

Doppler and Contrast-Enhanced Ultrasonography of Testicles in Adult Domestic Felines

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Contents

The objective was to characterize the vascular patterns of testicular blood flow of adult cats, measuring the systolic velocity (SV), diastolic velocity (DV), resistance index (RI), gate time (wash-in) peak enhancement and output time (wash-out) of the contrast and addition of tissue fill characteristics. Forty-five adult cats were selected, and the echotexture, echogenicity, size, contours and margins of testicles were assessed via ultrasound. By Doppler were evaluated the blood flow and determined of vascular index in testicular artery (SV, DV and RI) and via contrast-enhanced ultrasonography determine the time for phases: wash-in, wash-out and peak enhancement. Sonographic findings presented normal. Testicular artery was observed in the spermatic cord with tortuous patten and showed monophasic-patterned waves and low vascular resistance and with systolic peak evident. Values of indices vascular were as follows: SV = 6.73 cm/s, DV = 2.8 cm/s and RI = 0.54 for left testicles; and SV = 6.23 cm/s, DV = 2.77 cm/s and RI = 0.53 for right testicles. Contrast filled the subcapsular vascular structures and after a few seconds, a homogeneous moderate enhancement of the parenchyma, with parenchymal vessels still distinguishable and after the peak phase, a rapid homogeneous decrease in echogenicity. Values of time for contrast-enhanced ultrasonography were as follows: wash-in = 8.78 s, peak enhancement = 21.62 s and wash-out = 75.36 for left testicles; and wash-in = 10.76 s, peak enhancement = 21.50 s and wash-out = 81.81 for right testicles. Doppler and contrast-enhanced ultrasonography of the testicles in healthy adult cats was easily implemented and may provide baseline data for this organ to allow the use of these techniques as a diagnostic tool for evaluating testicular abnormalities in sick cats.

Introduction

The testicles are the male sex organs whose function is the production of sperm and sex hormones, especially testosterone (Feldman and Nelson 1987). The use of ultrasound to determine the normal range of male gonads and to detect the testicular disorders is important for the early diagnosis of diseases and for the selection of breeding and maintenance of domestic and wild feline species (Domingos and Salomão 2011).

Among the imaging methods for the evaluation of animal reproductive system, the B-mode ultrasonography in testicular animals allows the determination of biometric values such as size and volume, assessment of topographic and parenchymal features, position and its internal constitution (Brandão et al. 2006). In cats, the testes are easily located by ultrasound within the

scrotum, showing uniform echotexture and echogenicity similar to the spleen (Davidson and Tomas 2009).

Doppler ultrasound method allows to study the anatomical features of the vessels and their functional characteristics related to vessel blood flow and functional data for blood flow (presence or absence, direction and flow velocity) (De Souza et al. 2014). In medicine, testicular Doppler is routinely used to determine the blood flow of the testicular artery, the diagnosis of testicular diseases and the study of spermatogenesis (Pinggera et al. 2008). In veterinary science, few studies are available with reports on the evaluation of stallions testicles (Pozor and McDonnell 2004) and dogs (Carrillo et al. 2012; Zelli et al. 2013), and few studies, with no reports on its use in the evaluation of cats.

The microbubble ultrasound contrast is a new technique in veterinary science. It uses contrast media of encapsulated inert structures, highly reflected by the apparatus that improve the colour and spectral Doppler signal (King 2006). In humans, this technique has already been used for the diagnosis of diseases affecting the testes; when ultrasound findings are inconclusive, this technique is used to obtain a higher degree of diagnostic accuracy (Souza and Silva 2014). In animals, the first study on the use of the technique was performed in dogs (Volta et al. 2014) in the evaluation of males with scrotal abnormalities; there are no studies on the technique in other species.

Considering the novelty of using the Doppler technique and contrast-enhanced ultrasonography in testicular evaluation of cats, the aim of this study was to characterize the vascular patterns of testicular blood flow of adult cats, measuring the systolic velocity (SV), diastolic velocity (DV), resistance index (RI), gate time (wash-in) peak enhancement and output time (wash-out) of the contrast-enhanced ultrasonography and addition of tissue fill characteristics (homogeneous and heterogeneous).

Material and Methods

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Universidade Estadual Paulista, Campus Jaboticabal, Brazil (protocol no 018.897/13). Forty-five adult, male,

domestic, shorthair cats, 3–5 years of age (mean age = 4 ± 0.78 years) and weighing between 2.7 and 4.9 kg (mean = 3.82 ± 0.7 kg), were selected for this study. General and specific physical examinations (inspection of the scrotum and testicular palpation) were performed on all animals to determine which animals were healthy and met the inclusion criteria of the study.

After the animals were selected, the scrotal sac was clipped for ultrasonography. Before the examination, gel was applied for the ultrasound. No sedation was needed.

The ultrasonography was performed by a single evaluator experienced in ultrasonographic examinations. B-mode ultrasonography was performed with a 9.0-MHz linear transducer using ACUSON S2000/SIEMENS ultrasound equipment (Siemens, Munich, Germany). The echotexture (homogeneous or heterogeneous), echogenicity (hypo-, hyperechoic or mixed) of the parenchyma, size (increased, decreased or normal), contours and margins (regular or irregular) of the testicles (right and left) were assessed and categorized via B-mode ultrasound in longitudinal and transverse sections.

Doppler analysis was performed after locating the largest and possibly longitudinal or oblique section of testicular artery, and the angle between the Doppler beam and the long axis of the vessel never was over 60° . Colour gain was adjusted to reduce the excessive colour noise when blood flows were too slow. A gate between 2 and 3 mm (equivalent to $2/3$ of the vessel's diameter) was positioned in a central area of the vessel with apertures to measure the spectral trace of flow, spectral curve and vascular index, which was obtained automatically following software identification of the ultrasonic scanner for each waveform. A minimum of three subsequent waves were obtained to perform its evaluation. The parameters studied in the testicular artery were SV (cm/s), DV (cm/s) and resistive index ($RI = (V_{\max} - V_{\min}) / V_{\max}$).

For contrast-enhanced ultrasonography was used the same ultrasound machine with harmonic imaging software developed for use with contrast media (Cadence) and convex transducer 9 MHz with Harmonic ultrasound system. The images were evaluated after the exams, verifying the tissue filling by the contrast-enhanced ultrasonography (homogeneous or heterogeneous), and determine the time for incoming phases (wash-in), output (wash-out) and peak enhancement of contrast-enhanced ultrasonography in testicular tissues (Takeda et al. 2012).

The contrast-enhanced ultrasonography used was SonoVue (Arm, Switzerland), 0.01 ml per assessed structure, administered by venous catheter in the cephalic vein, followed by the administration of five millilitres of saline (saline 0.9% saline).

After imaging techniques were carried out, the animals were submitted to orchiectomy and testicular structures were collected to verify the normality of these tissues. The collected fragments were fixed in 10% formalin solution, (phosphate buffered, pH 7.4) and routinely processed to

paraffin embedding. Five-micrometre sections were made in microtome and then stained with haematoxylin and eosin. In the analysis of light microscopy, histological features of biopsied tissues were observed.

R Software® (R Foundation for Statistical Computing, Vienna, Austria) was used for the statistical analysis. Results were initially analysed for normality (Shapiro test). Raw or transformed data were evaluated using *t*-test to compare the right and left values, once comproved equality, the general average, confidence interval and descriptive statistical were calculated. Pearson correlation and linear regression analyses between the quantitative values of Doppler and contrast-enhanced ultrasonography were carried out. The significance level used was of 5% and fixed for all tests.

Results

At clinical examination, the animals were healthy, and specific examinations of the testicles of all animals studied showed a firm consistency, with thin scrotal skin and without abrasions. In addition, the macro- and microscopic histologic parameters of testicles after castration were normal.

The B-mode ultrasound showed that the testicular echotexture and echogenicity of the studied animals were homogeneous and normal (hypo-echoic in relation to adjacent tissue), with clear visualization of the mediastinum testis as a thin hyperechoic central line in the longitudinal plane and as a hyperechoic central point when viewed in the transverse plane.

The Doppler and contrast-enhanced ultrasonography were reliably performed in all felines, yielding images of good quality. No adverse effects were noted in any animal during the procedures.

On colour Doppler, the testicular artery was observed in the spermatic cord and exhibited a tortuous pattern. Spectral Doppler of the testicular artery showed monophasic-patterned waves and low vascular resistance and with systolic peak evident (Fig. 1). The values for the indices vascular of right and left testicular artery in felines are shown in Table 1.

The testicular artery in its spermatic cord portion (of homogeneous form) was quickly filled by the contrast

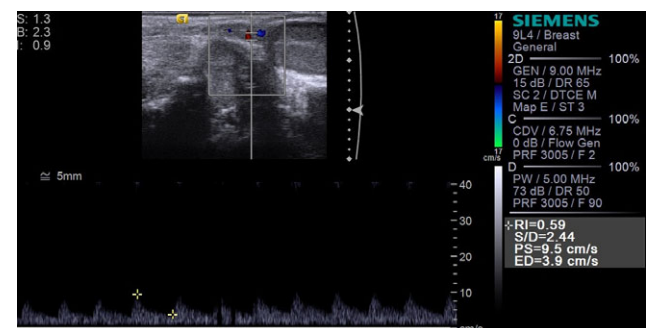


Fig. 1. Colour and spectral Doppler ultrasound image in testicle of cat. Note the Doppler assessments to determinate of vascular indices in testicular artery

Table 1. Mean values and standard deviation for vascular indices of the testicular artery and wash-in, peak enhancement and wash-out of contrast-enhanced ultrasonography in testicular parenchyma of felines

	Left testicle	CI 95%	Right testicle	CI 95%	<i>P</i> 5%
Doppler ultrasonography					
Systolic velocity (cm/s)	6.73 ± 2.78	5.89–7.58	6.23 ± 2.34	5.52–6.95	0.36
Diastolic velocity (cm/s)	2.80 ± 1.50	2.34–3.25	2.77 ± 1.36	2.35–3.18	0.92
Resistance index	0.54 ± 0.12	0.50–0.58	0.53 ± 0.12	0.49–0.57	0.67
Contrast-enhanced ultrasonography					
Wash-in (s)	8.78 ± 3.58	7.589–9.97	10.76 ± 4.04	9.41–12.11	0.038*
Peak enhancement (s)	21.62 ± 6.24	19.44–23.80	21.50 ± 5.81	19.47–23.53	0.76
Wash-out (s)	75.36 ± 20.20	68.53–82.20	81.81 ± 24.5	73.52–90.10	0.49

CI, confidence interval 95%; cm, centimetres; s, seconds.

*5% of significance level.

ultrasonography. Then, the contrast filled the subcapsular vascular structures, and after a few seconds, a homogeneous moderate enhancement of the parenchyma was observed, with parenchymal vessels still distinguishable. After the peak phase, a rapid homogeneous decrease in echogenicity was detected. After approximately 90 s, only few microbubbles were visible in the testicular parenchyma (Fig. 2). The values for the wash-in, peak enhancement and wash-out of right and left testicles in felines are shown in Table 1.

By comparing the values of vascular indices and contrast ultrasound measurements of the right and left testicular structures, it was possible to verify that no-statistic difference ($P > 0.05$). To correlate these variables ($P > 0.005$), except for wash-in, it was possible to

check a faster time in the left testicle, compared to the right ($P = 0.038$).

Discussion

Testicular Doppler is a very important method for the study of vascular features, providing real-time information of the vascular and hemodynamic aspects of testicular artery architecture (Carvalho et al. 2008) of the reproductive organs of humans and animals, especially in helping detecting the conditions that may cause fertility problems (Silva et al. 2012). Contrast-enhanced ultrasonography improves the representation of parenchymal organ vascularization, intensifying the Doppler signal, allowing the detection and characterization of parenchymal lesions with greater sensitivity and specificity, aiding in the diagnosis of testicular diseases in humans (Valentino et al. 2011; Souza and Silva 2014). This is the first study describing the use of contrast ultrasound Doppler and in healthy testicular evaluating feline, thus providing important reference values for the reproduction of veterinary obstetrics and cats.

Using the colour Doppler, the detection of feline testicular artery was consistent, identified dorsally, between testicular and epididymal structures and tortuous pattern, as described in the literature in canines (Nyland and Mattoon 2002; Carrillo et al. 2012; De Souza et al. 2014), stallions (Pozor and McDonnell 2004) and humans (Setchell and Breed 2006) unlike that observed in dogs (Carrillo et al. 2012; De Souza et al. 2014; Souza et al. 2014). The identification of marginal and intratesticular portions of the testicular artery in cats was limited, probably due to the small testicular volume, compared with the canines.

During the spectral Doppler exam, the testicular arteries of cats exhibited a pattern similar to that described in humans (Middleton et al. 1989) and in dogs (De Souza et al. 2014; Carrillo et al. 2012). According to Carvalho et al. (2008), the testicular artery showed characteristics waves of low resistivity, with low pulsatility and resistance, featuring flows with large and continuous systolic peaks and high-speed flow during diastole, typical of organs with continuous demand for blood.

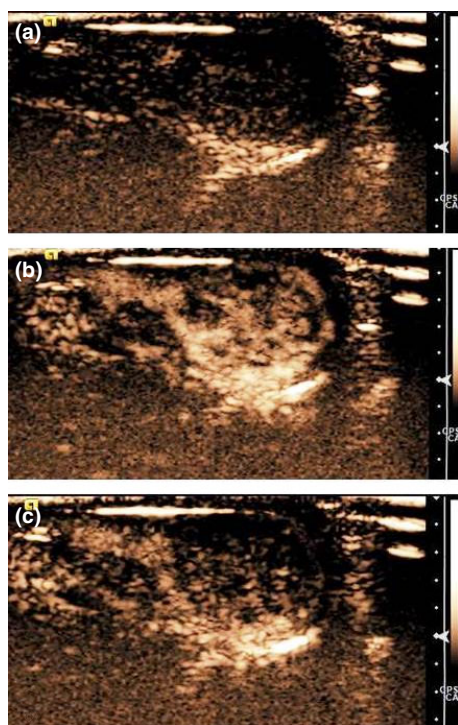


Fig. 2. Contrast-enhanced ultrasonography image of the testicle of cat in phases: (a) wash-in, (b) peak enhancement, and (c) wash-out

Regarding the vascular indices of the testicular artery in cats, the values for systolic and diastolic velocities are lower than those observed in canines (SV: 21.06 ± 1.3 cm/s and DV: 4.85 ± 0.44 cm/s, Carrillo et al. 2012; De Souza et al. 2014; Souza et al. 2014), justified by its smaller testicular structures, which requires less blood supply than the canine testicular tissue. But the RI of assessed vessels is similar to canines (Carrillo et al. 2012; De Souza et al. 2014; Souza et al. 2014), as this feature depends on other factors such as vessel diameter and blood flow region, with no influence of the tissue evaluated size.

According to Schurich et al. (2009), contrast ultrasound is a recent technique that allows the evaluation of macro- and microvascular tissue in animals and humans, providing relevant information on testicular perfusion. The first study of the technique in testicular evaluation of small animals was reported by Volta et al. (2014) in dogs, so the present study brings the novelty of the application of contrast ultrasound in the study of testicular perfusion in cats.

As in canines (Volta et al. 2014), contrast ultrasound technique was performed reliably and safely, producing images of good quality and observing fast testicular tissue filling by the contrast. According to Volta et al. (2014), the contrast flow is fast in the testes due to the testicular artery being a direct branch of the abdominal aorta.

In the present study, it was observed that the left testicular structures had less contrast entry time than the right testicles of felines. In dogs, that feature also occurs and is explained by the difference of the observed testicular volume (left testicle greater volume), requiring a greater blood flow to this structure. In this context, even if it was not statistically different in the speed of blood flow to the right testicular structure and left in cats, you can see a trend of a greater flow to the left testicle of these animals (see Table 1).

It is important to comment some considerations and limitations observed in this study:

1 Acquisition of the measurements: the Doppler and contrast-enhanced ultrasonography of the testes in felines were performed with no difficulty; the disposition of the animals allowed us to perform the

examinations without the need for sedation; and due to location of the evaluated structures, there was no interference from movements (e.g. respiratory) that hindered the acquisition of the measurements.

2 Age variation: age variation suggests that further studies must be conducted including testicular evaluation of animals of different age groups. However, this study provides important information about the validation of the technique and about obtaining reference values for adult male cats.

3 Animals with testicular disorders: it is necessary to assess the applicability of these imaging techniques in the diagnosis of testicular diseases of cats. Both Doppler and ultrasound contrast technique may be used to detect the abnormalities in testicular canine testis (Domingos and Salomão 2011; Volta et al. 2014).

Conclusion

Doppler and contrast-enhanced ultrasonography of the testicles in healthy adult cats were easily implemented and may provide baseline data for this organ to allow the use of these techniques as a diagnostic tool for evaluating testicular abnormalities in sick cats.

Acknowledgements

The authors would like to thank FAPESP for the financial support to the research group and support scholarships (processes 2012/16635-2 and 2013/06443-1).

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contribution

Brito MBS and Feliciano MAR designed the research; participated in acquisition, analysis and interpretation of data; and drafted and critically revised the manuscript. Coutinho LN, Uscategui, RR, Simões APR, Maronezi MC Almeida VT, Crivelaro RM, Gasser B and Pavan L participated in acquisition, analysis or interpretation of data. Vicente WRR also involved in designing the research and drafting and critical revision of the manuscript.

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Submitted: 2 Apr 2015; Accepted: 18 May 2015

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