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Research article

THE USE OF DOPPLER ULTRASONOGRAPHY IN THE EXAMINATION OF TESTICLES IN DOGS

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Doppler ultrasonography is method suitable for observing and measuring the blood supply of many organs. Its principle is the change in the frequency of wave blood that moves with respect to the source of the wave (probe). In a testicular examination, the use of Doppler can also be very useful for detecting abnormalities or pathological conditions. The aim is just to characterize the flow in *arteria testicularis* in clinically healthy dogs aged 1-5 years. Twelve dogs were included in the study, of which 6 were rough collies weighing approximately 23 kg and 6 were standard dachshunds weighing approximately 9 kg. The pulsatility index (PI) and resistance index (RI) of *a.testicularis*, as well as the dimensions of the testicles - height and depth, were assessed by ultrasonographic examination using the ALOKA ProSound Alpha 6 ultrasonographic device (Hitachi-Aloka, Japan). The statistical evaluation of the results shows that the differences between the averages of both measurements of PI and RI indices are highly significant, so the weight of the individual had a high effect on the measured values of PI and RI indices. The depth of the testicles was also directly related to the increasing values of PI and RI indices and to the weight of the dogs, in contrast to their height, which did not show any statistical significance in this respect. The measured values and findings may in the future also serve as a basis for determining reference values in testicular ultrasonography according to the weight of dogs, which may be beneficial in clinical status assessment, diagnosis of various pathological conditions, or predicting spermatogenesis and fertility of the individuals.

Keywords: dog, testicle, ultrasound, Doppler

INTRODUCTION

Ultrasonographic examination of the testes allows the study of the anatomy, parenchyma, and adjacent structures that are important for the detection of anatomical abnormalities and subsequent diagnosis [1]. Two-dimensional testicular ultrasonography can assess echogenicity and echotexture, as well as to measure the testes, and can be used to assess the physiological development of testes or to detect

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testicular diseases [2]. The testes are supplied with blood by the *arteria testicularis*, which is an unusually long artery [3], with high resistance to flow, with the result that intratesticular pressure is lower than in any other organ and at the same time only slightly higher than the pressure in the veins [4]. The ability to visualize the normal vascular anatomy, as well as the presence or absence of blood flow in pathological conditions, may not only improve the diagnosis of testicular disorders but also allow monitoring of the therapeutic effects [5]. In human medicine, monitoring of blood circulation in the testes has been found as a predictor of testicular function and thus spermatogenesis [6,7], and has also been used in differentiating the various causes of dispermia [8,9].

The Doppler principle is based on the assumption that depending on the speed of the source's movement towards the listener, the frequency of waves transmitted by the moving source also changes [10]. Doppler ultrasonography is a non-invasive real-time impulse recording technique and Color Doppler ultrasonography has become the method used to evaluate the vessels of various organs, including the testes [11]. Currently, Doppler ultrasonography is commonly used in reproductive diagnostics [12]. In humans, the study of testicular vascularization by Doppler ultrasonography is already well known [13,14]. There have been several studies specifically targeted at dogs [15-17], but most of veterinary studies are focused on horses [18-22]. The results in these studies indicated that the blood flow in the testis is best defined by RI and PI indices. Though all these studies did not identify the possible differences depending on the size of the animal, there were no significant variations in size in the examined group of dogs. The aim is to determine the physiological parameters for a given species within certain sizes. Any deviations from the established values of blood flow in the testicles could lead to any pathological process even before the onset of clinical symptoms.

Despite the fact that other diagnostic methods (such as CT, angiography, MRI angiography) have been developed in the last decade, color Doppler ultrasonography is becoming the method of choice for its accessibility, simplicity, non-invasiveness and patient safety [23]. The use of Doppler ultrasonography in testicular examination allows the study of their anatomy, parenchyma, and accessory structures, which is important for the detection of anatomical abnormalities and for the selection of next suitable diagnostic methods [1], and also for diagnosis of some diseases, including testicular torsion and varicocele [24], as well as obstructive and non-obstructive azoospermia, which may reflect infertility problems [25]. Triplex Doppler involves the use of two-dimensional ultrasound and color Doppler, which allows the collection of anatomical data on blood vessels and functional data on blood flow, including the presence or absence, direction, and speed of the blood flow. It is a method for determining the vascularity of the testes and other organs [26,27]. In order to determine the suitable ultrasonographic parameters, it is important to identify the possible differences depending on the size of the animal and to determine the physiological parameters for a given species within certain sizes. Doppler ultrasonography is one of the simplest and

most accurate methods for measuring blood flow, combining anatomy with dynamic flow parameters [8]. Doppler ultrasonography in dogs was used to study blood vessels that demonstrated the importance of pulsation index (PI) and resistance index (RI) in determining healthy blood circulation in dogs with high weight variability [16]. It has been shown that RI and PI could be potential markers of seminal quality in dogs, as a negative correlation existed with total and progressive motility, and with the percentage of membrane intact sperms [28]. Infertile dogs showed lower peak systolic velocity (PSV) and end-diastolic velocity (EDV) measured in the distal supratesticular artery, marginal testicular artery, and intratesticular artery when compared to fertile ones. Notably, RI and PI did not differ between infertile and fertile dogs [29]. Testicular artery RI and PI were not predictive of future total sperm output or proportions of live normal sperm in dogs [30].

The aim of our work was to characterize the flow in *a. testicularis* in clinically healthy dogs aged 1-5 years.

MATERIAL AND METHODS

The study was carried out at the University of Veterinary Medicine and Pharmacy in Košice. The research period was 18 months between years 2017 and 2018. All the animals were presented at the Small Animal Clinic at the University of Veterinary Medicine and Pharmacy in Košice.

Animals

In this study, three groups of dogs were used:

– a group of rough collies, including 6 individuals weighing approximately 23 kg
– a group of standard dachshunds, including 6 individuals weighing approximately 9 kg
– 3 more dogs to compare with the results of two different weight groups, Rhodesian Ridgeback, Hungarian Vizsla, and German pointer

All subjects were aged from 1 to 5 years. They were kept in different conditions, fed with commercial dry food with unrestricted access to drinking water. There were no changes in the health or reproduction of the subjects during the study. Clinical examination of the testicles by palpation confirmed a firm consistency of the testicles with thin uninjured skin in all dogs.

Ultrasound examination

The ultrasonography was performed using the ALOKA ProSound Alpha 6 ultrasonographic system (Hitachi-Aloka, Japan), using a linear probe up to 16 MHz. The testicular examination by Doppler ultrasonography took place in a quiet room without disturbing influences with the owner's assistance. The examined individuals

were placed in a latero-lateral position without the use of sedation, only with the help of fixation. SONO-AQUAGEL gel was applied to the skin of the testicles and the probe was applied to the testicle afterwards.

B-mode

Initially, the echogenicity, echotexture and size of the testis were examined in the basic B-mode. Subsequently, the height and depth of the testicle in the longitudinal plane were measured. The correct imaging of the *mediastinum testis* was used as a reference point for accurate imaging of the entire testis. Each measurement of the right and left testes was performed three times and the arithmetic mean was then calculated from the measurements (Figure 1 and 2).

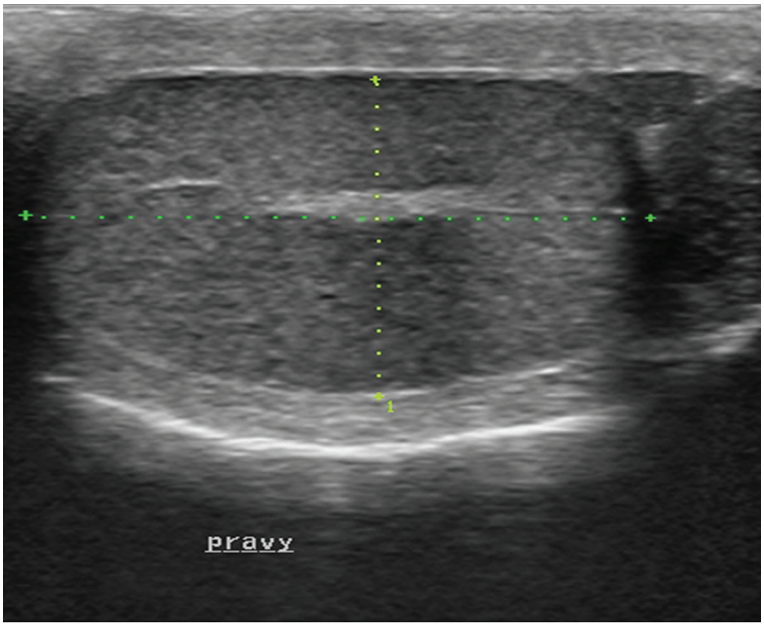

Figure 1. Ultrasonographic imaging of the right testicle in B-mode, followed by measurement of its height and depth. B-mode is also suitable for evaluating the echogenicity and echotexture of the testis.

Doppler ultrasonography

The blood flow of the a. testicularis was measured in each dog on both testicles in the *marginal region* (Figure 3). After accurate imaging of the testicle in B-mode, the duplex mode was activated and used to display the testicular blood supply. The testicular artery was visualized with alternating blue and red areas. This color change was caused by the detection of a flow of blood towards and away from the probe. After activating the triplex mode, following blood flow parameters were measured: PSV (peak systolic velocity), EDV (end-diastolic velocity), RI (resistance index), and PI (pulsatility index).

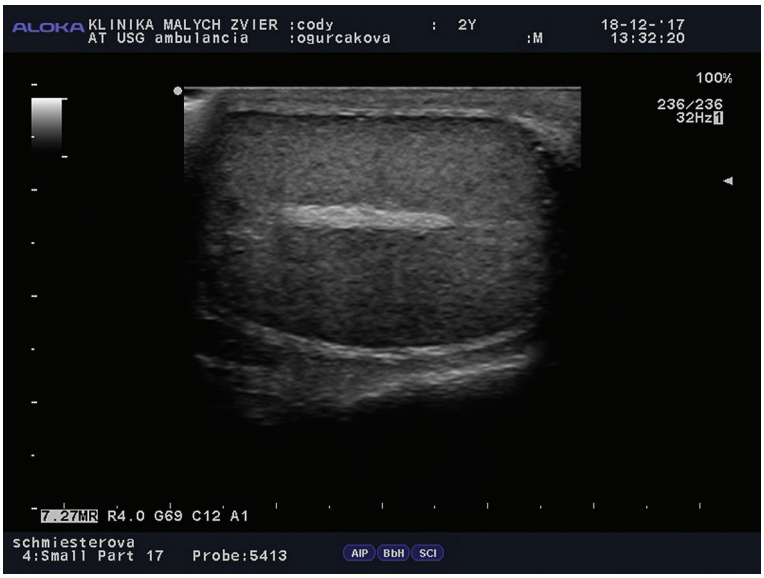

Figure 2. Correct performance of ultrasonographic examination of the testes in B-mode in the longitudinal plane showed us a clearly visible and formed mediastinum testis, which is visualized as a thin, hyperechogenic line located centrally in the testis.

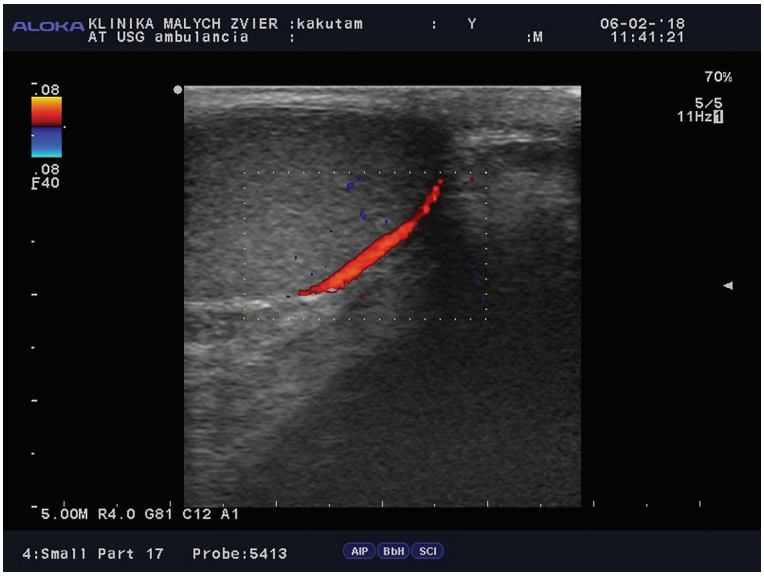

Figure 3. Doppler testis examination: testicular artery in the peripheral region observed along the dorsal edge of the testicles

The value was obtained by recording three waves, of which the middle wave was used for measurement. All measurements were obtained with a maximum correction angle of up to 60 degrees. The color intensity has been adjusted to a level that sufficiently

reduces the appearance of possible artefacts. Each measurement was performed by the same examiner (Figure 4).

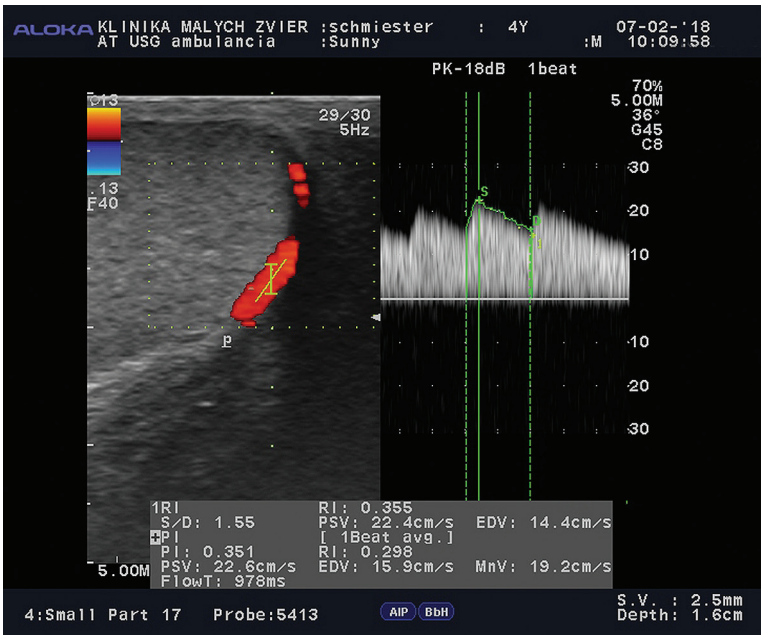

Figure 4. Imaging of the testicular artery using Doppler ultrasonography. The picture shows three waves, of which the middle one is used to measure values. Subsequently, the blood flow parameters are calculated: PSV, EDV, RI, and PI

Statistical analysis

The data were analyzed in the statistical program GraphPad Prism Version 9.0.0. (GraphPad Software, San Diego, USA). After analyzing the normality of the data sets using the Shapiro - Wilk test, the data were evaluated by unpaired t test. The correlation between dog weights and PI and RI indices was tested using Pearson correlation.

Ethical approval

Before every single examination an informed consent statement was obtained from all the owners of each dog. We hereby declare that it was only a non invasive examination (ultrasonographic examination). All the dogs present in this study did undergo only a non invasive medical examination such as an ultrasonographic examination is.

RESULTS

The average time needed for the ultrasonographic examination was 30 minutes (depending on the circumstances 20-45 minutes). In the B-mode ultrasonographic

examination in the longitudinal plane, a homogeneous structure was observed in all testicles with a clearly visible and shaped mediastinum, which was visualized as a thin hyperechogenic structure in the central line.

Doppler ultrasonography enabled the identification of *a. testicularis* at all sites in the left and right testicles.

From our own observations, increased values when measuring testicular dimensions in B-mode depth and height, as well as PI and RI values using Doppler imaging in actively used stud dogs were found. In Doppler ultrasonography of the testicles, significant differences in the measured blood-flow values between the left and right testicle were not found.

Testicular dimensions

The dimensions of the testicles are recorded in Table 1. All measurements were performed in B-mode, by marking the outermost parts of the testicles.

Table 1. Measured values of testicles height and depth in the group of dogs with higher weight (rough collies) and in the group of dogs with lower weight (dachshunds)

	LEFT TESTICLE		RIGHT TESTICLE	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
	Height (mm)	**Depth (mm)**	**Height (mm)**	**Depth (mm)**
Rough collies	32.5 ± 4.0	19.6 ± 2.5	31.7 ± 2.8	19.2 ± 2.2
Dachshunds	33.0 ± 1.7	16.3 ± 1.9	31.6 ± 1.8	16.0 ± 0.9
Unpaired t test	P = 0.7784	P = 0.0297	P = 0.9479	P = 0.0089
P value summary	ns	*	ns	**

The height of the left testicle in rough collies ranged from 28.87 to 39.77 mm with an arithmetic mean 32.5 ± 4.0 mm. The values of the left testicle height from 30.67 to 33.8 mm were measured for dachshunds with an arithmetic mean of 33.0 ± 1,7 mm. After statistical evaluation of left testicular height, no relevant statistical significance was noticed. The depth of the left testicle in rough collies reached 16.95 - 24.2 mm with an arithmetic mean of 19.6 ± 2.5 mm and in dachshunds from 12.87 to 18.13 mm with an arithmetic mean of 16.3 ± 1.9 mm. The height of the right testicle was in the range of 27.05 - 35.43 mm for rough collies with an arithmetic mean of 31.7 ± 2.8 mm and we measured values of 29.17 - 34.4 mm for dachshunds with an arithmetic mean of 31.6 ±1.8 mm. Also in the case of statistical evaluation of the right testicle height, no relevant statistical significance was noticed. The depth of the right testicle in rough collies had values of 15.6 - 22.43 mm with an arithmetic mean of 19.2 ± 2.2 mm and at dachshunds 15.1 - 17.67 mm with an arithmetic mean of 16.0 ± 0.9 mm. After statistical evaluation of the measured values, a significant difference was found in the depth of both left and right testicles between dog breeds, so the weight of the

individual had a great influence on the depth of the testicles, but not on their height (Table 1).

Measurements of PI and RI indices using Doppler ultrasonography

Doppler imaging of the testicular artery was possible in all cases of the examined individuals. The testicular artery in the peripheral region in which PI and RI indices were measured, had a linear pathway and was observed in the longitudinal plane along the entire length of the testicles. Observation of the peripheral part of the *a. testicularis* along the dorsal edge of the testes allowed the identification of a curve around the caudal pole. From there, the branches of the intratesticular part of the *a. testicularis* leading to the testicular parenchyma, directed to the mediastinum testis, were subsequently identified.

Doppler ultrasonography showed that the *a. testicularis* blood-flow curve was monophasic in each of three separate areas with systolic peaks, continuous diastolic flow, and low vascular resistance. In 1-year-old dogs, the systolic peak was milder than in older dogs.

PI (pulsatility index)

In the group of rough collies, the PI values of the left testicle were measured in the range of 0.390 - 0.604 with an arithmetic mean of 0.470 ± 0.072. In the group of dachshunds, the PI values of the left testicle ranged from 0.215 to 0.375 with an arithmetic mean of 0.269 ± 0.053.

In the case of the right testicle in rough collies, the PI index values ranged from 0.324 to 0.561 with an arithmetic mean of 0.456 ± 0.105 and in dachshunds it was in the range of 0.213 to 0.394 with an arithmetic mean of 0.288 ± 0.068 (Table 2).

Table 2. The PI index values of the testes of rough collies and dachshunds

	LEFT TESTICLE	RIGHT TESTICLE
	Mean ± SD	Mean ± SD
Rough collies	0.470 ± 0.072	0.456 ± 0.105
Dachshunds	0.269 ± 0.053	0.288 ± 0.068
Unpaired t test	P = 0.0005	P = 0.0132
P value summary	***	*

Since the PI values of the left as well as right testicles of both dog breeds differ significantly, it can be concluded that the weight of the examined dogs had a highly significant effect on the PI values.

RI (resistance index)

In the group of dogs with higher weight (rough collies), the RI values of the left testicle were measured in the range of 0.33 - 0.45, with an arithmetic mean of 0.373 ± 0.043 and in the group of dogs with lower weight (dachshunds), it reached only from 0.195 to 0.316 with an arithmetic mean of 0.240 ± 0.038.

Because the difference between the averages of the two measurements is statistically highly significant ($p < 0.01$), the weight of the examined dogs had a highly significant effect on the RI value of the left testis.

In the group of dogs with higher weight (rough collies) the RI values of the right testicle were measured in the range of 0.284 - 0.429 with a subsequent arithmetic average of all values of 0.390 ± 0.056. In the group of dachshunds, the values of the RI of the right testicle ranged from 0.208 to 0.331, followed by the arithmetic mean of all values of 0.271 ± 0.050 (Table 3).

Table 3. The RI index values of the testes of rough collies and dachshunds

	LEFT TESTICLE	RIGHT TESTICLE
	Mean ± SD	**Mean ± SD**
Rough collies	0.373 ± 0.043	0.390 ± 0.056
Dachshunds	0.240 ± 0.038	0.271 ± 0.050
Unpaired t test	P = 0.0004	P = 0.0053
P value summary	***	**

Likewise, the difference between the averages of both measurements of RI values on the right testicle is statistically highly significant ($p < 0.01$), and thus it can be stated that the weight of the examined dogs had a highly significant effect on the RI value.

Comparison of PI and RI indices with other individuals of different weight categories

In addition to the values determined in the two selected groups of dogs, PI and RI indices were measured in several other individuals of different weight categories (Table 4). After the subsequent ranking of breeds by weight, the measured parameters showed a relation between PI and RI indices with the weight of individuals. All measured values of PI and RI indices had an increasing tendency on both left and right testes, directly proportional to the weight. The hypothesis was confirmed by correlation analysis, which included all tested dogs - Collies (n = 6), Dachshunds (n = 6) and other breeds (n = 3). A higher degree of dependence on the weight of the dogs was recorded in the case of the left testicle, where the Pearson coefficient in the case of the PI index reached 0.894 and in the case of the RI index 0.896. The dependence on the weight of the dogs was slightly lower in the right testicles, as the Pearson coefficient in the PI index was 0.776 and in the RI index r = 0.771.

Our hypothesis, that the values of PI and RI indices measured in the marginal area of *a. testicularis* are also affected by the weight of the dog was confirmed.

DISCUSSION

The correct display the homogeneous testicular echotexture was possible and the *mediastinum testis* was presented as a thin, centrally located, hyperechogenic line. When Doppler was activated, the testicular artery was easily visualized in the marginal region, so it was chosen as the localization of measurement. Gumbsch et al. [16] also described this area as suitable for measuring PI and RI indices and used it in their research. The latest studies also agree on the fact that B-mode and Doppler examination of the testicles of dogs provide an important picture and useful information about their current state [28,29,31].

Souza et al. [27], confirmed in their scientific article on Doppler examination of testes of dogs of different sizes that the observed values of PI and RI indices differ with respect to the size and weight of the dog. The larger and heavier breeds of dogs show different values of velocimetric parameters than smaller breeds of dogs.

Souza et al. [27], report the results of measured PI index values in the range of 0.3 - 0.5 and RI index values in the range of 0.2 - 0.4. In 2015, another study, that included measuring and evaluating the velocimetric parameters of *a. testicularis* was conducted by Souza. In this study, the results of PI indices ranged from 0.4 to 0.7 and RI indices ranged from 0.3 to 0.6 [29]. Moxon et al. [31] in their study of velocimetric parameters of dog testes measured PI index values in the range of 0.2 - 1.0 and RI index values in the range of 0.2 - 0.6. Based on these results, it can be stated that the PI values measured by us in the range of 0.2 - 0.7 and the measured RI index values in the range of 0.2 - 0.5 were consistent with the studies of other authors.

The first research investigating the influence of blood supply by the *a. testicularis* as a prerequisite for spermatogenesis in healthy, adult dogs was published in 2013 [28]. Its importance is in the uniformity of the results and the selection of the specific region of the *a. testicularis* used to measure PI and RI indices. Doppler examination of canine testis has also shown that Doppler parameters are location-depended [16,17,27,32]. Moreover, there are not only regional but also significant pubertal differences in the blood flow of the testicular artery [27]. Souza et al. [27] evaluated that Doppler velocimetric parameters show differences depending on the localization and site of measurement of the *a. testicularis:* RI and PI have the highest values when measured at the site of the spermatic cord *(funiculus spermaticus)* and the lowest when measured in the intratesticular branches in the parenchyma of the testis. Lower values of PI and RI indices in the peripheral region than in the *funiculus spermaticus*

were also found by Gumbsch et al. [16] in a study of canine testes as well as by Pozor and McDonnell [18] in a study of stallion testes. Gumbsch et al. [16] also wrote that the measurement of *a. testicularis* blood flow in the *funiculus spermaticus* region is not

very accurate, due to its complex course. This hypothesis was confirmed by Souza et al. [27], since their measurements of PI and RI indices in the *funiculus spermaticus* region showed different results. Setchell [33] justified the higher values in the *funiculus spermaticus* than in the marginal region by the fact that the testicular artery in the marginal region has thinner walls and a larger internal diameter than in the seminal vesicle, because the testicular artery in the *funiculus spermaticus* region has to stretch more than usual. Biagotti et al. [9] also state that a limiting factor of ultrasonography in measuring PI and RI indices to assess blood flow *a. testicularis* is their independence from the correction angle.

Both PI and RI indices have been evaluated in several human and veterinary studies, under both physiological and pathological conditions [34-37]. Two studies have also reported a slight difference in PI and RI index values between the left and right testis [34,38], but these differences were not detected in our measurements.

The blood supply of the testes via the *a. testicularis* is also important in view of the knowledge that the oxygen concentration in the seminiferous tubules is very low [33]. Therefore, a reduction in blood supply may cause ischemic damage leading to impaired spermatogenesis. In addition, Pinggera et al. [6] found that PI and RI indices are potentially relevant markers in determining future semen quality in both dogs and humans. The research on the measurement of the testicle performed by Foresta, in 1998, stated that the part that showed the highest perfusion of blood flow also represented the place where the production of sperm cells was the highest [25]. However, since the studies are still in their early stages, more extensive research and measurements of the limiting values of PI and RI indices in pathological conditions are still needed to confirm this.

It has been published in human medicine that knowing the RI values of the testicular artery can be useful in differentiating the various causes responsible for both obstructive and non-obstructive azoospermia [9].

CONCLUSION

Doppler ultrasonography can be used to examine the testes of dogs of different breeds and weights. Determination of PI and RI values may be important for the assessment of testicular function. However, when measuring and evaluating, it should not be forgotten that the values of PI and RI indices measured in different regions of the testicular artery vary. The highest PI and RI values are observed in the *funiculus spermaticus* region, lower values in the peripheral region of the testicular artery, and the lowest values are measured in the intratesticular region.

Statistical analysis showed a highly significant effect ($p<0.01$) of dog weight on PI and RI values. The same was confirmed when examining testicular depth, which was directly related to and increased with the weight of the dogs, and thus with the PI and RI index values observed and examined. In contrast, no relevant statistical significance was found when testicular height was measured in relation to the weight of the dogs.

Our study highlights the importance of velocimetric parameters, which are influenced by many factors. The results of our research may also serve as a basis for establishing reference values in future scientific research works in testicular ultrasonography, as the knowledge of reference values may be useful in the evaluation of health status, finding and diagnosis of various pathological conditions, or in the prediction of spermatogenesis and fertility of an individual. The testicular artery plays an irreplaceable role in the blood supply to the testicles of dogs, since it is the main route for the transport of nutrients, hormones, and secretory products to the testes, and thus the reproductive capacity of the individual is dependent on its function.

To conclude, the results of our study confirm that Doppler ultrasonographic examination of the testes is a non-invasive, highly sensitive method that can detect and measure blood flow in the testicular artery.

Other factors that affect testicular blood supply, such as age, sexual activity, or pathologies of a different nature should be researched.

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Authors' contributions

ES drafted the manuscript. AV designed the study and performed the statistical analysis. KS carried out the clinical study and USG examinations. MF responded to reviewer comments. LH conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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UPOTREBA DOPLER ULTRASONOGRAFIJE U PREGLEDU TESTISA PASA

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Dopler ultrasonografija predstavlja metodu koja je pogodna za posmatranje i merenje prokrvljenosti mnogih organa. Princip na kojem je zasnovana se bazira na merenju promene frekvencije talasa krvi koji se kreće u odnosu na izvor talasa (sonda). Prilikom pregleda testisa, upotreba doplera može da bude korisna za detekciju abnormalnosti i patoloških stanja. Cilj je bio da okarakterišemo protok kroz *Arteria testicularis* kod klinički zdravih pasa starosti od 1 do 5 godina. U studiju je bilo uključeno 12 pasa, od kojih 6 rase oštrodlaki koli, prosečne težine 23 kg i 6 standardnih jazavičara prosečne težine 9 kg. Indeks pulsiranja (PI) i indeks rezistencije (RI) *a.testicularis*, kao i dimenzije testisa - visina i dubina, procenjeni su ultrazvučnim pregledom na ALOKA ProSound Alpha 6 ultrasonografskom aparatu (Hitachi-Aloka, Japan). Statistička procena rezultata pokazuje da su razlike između prosečnih vrednosti PI i RI indeksa značajne, pa je težina jedinke imala veliki uticaj na izmerene vrednosti PI i RI indeksa. Dubina testisa je takođe bila u direktnoj vezi sa povećanjem vrednosti PI i RI indeksa i sa težinom pasa, ne i u vezi sa visinom, koja u ovom pogledu nije pokazala statističku značajnost. Izmerene vrednosti i nalazi mogu u budućnosti da posluže i kao osnova za određivanje referentnih vrednosti u ultrasonografiji testisa prema težini pasa, što može biti od koristi u proceni kliničkog statusa, dijagnozi različitih patoloških stanja, ili predviđanju spermatogeneze i plodnosti pasa.