cardinali L, Polisca A: Power and pulsed doppler evaluation of prostatic artery blood flow in normal and benign prostatic hyperplasia-affected dogs. *Reprod Domest Anim,* 48 (5): 768-773, 2013. DOI: 10.1111/rda.12159

34. Claus LAM, Barca Junior FA, Junior CK, Pereira GR, Fávaro PDC, Ferreira FP, Galdioli VHG, Seneda MM, Ribeiro ELA: Testicular shape, scrotal skin thickness and testicular artery blood flow changes in bulls of different ages. *Reprod Domest Anim,* 56 (7): 1034-1039, 2021. DOI: 10.1111/rda.13947