RESUMO.- [Acurácia da elastografia ARFI na diferenciação dos estágios da catarata em cães.] O objetivo foi avaliar a precisão da elastografia na diferenciação entre lentes normais e de catarata. Dez cães foram submetidos à facoemulsificação. Parâmetros biométricos, ecogenicidade e padrões de ecotextura das câmaras anterior, posterior e vítrea, lente e complexos retina-coróide-esclera foram avaliados por ultrassonografia ocular nos modos A e B. A elastografia mostrou-se viável para avaliar a lente do cão, caracterizando os tipos de catarata e demonstrando aumento na rigidez das lentes afetadas.
deformability and the coloration (cor azul = indicou estruturas menos rígidas, cor vermelha = estruturas mais rígidas) das lentes foram avaliadas pelo elastograma. A velocidade da onda de cisalhamento (SWV; m/s) foi calculada em três regiões da lente, tanto no córtex quanto no núcleo. A SWV do núcleo foi estatisticamente diferente entre as lentes normais e com catarata e entre os estágios da catarata (P<0,001). Lentes saudáveis e cataratas incipientes tinham um núcleo mais rígido. Cataratas maduras apresentaram menor rigidez nuclear (P<0,01). Na região cortical, a SWV foi significativamente maior (P<0,01) nas cataratas intumescentes e incipientes. Uma SWV menor que 2,67m/s indica catarata com sensibilidade de 72% e especificidade de 94%. Valores inferiores a 2,23m/s sugerem catarata madura, com sensibilidade de 71% e especificidade de 76%. Uma SWV superior a 2,66m/s está associada à catarata normal ou incipiente, apresentando sensibilidade de 94% e especificidade de 84%. O método qualitativo permitiu a diferenciação entre lentes normais de olhos saudáveis e afetadas e a classificação dos estágios evolutivos. A elastografia se mostrou uma ferramenta viável para avaliar as lentes de cães, caracterizando os tipos de catarata e demonstrando maior rigidez das lentes doentes.

**INTRODUCTION**

Cataracts are considered as the greatest cause of blindness in both humans and dogs (Temporini & Kara 2004, Ofri 2013). Since, higher life expectancy is one of its causes, studies on the stages of cataract, lens characteristics in cataract, and especially about surgical treatment are required to determine the prognosis of the functional vision (Adkins & Hendrix 2005, Pigatto et al. 2007, Safatle et al. 2010).

Elastography is based on the principle of elasticity (Young’s modulus), in which the concerned tissue is subjected to a force. The degree of deformation depends on the tissue’s stiffness. In general, more rigid tissues suffer less deformation and after cessation of the applied force, the tissue returns to its original shape. This technique is performed in real-time, non-invasively, and more often does not require sedatives or anesthetics (Carvalho et al. 2015, Abreu et al. 2018). Currently there is no study that uses the elastography for the diagnosis of cataracts in animals or humans.

The developmental stages of cataract are identified by slit lamp biomicroscopic, after administration of mydriatic eyedrops, and a subjective evaluation of lenticular opacity (Mould 2002). Although it is possible to infer the degree of lens opacity based on its echogenicity and echotexture by A and B-mode ultrasonography, it does not support the measurement of rigidity (Martins et al. 2010). Therefore, the methods used in the classification of the stages of cataract are subjective and limited, and can affect the choice of the most appropriate therapy, as well as establishing prognostic value of surgery.

Most methods proposed for the investigation of lens stiffness are performed *in vitro* and did not include canine subjects (Antunes et al. 2006, Czygan & Hartung 1997). The methods include the automated hydrostatic guillotine, which cuts the lens with water pressure measuring the force required for its separation; linear compression plates; penetrating inventor; and high-frequency ultrasonic needle transducer (Assia et al. 1997, Czygan & Hartung 1997, Smith et al. 2002, Huang et al. 2009). Studies have also reported association between images obtained from an ocular ultrasound with the results of guillotine test to determine the relationship between the density of the tissue and its acoustic characteristics (Tabandeh et al. 2000).

The success of phacoemulsification surgery depends on the physical properties of the lens, and given the lack of an *in vivo* method to evaluate hardness of cataractous lenses. The aim of this study was to investigate acoustic radiation force impulse (ARFI) elastography in differentiating between cataractous and normal lenses; and to classify the stages of cataract in dogs. We hypothesized that these findings can be useful not just as a diagnostic measure, but also as a reliable method to establish the correct prognosis for patients undergoing phacoemulsification surgery.

**MATERIALS AND METHODS**

This study was approved by the institution’s ethics committee under the protocol number 005572/17. The owners of the dogs selected for this study authorized the participation of their dogs by signing a free and informed consent term. All dogs included in the study were from the “Serviço de Odontologia Veterinária” of the “Hospital Veterinário”, “Departamento de Clínica e Cirurgia Veterinária” (FCAV), “Universidade Estadual Paulista ‘Júlio de Mesquita Filho’” (Unesp, Jaboticabal).

The canine subjects were classified into six groups according to the developmental stage of cataract as healthy, incipient cataract, immature cataract, mature cataract, intumescent cataract, and hypermature cataract. The study of the different developmental stages of cataract such as incipient, mature, hypermature, and intumescent, is justified considering that this classification is commonly used in studies involving lenses (Ofri 2013). Considering a statistical significance level of 5%, a minimum sample size of 20 eyes was established for each of the developmental stage and healthy eyes group.

Physical, laboratorial (complete blood count, creatinine, and alanine aminotransferase - ALT), and ophthalmic examinations were performed for all the subjects in the study. Ocular examination included direct and consensual pupillary reflexes, menace response, Schirmer’s lacrimal test, slit-lamp biomicroscopic, applanation tonometry, binocular indirect ophthalmoscopy, fluorescein and tear-film rupture test, and ocular ultrasonography on A and B-mode. Dogs of the “healthy” group, presented with no abnormalities on physical, laboratorial, and ophthalmic examinations. The dogs that presented with cataract were classified according to the development stage based on evaluation of the lenses with menace response, slit-lamp, and ultrasonography (A and B-mode).

Canine subjects classified under “incipient cataract” presented with positive menace response, corneal opacity >15% of the lens at slit-lamp, low peak formation, and a small region of hypechogenicity on ocular ultrasonography. The “immature cataract” group had decreased menace response; lenticular opacity <15%, especially in the central region; moderate peak formation; and incomplete hypechogenicity of the lens on ocular ultrasound. In the “mature cataract” group, patients showed negative menace response, complete lenticular opacity, peak formation, and complete hypechogenicity of the lens on ocular ultrasound (Ofri 2013).

Eyes of the canine subjects classified as “intumescent cataracts” presented with negative menace response, complete opacity of the lens, peak formation, and hypechogenicity associated with hypechogenicity observed throughout the lens extension on
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evaluation of the ultrasonography, lenticular thickness >7 mm, and anterior chamber decrease. The canine patients of the “hypermature cataract” group showed negative menace response, complete opacity and capsule roughness prior to slit-lamp evaluation, peak formation, hyperechogenicity throughout the lens on ultrasonographic evaluation, and presence of irregularities in the anterior capsule (Ofri 2013).

To perform ocular ultrasonography and elastography, the corneas were desensitized with proxymetacaine hydrochloride (0.5%) eyedrop (Anestalcon, Alcon, SP, Brazil). Sterile acoustic gel (Supra Gel, Adlin Plásticos LTDA, Jaraguá do Sul/SC, Brazil) was used as contact medium for the transcorneal method. Chemical restraint was not needed.

The A and B-mode ocular ultrasounds were performed with Ultrascan A/B equipment (Alcon Laboratories, INC-USA) using a 20MHz probe in axial position by a trained examiner. Biometric parameters (diameter, length, and thickness measured in mm), echogenicity, and echotexture patterns of the anterior, posterior, and vitreous chambers, lens, and retinal-choroid-sclera complexes were evaluated.

The device employed for elastography was the Acuson S2000/Siemens (Siemens Medical Solution, USA, INC-USA) using the ARFI method (Virtual Touch software) with a 9.0MHz transducer by an experienced sonographer. For the qualitative study, deformability of the lenses was assessed (deformable or non-deformable) from the different colors of the elastogram. Blue color indicated fewer rigid structures than those of green color (intermediate rigidity), and the red color showed the most rigid structures. The quality of the images was tested using a display device in which homogeneous and greenish images indicated high quality, while heterogeneous and yellowish images indicated low quality. After the qualitative analysis, three regions of interest, covering the entire length of the lens both in the cortex and in the nucleus were defined by positioning the caliper over these areas. Using this methodology, the software automatically calculates the shear wave velocity (SWV) in m/s of each region and the mean of which was calculated for statistical analysis (Abreu et al. 2018).

Statistical analysis was performed using R software (RTM Foundation for statistical computing, Austria). SWVs of the cortex and lens nucleus were compared between the cataract stages of the groups using the Kruskal Wallis test and the Dunn post-test, and the results were presented as median ± interquartile range (IQR). Parameters with significant differences (P<0.05) between cataract stages were, subsequently, submitted to a discriminative power analysis through receiver operating characteristic (ROC) curves and cutoff value, sensitivity, specificity, and area under the curve (AUC) were calculated using a logistic regression model. Significance level was set at 5% (P<0.05) for all tests.

RESULTS

Twenty eyes with healthy lenses were studied. In the cataract groups, the following numbers were acquired: 20 incipient, 20 intumescent, 20 immature, 45 mature, and 20 hypermature. The study included 145 eyes of 98 dogs of different breeds, adults from two to 12 years (mean age 5.85±2.88 years), males (23.81%), and females (76.19%).

In the elastogram, normal lenses presented with poor image quality and no homogeneity in color on evaluation. In general, cataractous lenses showed high image quality and homogeneity in the color patterns, especially for regions with lenticular opacity (Fig.1-10). Lenses with incipient cataracts were blue for the affected regions and showed poor image quality for the non-affected regions. Intumescent cataracts presented with blue cortex and some small regions of green. However, on examination, the nucleus showed as having poor image quality. In immature cataracts, there was a predominance of blue and some areas of green, although high image quality was observed only for regions of opacity. Mature cataracts had high image quality throughout the lens with predominant blue tone and some nuclear regions as green. In case of hypermature cataracts, the lenses were presented as high image quality showing a predominance of blue with some greenish and reddish tones.

Table 1 shows the minimum, maximum, median, IQRs and Confidence intervals (CI-95%) of cortical and nuclear SWVs of healthy lenses and the different stages of cataract. Whereas, in the healthy lenses, it was possible to obtain values related

Fig.1-2. (1) B-mode and (2) elastogram of a dogs with cataract showing different colours of the lens. Dog with incipient cataract: (1) hyperechogenicity of anterior capsule (yellow arrow) coincident with ophthalmic opacity and (2) blue colour (yellow arrow) (soft) in the region of opacity due to cataract.
Fig.3-4. (3) B-mode and (4) elastogram of a dog with cataract showing different colours of the lens. Dog with intumescent cataract: (3) areas of hyperechogenicity (yellow arrow) and anechogenicity (red arrows) (regions with aqueous content) and (4) blue colour (soft) in the region of opacity caused by the cataract (yellow arrow) and absence of colour due to the aqueous content (red arrows) characteristic of this stage.

Fig.5-6. (5) B-mode and (6) elastogram of a dog with cataract showing different colours of the lens. Dog with immature cataract: (5) hyperechogenicity in almost the entire lenticular surface and (6) blue colour (soft) throughout the lens extension.

Fig.7-8. (7) B-mode and (8) elastogram of a dog with cataract showing different colours of the lens. Dog with mature cataract: (7) hyperechogenicity throughout the lens and (8) green colour throughout the lens.
Table 1. Minimum, maximum, median, and interquartile range (IQR) of the cortical and nuclear shear velocities (m/s) of healthy lenses and of different stages of cataract

| Variable       | Stage      | Minimum | Median  | Maximum | IQR  | Mean - CI          | P-value |
|----------------|------------|---------|---------|---------|------|--------------------|---------|
|                | Hipermature| 1.020   | 2.480b  | 2.990   | 0.893| 2.1 - 2.8          | <0.001  |
| SWV - Nucleus  | Mature     | 1.930   | 2.360b  | 3.00    | 0.387| 2.3 - 2.6          |         |
|                | Incipient  | 2.230   | 2.850a  | 3.100   | 0.177| 2.8 - 2.9          |         |
|                | Intumescent| 1.100   | 2.160b  | 3.680   | 0.542| 2.1 - 2.6          |         |
|                | Mature     | 1.090   | 2.050a  | 3.460   | 0.780| 1.8 - 2.2          |         |
|                | Healthy    | 2.230   | 2.850a  | 3.100   | 0.177| 2.8 - 2.9          |         |
| SWV - Cortex   | Hipermature| 1.260   | 2.370b  | 3.140   | 0.720| 1.9 - 2.6          | <0.001  |
|                | Mature     | 1.500   | 2.205b  | 3.390   | 0.435| 2.1 - 2.5          |         |
|                | Incipient  | 1.750   | 2.505a  | 2.950   | 0.302| 2.4 - 2.6          |         |
|                | Intumescent| 1.650   | 2.730a  | 4.070   | 0.690| 2.4 - 3.0          |         |
|                | Mature     | 1.180   | 2.110b  | 6.790   | 0.810| 1.8 - 2.4          |         |
|                | Healthy    | N/A     | N/A     | N/A     | N/A  | N/A                | <0.001  |

m/s = Meters per second, CI = confidence interval, N/A = measure not available; a,b,c Median stages of cataract with different superscript letters are significantly different (P<0.01).

Fig. 9-10. (9) B-mode and (10) elastogram of a dogs with cataract showing different colours of the lens. Dog with hypermature cataract: (9) hyperechogenicity of the entire lens and anterior capsule irregularity (yellow arrow) and (10) blue colour (red arrow) of the entire lens, some greenish regions (orange arrow) (intermediate stiffness), and the others reddish (gray arrow) (high stiffness).

to the nucleus only, since the cortical region had an SWV of XX m/s, that is assumed to be indicative of a value below or above the limit and, therefore, was not measurable by the equipment. On the other hand, in the incipient cataracts, it was possible to measure the SWVs of the cortex only due to the opacity and, therefore, displayed as good image quality on examination.

The difference in the SWVs of the nucleus between the different stages of cataract, and that between the healthy lenses and the affected ones was statistically significant (P<0.001; Fig.11-13). Healthy lenses and those with incipient cataracts had a more rigid nucleus (P<0.001) than those with hypermature, immature, and intumescent cataracts. Mature cataracts presented with lowest nuclear rigidity when compared to the others (P<0.001). The cortical SWV was significantly higher (P<0.001) for the intumescent and incipient cataracts than for the other stages (Fig.11-13).

Based on an elastography evaluation, an SWV of the lens nucleus <2.67 m/s is indicative of cataract. The capacity of elastography to predict a cataract stage showed a sensitivity of 72%, specificity of 94%, and AUC of 82% (Fig.11-13). Additionally, a value <2.23 m/s indicates a mature cataract, with sensitivity of 71%, specificity of 76%, and AUC of 76%. Furthermore, an SWV >2.66 m/s is suggestive of a lens that is healthy or has incipient cataract, with a sensitivity of 94%, specificity of 84%, and AUC of 89%.
structures with large aqueous content are presented as poor
et al. 2000, Huang et al. 2007). (Czygan & Hartung 1997) and ultrasonography (Tabandeh
compression plates (Smith et al. 2002), penetrating indentor
humans using the guillotine system (Assia et al. 1997), linear
stages of cataract, which corroborates with previous studies in
healthy lenses and different developmental stages of cataract, may be
explained by the composition and organization of the lenses,
emphasizing the importance of elastography in such cases. Healthy
lenses are basically composed of aqueous content and 33% of protein fibers organized in the lamellae (Kuszak et al. 2004a, 2004b). During the development of cataract, there is a disorganization of the lamellar arrangement (Galego et al. 2012) and dehydration of the lens, leading to opacity, which results in variable visual deficiency, according to the stages of development of the condition (Czygan & Hartung 1997, Kleiner 2007, Gelatt & Wilkie 2011, Galego et al. 2012).

Tissues affected by different diseases present with changes in their components and/or molecular organizations, that directly interferes with its biomechanics (Shah et al. 2007), and consequently, with the rigidity of the tissue, as was observed in the present study (Paszek et al. 2005).

The distinction in stiffness observed between the nucleus and the cortical areas may be explained by the anatomical and physiological characteristics of the lens. The epithelial cells below the anterior capsule are responsible for the production of lenticular fibers during its life, forming the cortex and the nucleus (Dick et al. 2008). Fibers from the nuclear region are called primary fibers and are older than the secondary fibers present in the cortex (Kuszak et al. 2004a, Dick et al. 2008). The authors suggest that such structural variations within the lens may interfere with the results of an elastography, explaining the differences of the nuclear region (primary and old fibers) than the cortex region (secondary and new fibers).

Elastogram revealed variations in rigidity for different stages of cataract, which corroborates with previous studies in humans using the guillotine system (Assia et al. 1997), linear compression plates (Smith et al. 2002), penetrating indentor (Czygan & Hartung 1997) and ultrasonography (Tabandeh et al. 2000, Huang et al. 2007).

According to a colored elastogram, it is to be noted that structures with large aqueous content are presented as poor quality on examination. Considering that incipient cataracts have up to 15% opacity of the lens (Ofri 2013), the images with blue shades occurred only in the small region affected by opacity, whereas, the healthy areas that maintained enough aqueous content showed as poor quality on examination. Intumescent cataracts are more frequent in diabetic patients and are characterized by higher aqueous content due to the osmotic gradient facilitated by sorbitol (Galego et al. 2012); that explains the mixed patterns of low stiffness (blue color) and poor image quality (aqueous content) observed for this stage. In this study all patients with intumescent cataracts were diabetic. In immature cataracts, opacity occurs partially (Gelatt & Wilkie 2011). In the elastogram it was noted that the lenses presented with no color in regions with aqueous content and regions of green indicating an intermediate level of stiffness. On the other hand, in mature cataracts the opacity is complete and presents with visual deficit (Rodrigues et al. 2010), corroborating with the qualitative evaluation in which the entire lens was colored, representing both low and intermediate degrees of rigidity. The same was observed for the hypermature stage; however, due to the higher composition of compact fibers and loss of aqueous content (Gelatt & Wilkie 2011), some areas presented with high stiffness (red).

Studies on lenticular stiffness in humans are commonly performed ex vivo (Assia et al. 1997, Czygan & Hartung 1997, Smith et al. 2002) and in vitro (Huang et al. 2007); however, fewer are performed in vivo (Tabandeh et al. 2000). Compared to these studies which showed that the more advanced cataract showed greater rigidity of the lens due to increased protein concentration and disorganization of the lenticular fibers (Kurapkiené et al. 2005), in our study, it was observed that normal and incipient cataracts presented higher stiffness on the nuclear region (higher SWVs) than the other stages. This finding is justified from the poor image quality obtained on examination of the healthy lenses and incipient cataracts. The authors of this study have suggested that the equipment is more reliable in the evaluation of advanced cataract (mature and hypermature) due to lower aqueous content, thereby justifying the difference in our results and in vitro studies.

**CONCLUSIONS**

Qualitative method was useful in the study of stiffness in each region of the lens and could be useful as a prognostic
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