

ARFI Elastography of Healthy Adults Felines Testes

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ABSTRACT

Background: Elastography is a promising ultrasound technique that evaluates tissue elasticity, characteristic related to the ability of a body or substance to return to its original size or shape after it is deformed by an external force. In veterinary, the research conducted for the standardization of elastography in tissue evaluation of the animals are recent and given the importance of this new ultrasound technique for studying the stiffness of various tissues and its recent application in veterinary medicine, the aims of this study were to describe the use of ARFI elastography for evaluating the testicular stroma in adults felines and to establish normal reference values for this tissue.

Materials, Methods & Results: Forty five male cats were submitted to B-Mode ultrasonography and ARFI Elastography, by qualitative and quantitative analysis to describe baseline data for the study of domestic feline testes. The echotexture (homogeneous or heterogeneous) ecogenicity (hypo, hyperechoic or mixed), size (increased, decreased or normal) and contours and margins (regular or irregular) of the testes (right and left) were assessed and categorised via B-Mode ultrasound in longitudinal and transverse sections for research of structural changes, such as the presence of cystic areas or masses. Qualitative and quantitative elastography of the testes in felines was performed without difficulty and without sedation. Due to the location of the evaluated structures, there was no interference from movements that hindered the acquisition of the measurements. During qualitative elastography, the felines demonstrated a homogenous and not pliable testicular stroma. The rigidity observed in the testicles of felines was lower compared to the adjacent tissue. The quantitative elastography, the mean shear velocity values were 1.51 m/s (95% confidence interval: 1.42 and 1.59 m/s) for the right testicle and 1.48 m/s (95% confidence interval: 1.41 and 1.54 m/s) for the left testicle of the felines. There was no significant difference when comparing the right and left testicular structures.

Discussion: The cats testicles are round or oval structures located ventrally to the anus and dorsally to the foreskin, visible only when the animal tail is raised. The normal testicular ultrasound image showed a pattern of homogeneous echotexture and echogenicity similar to the spleen. Overall, feline testicular ultrasound allowed achieving normal organ morphology, provide volume measurement, and enables the detection of testicular changes that may promote infertility of animals. Quantitative and qualitative ARFI elastography of the testes in felines was easily implemented, and this study provide baseline data for the study of these organs with ARFI. In this context, the feline testicular homogeneity and stiffness values obtained from qualitative ARFI in this study can aid the future use of elastography in the detection of tissue heterogeneity (soft or hard areas) resulting from pathological processes in felines as a noninvasive method of diagnosis. After standardising the reference values for testicular elastography in healthy cats, the differences in shear velocity values of diseased tissues can be evaluated to differentiate between benign and malignant tumours in felines, once the definitive diagnosis of benign or malignant lesions is today made only by histopathology after castration, considered a invasive method, which promotes the loss of the reproductive value of animals.

Keywords: feline, stiffness, testicular ultrasound.

INTRODUCTION

Elastography is a new ultrasound technique, first developed in the early 1990s for studying the hardness of tissues. This technique has been used in humans for identifying and differentiating between mammary tumours, diagnosing prostate tumours, assessing the testes [4,8] and, recently, evaluating mammary neoplasms in female dogs [10], spleen in cats [9] and dogs [16]. Acoustic radiation force impulse (ARFI) elastography is an imaging technique that provides quantitative and qualitative measurements of tissue stiffness with reduced inter-observer variability.

In humans, elastography has 100% sensitivity in detecting testicular tumours when values indicate an increased stiffness of the testicular stroma and facilitates the diagnosis of cystic alterations, haematomas, calcification, necrosis and other alterations that disrupt the homogeneity of the testicular tissue [1] and can detect malignant lesions [15]. These results demonstrate the importance of elastography as a diagnostic tool for assessing and qualitatively characterising images and for determining normal shear velocity reference values in the testes of felines.

In veterinary, there are no reports of the use of elastography in the evaluation of the reproductive system of felines. In canines, recently, the first study [11] on the technique for testicular assessment demonstrated that the quantitative and qualitative ARFI elastography of the testes in dogs was easily implemented obtaining important reference values for the species.

MATERIALS AND METHODS

Forty five healthy, adult, entire male, domestic, shorthair cats, aged three to five years (mean age = 4 ± 0.78 years) and weighing between 2.7 and 4.9 kg (mean = 3.82 ± 0.7 kg) were included in 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 for the study.

After the animals were selected, the scrotal sac were 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 ultrasound equipment

(ACUSON S2000)¹. The echotexture (homogeneous or heterogeneous) ecogenicity (hypo, hyperechoic or mixed), size (increased, decreased or normal) and contours and margins (regular or irregular) of the testes (right and left) were assessed and categorised via B-Mode ultrasound in longitudinal and transverse sections.

For elastography, qualitative and quantitative analysis was performed using the ARFI software and method (Virtual Touch Tissue Quantification) with a 9.0 MHz linear matrix transducer [11].

After B-mode ultrasonography, qualitative ARFI was performed to obtain greyscale images of the testicular tissue in longitudinal sections. The images were evaluated for the presence of deformities, white areas (indicative of more elastic tissue that is less rigid, softer and more pliable) and dark regions (more rigid, harder and not pliable).

The quantitative analysis was performed after scanning the testes with the B-mode ultrasound to determine the mean shear velocity values for these tissues. To obtain the shear velocity, the calliper was positioned in the right and left testicular stroma in the longitudinal section (excluding the mediastinal portion). Six measurements were recorded in each region and the depth utilised for the testicular measurements was 0.5 to 1.0 cm.

The data were tested for normality and homogeneity of variances (F test). The gross and transformed mean were evaluated by analysis of variance. In addition, a one-way analysis of variance for paired samples was applied to all shear velocities (values of left and right testicular). A P value < 0.05 was considered significant. Non-parametric data (echotexture, echogenicity size, greyscale images and the presence of deformities of the testes) were evaluated using descriptive analysis.

RESULTS

None of the owners reported or observed alterations that would compromise the health of the animals during the review of the medical history or the clinical examination. Assessment of the testes in all felines using B-mode ultrasound revealed no abnormalities in the size, contours and margins and echogenicity and echotexture of the stroma.

During qualitative elastography, the felines demonstrated a homogenous and not pliable testicular

stroma. The rigidity observed in the testicles of felines was lower (light gray, soft) when compared to the adjacent tissue (tunica - darker gray, hard) [Figure 1A].

Using quantitative elastography, the mean shear velocity values were 1.51 m/s (95% confidence interval: 1.42 and 1.59 m/s) for the right testicle and 1.48 m/s (95% confidence interval: 1.41 and 1.54 m/s) for the left testicle of the felines (Figure 1B). There was no significant difference when comparing the right and left testicular structures ($P = 0.5438$).

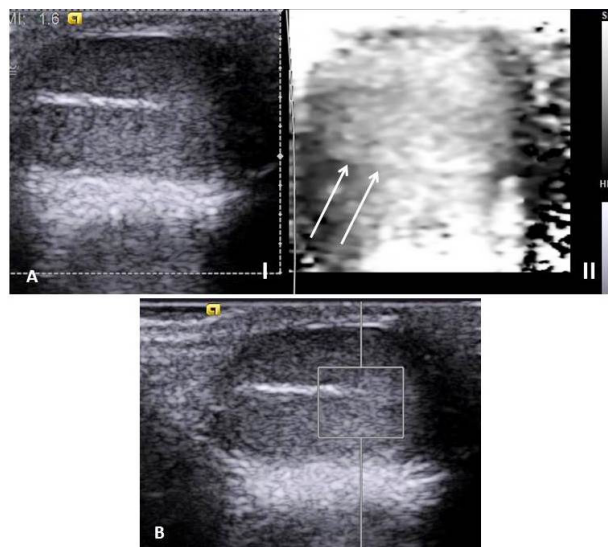


Figure 1. (A) Image of the qualitative ARFI elastography analysis showing the stiffness in the testicular stroma of the feline. (I) B-mode ultrasound image and (II) qualitative ARFI elastography (arrows) of the testicle of a feline showing a homogeneous image (light-grey) that is typical and not pliable. (B) Image of quantitative ARFI elastography in the testis of a feline; Note the presence of the calliper within the testicular stroma for measuring the shear velocity.

DISCUSSION

Elastography is a promising ultrasound technique that evaluates tissue elasticity, characteristic related to the ability of a body or substance to return to its original size or shape after it is deformed by an external force. The amount of deformity is inversely proportional to the stiffness and breakthrough times for tissue repair [12]. Qualitative ARFI uses short, high intensity acoustic pulses to deform the tissue and create a static greyscale map (elastogram) that represents the relative stiffness of the tissue in the imaged region. The elastogram can then be compared to the corresponding conventional ultrasound image. In general, lighter areas represent more deformable tissue compared with darker areas [9,12].

Quantitative ARFI involves directing a primary acoustic pulse towards a region of interest to create propagating pressure waves that are capable of quantitatively deforming the tissues (pressure wave velocity of propagation or shear velocity). The velocity of propagation and attenuation of the waves are related to the rigidity and viscoelasticity of the tissue [3,9]. In veterinary, the research conducted for the standardization of elastography in tissue evaluation of the animals are recent, and this is the first study on the use of ARFI elastography in testicular assessment of felines.

Tissue lesion of any nature (physiological or pathological) immediately promotes a series of events related to tissue repair and can present itself in two forms: regeneration with tecidual recomposition or formation of a fibrotic matrix [11]. In cases of chronic and neoplastic lesions, the healing process negatively interferes and promotes a loss of normal functional tissue patterns [2]. This cicatricial characteristic is related to tissue stiffness and can be studied by qualitative and quantitative AFRI elastography allowing the differentiation between benign and malignant processes in testicular tissues of felines, using the reference values obtained in this study.

Testicular ultrasonography can demonstrate lesions too small or inaccessible for detection via palpation, and permits differentiation of soft tissue details not recognized with radiography [6]. However, this technique does not permit the differentiation of testicular damage and is necessary the realization of techniques considered invasive as fine needle aspiration cytology, testicular biopsy and histopathological examination after castration [7]. In this context, the feline testicular homogeneity and stiffness values obtained from qualitative ARFI in this study can aid the future use of elastography in the detection of tissue heterogeneity (soft or hard areas) resulting from pathological processes in felines as a noninvasive method of diagnosis.

The shear velocities obtained for the felines testicles were higher than those observed in humans (0.62 to 1.01 m/s) [4] and dogs (juvenile = 1.28 m/s; adult = 1.23 m/s; and senior = 1.23 m/s) [11], most likely because dogs have more testicular fibrous tissue.

In humans, the elastography could be important for the detection and characterisation of these tissues allowing the early diagnosis of pathological testicular alterations [1]. The reference values determined for

the shear velocity of the felines testicles can assist the diagnosis of malignant lesions in these tissues by favouring early diagnosis and accelerating the institution of therapy in diseased animals. This statement is corroborated by data in human and veterinary studies [4,8,10] which quantitative ARFI techniques were used to differentiate between benign and malignant tumours (rigid and nonpliable tissues with high shear velocity values are indicative of malignancy).

It is important to comment on some of the considerations and limitations observed this study: - Qualitative and quantitative elastography of the testes in felines was performed without difficulty and without sedation. Due to the location of the evaluated structures, there was no interference from movements (e.g. respiration) that hindered the acquisition of the measurements as cited in literature [13] during abdominal elastography in canines; - It is necessary to conduct a study with animals of different ages to verify how this variable affect the elastography values in the testes of felines; and - Only one person was responsible for imaging, and future studies should verify any inter- or intra-observer variability in the acquisition and values obtained for qualitative and quantitative elastography of the feline testes.

After standardising the reference values for testicular elastography in healthy cats, the differences in shear velocity values of diseased tissues can be evaluated to differentiate between benign and malignant tumours in felines.

CONCLUSION

Quantitative and qualitative ARFI elastography of the testes in felines was easily implemented, and this study provide baseline data for the study of these organs with ARFI.

MANUFACTURER

¹Siemens Company. Munich, Germany.

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Ethical Approval. The trial was conducted following approval (process nº018.897/13) by the Animal Welfare and Ethics Committee of the School of Agrarian and Veterinary Sciences of the São Paulo State University (FCAV/UNESP - Jaboticabal, SP, Brazil).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- 1 Aigner F., De Zordo T., Pallwein-Prettner L., Junker D., Schäfer G., Pichler R., Leonhartsberger N., Pinggera G., Dogra V.S. & Frauscher F. 2012. Real-time sonoelastography for the evaluation of testicular lesions. *Radiology*. 263: 584-589.
- 2 Balbino C.A., Pereira L.M. & Curi R. 2005. Mecanismos envolvidos na cicatrização: uma revisão. *Brazilian Journal of Pharmaceutical Sciences*. 41: 27-51.
- 3 Comstock C. 2011. Ultrasound elastography of breast lesions. *Ultrasound Clinics*. 6: 407-415.
- 4 D'Anastasi M., Schneevoigt B.S., Trottmann M., Crispin A., Stief C., Reiser M.F. & Clevert D.A. 2011. Acoustic radiation force impulse imaging of the testes: a preliminary experience. *Clinical Hemorheology and Microcirculation*. 49: 105-114.
- 5 Da Silva L.D.M., De Souza M.B., Pereira B.S., Monteiro C.L.B. & De Freitas L.A. 2012. Bi-dimensional-ultrasonography and Doppler to evaluate the reproductive tract of small animals. *Ciência Animal*. 22: 339-353.
- 6 Davidson A.P. & Baker T.W. 2009. Reproductive ultrasound of the dog and tom. *Topics in Companion Animal Medicine*. 24: 64-70.
- 7 Domingos T.C.S. & Salomão M.C. 2011. Diagnostics tools of testicular disorders in dogs: review. *Revista Brasileira de Reprodução Animal*. 35: 393-399.
- 8 Dudea S.M., Giurgiu C.R., Dumitriu D., Chiorean A., Ciurea A., Botar-Jid C. & Coman I. 2011. Value of ultrasound elastography in the diagnosis and management of prostate carcinoma. *Medical Ultrasonography*. 13: 45-53.
- 9 Feliciano M.A.R., Maronezi M.C., Crivellenti L.Z., Crivellenti S.B., Simões A.P., Brito M.B.S., Garcia P.H.S. & Vicente W.R.R. 2015. Acoustic radiation force impulse (ARFI) elastography of the spleen in healthy adult cats - a preliminary study. *Journal of Small Animal Practice*. 56: 180-183.
- 10 Feliciano M.A.R., Maronezi M.C., Pavan L., Castanheira T.L., Simões A.P., Carvalho C.F., Canola J.C. & Vicente

- W.R.R. 2014.** ARFI elastography as complementary diagnostic method of mammary neoplasm in female dogs - preliminary results. *Journal of Small Animal Practice*. 55: 504-508.
- 11 Feliciano M.A.R., Maronezi M.C., Simões A.P.R., Uscategui R.R., Maciel G.S., Carvalho C.F., Canola J.C. & Vicente W.R.R. 2015.** Acoustic radiation force impulse elastography of prostate and testes of healthy dogs: preliminary results. *Journal of Small Animal Practice*. 56: 320-324.
- 12 Goddi A., Bonardi M. & Alessi S. 2012.** Breast elastography: a literature review. *Journal of Ultrasound*. 15: 192-198.
- 13 Holdsworth A., Bradley K., Birch S., Browne W.J. & Barberet V. 2014.** Elastography of the normal canine liver, spleen and kidneys. *Veterinary Radiology & Ultrasound*. 55: 620-627.
- 14 Karmazyn B. 2010.** Escrotal ultrasound. *Ultrasound Clinics*. 5: 61-74.
- 15 Lorenz A., Ermet H., Sommerfeld H.J., Garcia-Schürmann M., Senge T. & Philippou S. 2000.** Ultrasound elastography of the prostate. A new technique for tumor detection. *Ultraschall in der Medizin*. 21: 8-15.
- 16 Maronezi M.C., Feliciano M.A.R., Crivellenti L.Z., Simões A.P.R., Bartlewski P.M., Gill I., Canola J.C. & Vicente W.R.R. 2015.** Acoustic radiation force impulse elastography of the spleen in healthy dogs of different ages. *Journal of Small Animal Practice*. 56: 180-183.