B mode ultrasonography and elastography in the evaluation of the pectineus muscle in dogs with hip dysplasia

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Abstract: The goal of this study was to describe and compare B mode and elastographic characteristics of the pectineus muscle of healthy dogs with dysplastic dogs. Thirty-one dogs (62 limbs) with hip dysplasia and 17 nondysplastic dogs (34 limbs) were evaluated. The hip dysplasia score was defined according to the Fédération Cynologique Internationale. Using B mode, echotexture and echogenicity of different regions of the pectineus muscle were evaluated. By means of ARFI elastography, qualitative (elastogram) and quantitative (shear wave velocity) tissue stiffness was assessed. B mode findings demonstrated a hyperechoic and heterogeneous pattern of the pectineus tissue in dogs with hip dysplasia, with compromised muscular delimitation and loss of its normal sonographic appearance, indicating the disease (P < 0.001). In the elastogram, it was observed that dogs with hip dysplasia showed less deformable pectineus muscle, with red colors (rigid). In quantitative evaluation, the different regions evaluated presented similar shear wave velocities; in dysplastic patients, shear wave velocities were higher compared to nondysplastic animals, with values higher than 2.85 m/s being strong indicators of the disease. Values of shear wave velocity were also influenced by the grade of dysplasia and age of the patients; however, there was no correlation with the depth of the evaluated area or body weight. It was concluded that pectineus muscle in dogs with hip dysplasia presents B mode and elastographic changes when compared to normal animals, demonstrating that these techniques might aid the evaluation of diseased dogs.

Key words: Canine, dysplasia, joint, elastography, ultrasound

1. Introduction
Hip dysplasia (HD) is an osteoarticular abnormality which has a multifactor character and involves genetic components associated with articular laxity [1]. The biomechanics of the canine hip is complex, and there are interactions of various osseous, muscular, and ligament structures, besides important neuromuscular mechanisms [2]. In dogs with hip dysplasia, limitation in the abduction of the affected limb is noted due to tension in the adductor muscle [3]. It is described that in order for the femur to move towards the body midline (joint reduction position), a series of muscles have to contract, including the adductor magnus, adductor longus, gracilis, and pectineus muscles [2].

Biomechanical evaluation of organic tissues can be studied with in vivo and ex vivo models, whether to simplify a real system or because it is impossible to execute a study without these models [4,5]. Regarding the methods for biomechanical studies, elastography is a safe and noninvasive technique that allows evaluation of stiffness/elasticity of various tissues through qualitative and quantitative data [6].

Given that the pectineus muscle is prominent and rigid in many dysplastic patients, this study proposed to analyze the applicability of Acoustic Radiation Force Impulse (ARFI) elastography in evaluating the stiffness of the pectineus muscle of dysplastic dogs [7]. Thus, the aim of the study was to describe and compare qualitative (B mode and elastogram) and quantitative (shear wave velocity) characteristics of the pectineus muscle of healthy and dysplastic dogs.

2. Materials and methods
This study was performed after the approval of the Ethics Committee in Animal Use of the School of Agricultural
and Veterinary Studies of the São Paulo State University, Brazil, under protocol no. 3212/17. All patients had previous authorization from their tutors by means of signed consent terms.

Forty-eight adult dogs (average age 4.3 ± 1.5) were studied, 50% males and 50% females, weighing from 20.2 to 49.9 kg (average weight 31.2 ± 8.3 kg). There were 16 different breeds (Table 1), but most animals were mongrels (n = 13), followed by Labrador Retriever (n = 9), Australian Cattle Dog (n = 5), and Golden Retriever (n = 4).

For patient selection, general and orthopedic physical examinations, as well as laboratory tests (hemogram, alanine aminotransferase, and creatinine) were performed. Orthopedic evaluation included palpation, extension, and flexion of all joints, cranial draw test, and tibial compression of both stifles as well as evaluation of possible patellar instability, to exclude any orthopedic disease concomitant of HD.

Patients with positive Ortolani test, pain/discomfort/pseudo crepitation during manipulation of the hip joint, history of dysplastic relatives, pain when getting up, exercise intolerance, hind limb lameness, and aggressiveness during manipulation of the pelvic region, together with radiographic hip joint abnormalities were included in the diseased group (DG – dysplastic patients), while the control group (CG – healthy animals) was composed of animals that did not present any of these changes.

For definitive diagnosis, patients underwent general anesthesia in order to obtain radiographic images of the hip and for confirmation of Ortolani test. Chlorpromazine 0.5 mg/kg was administered intramuscularly as preanesthetic medication. Anesthesia was induced with propofol intravenously at the dose necessary for loss of the laryngotracheal reflex (1.0–5.0 mg/kg), and the dogs were immediately intubated to provide 100% oxygen. General anesthesia was performed with continuous infusion of propofol, at the initial rate of 0.5 mg/kg/min, administered through an infusion pump.

Patients underwent mediolateral and ventrodorsal radiographic projections as recommended by the Orthopedic Foundation for Animals (OFA), using an 800-mA SIEMENS Triodoros 812E® x-ray generator (Siemens Medical Solutions, USA) and Agfa CR 30-X® scanner (Agfa Healthcare, NV, Mortsel, Belgium). The images were blindly evaluated by an experienced radiologist (10 years of experience) who was unaware of the patients’ health status.

Radiographic signs of each joint were classified according to the Fédération Cynologique Internationale (FCI) in 5 grades: A = no signs of hip dysplasia; B = near normal hip joints; C = mild hip dysplasia; D = moderate hip dysplasia; E = severe hip dysplasia [8].

After radiographic study and anesthetic recovery, ultrasonographic evaluation of the pectineus muscle

| Hip dysplasia score | Breed          | Age (Years) | Weight (kg) |
|---------------------|----------------|-------------|-------------|
| A                   | Rottweiller    | 25.4        | 5           |
| A                   | Blue Heeler    | 31.2        | 6           |
| A                   | Dalmata        | 28.3        | 6           |
| A                   | Mixed-breed    | 21.8        | 6           |
| A                   | Blue Heeler    | 22.4        | 5           |
| A                   | Border Collie  | 20.3        | 4           |
| A                   | Mixed-breed    | 26.8        | 4           |
| A                   | Blue Heeler    | 25.2        | 5           |
| A                   | Blue Heeler    | 22.8        | 6           |
| A                   | Mixed-breed    | 36.6        | 2           |
| A                   | Labrador       | 39.1        | 2           |
| A                   | Mixed-breed    | 33.5        | 5           |
| A                   | Labrador       | 24.5        | 2           |
| A                   | Labrador       | 23.2        | 4           |
| A                   | Blue Heeler    | 25.4        | 6           |
| A                   | Belgian Malinois | 23.5     | 2           |
| A                   | Labrador       | 37.2        | 4           |
| B                   | Mixed-breed    | 32.5        | 5           |
| B                   | Mixed-breed    | 24          | 4           |
| B                   | Mixed-breed    | 23          | 6           |
| B                   | Labrador       | 50          | 4           |
| B                   | Labrador       | 42          | 3           |
| B                   | Beagle         | 20.2        | 3           |
| C                   | Beagle         | 21.1        | 4           |
| C                   | Boxer          | 26.4        | 6           |
| C                   | Mixed-breed    | 24.1        | 4           |
| D                   | Bernese Mountain | 36.8     | 3           |
| D                   | Golden Retriever | 41.3    | 6           |
| D                   | Golden Retriever | 29.2    | 2           |
| D                   | Rottweiller    | 33          | 2           |
| D                   | Boxer          | 28          | 6           |
| D                   | Labrador       | 34.3        | 3           |
| D                   | German Shepherd | 32.4     | 4           |
| D                   | Golden Retriever | 43.8    | 6           |
| D                   | German Shepherd | 33          | 6           |
| D                   | Mixed-breed    | 25.2        | 3           |
| D                   | Fila Brasileiro | 49.8       | 6           |
| D                   | Mixed-breed    | 20.2        | 2           |
| D                   | Golden Retriever | 37.5    | 3           |
| D                   | Labrador       | 45          | 6           |
| D                   | Mixed-breed    | 43.3        | 4           |
| D                   | Rottweiller    | 33          | 2           |
| D                   | German Shepherd | 35          | 6           |
| D                   | Mixed-breed    | 27.2        | 2           |
| D                   | Golden Retriever | 40        | 6           |
| D                   | Pitbull        | 24.6        | 5           |
| D                   | Labrador       | 42.2        | 6           |
| E                   | Mixed-breed    | 22          | 6           |
was performed by an experienced operator (15 years of experience). The patients were physically restrained without sedation or anesthesia, in dorsal recumbency and semiflexed hind limbs. Ultrasonographic exams were performed using ACUSON S2000/SIEMENS (Siemens, Munich, Germany) with a 9.0 MHz multifrequency linear transducer.

In B mode, the pectineus muscle characteristics evaluated were echogenicity (compared with adjacent tissue) and echotexture (homogeneous or heterogeneous). Images were obtained in three different regions of the muscle: medial, central, and lateral (Figure 1).

The same machine was used to perform ARFI elastography with software for qualitative characterization and quantification with the ARFI method (VTIQ method of ARFI – Virtual Touch Tissue Imaging Quantification 2D-SWE technique) with a 9.0 MHz linear transducer. For quantitative ARFI, a minimum of 5 measurements were obtained for each muscular portion (medial, central, and lateral), and the mean values were expressed in shear wave velocity (SWV) m/s.

With elastogram, colored images of the muscle tissue of all patients were obtained to assess relative tissue stiffness and its deformity (deformable or nondeformable). Regarding the interpretation of elastographic images, blue regions demonstrate that the tissue was more elastic (less rigid and deformable) than green (intermediate stiffness, nondeformable) and red (rigid and nondeformable) regions. Additionally, tissue homogeneity was evaluated.

Statistical analysis was performed with R (RTM Foundation for Statistical Computing, Vienna, Austria) software. Measurements of different regions of the muscle were compared using Bland & Altman agreement method. Qualitative variables of B mode and elastography were compared with the presence of HD using chi-square test, while SWV were submitted to Student’s t-test to verify if there was an association with the disease. Those parameters that presented significant differences (P ≤ 0.05) were submitted to discriminatory power analysis (using HD radiographic diagnosis as a gold standard), using ROC curves, besides calculating cutoff value (CV), sensitivity, specificity, area under curve (AUC), and accuracy, using logistic regression.

SWV values were also analyzed in different grades of the disease by ANOVA test and correlated with other variables, such as body weight, age, and depth of assessment using Spearman test. Significance for all tests was set at 5% (P ≤ 0.05).

3. Results

In B mode scanning of pectineus muscle, a homogenous and hypoechoic pattern in healthy patients was observed, surrounded by a hyperechoic fascia and muscle fibers arranged as hyperechoic dots on transverse view. Heterogeneous patterns were seen in dysplastic animals, compromising muscle delimitation and loss of normal muscle sonographic pattern, characterizing the disease (P < 0.001). Another characteristic associated with the presence of the disease was the change in the muscular pattern from hypoechoic to hyperechoic (P < 0.001) (Figure 2).

In qualitative elastography, an increase in pectineus muscle stiffness was observed in the affected animals, where reddish tones were predominant in patients with advanced HD grades (Figure 3). It was also found that dysplastic animals showed less deformity of the pectineus muscle (Table 2), meaning that the muscle was less elastic.

Regarding shear wave velocity, it was found that the different muscle regions presented similar SWV (medial: 3.12 ± 1.07 m/s; lateral: 3.05 ± 1.00 m/s; P > 0.057), with a mean difference between regions of 0.079 ± 0.036 m/s, so a mean SWV value was used to study diagnostic accuracy.

Figure 1. A) Positioning of the dogs for sonographic evaluation of the pectineus muscle. B) Schematization of the three different studied regions: M, medial; C, central; and L, lateral.
Figure 2. Transverse view of the pectineus muscle of a healthy animal in B mode sonographic study (A) and of a grade D dysplastic animal (B). It is noted in (A), there is a hypoechoic muscle pattern with normal distribution of muscle fibers, represented as hyperechoic dots (*). In (B), it is noticed that there is a diffuse hyperechoic pattern with loss of normal muscle fiber architecture, giving a heterogeneous appearance to the image.

Figure 3. Qualitative ARFI elastography (elastogram) of the pectineus muscle in dogs with grade A (A), grade B (B), grade C (C), grade D (D), and grade E (E) hip dysplasia. It is noted that there is a predominance in reddish tones (greater tissue stiffness) in higher grades of hip dysplasia (D and E grades).
When comparing the values between the experimental groups, SWV of the pectineus muscle of the dysplastic animals (3.49 ± 1.00 m/s) was significantly higher than that of the healthy patients (2.41 ± 0.59 m/s) (P < 0.001). Additionally, it was found that SWV higher than 2.85 m/s might indicate HD, with 77% sensitivity, 79% specificity, 83% AUC, and 78% accuracy (Figure 4).

Pectineus muscle SWV was also influenced by the HD grade (P < 0.001), being significantly higher in C, D, and E, when compared with A (GC patients) and B (Figure 5). SWV did not show correlation with depth of the region assessed (P > 0.378) or body weight (P = 0.1728); however, a direct and weak correlation with age was found (r = 0.2124; P < 0.038). There was no statistical difference between right and left limbs (P > 0.624).

Table 2. Sensitivity (Se), specificity (Sp), and accuracy (Ac) of qualitative sonographic evaluation of the pectineus muscle as indicator of hip dysplasia in dogs.

| Characteristics | Classification | Dysplasia indicator | P-value | Se (%) | Sp (%) | Ac (%) |
|-----------------|-----------------|---------------------|---------|--------|--------|--------|
| Echogenicity     | Hypoechoic      | Hyperechoic         | 0.0011* | 44     | 91     | 60     |
| Echotexture     | Homogenous      | Heterogeneous       | 0.0006* | 45     | 91     | 62     |
| Deformability    | Deformable      | Nondeformable       | 0.0001* | 81     | 71     | 77     |

*Statistically significant.

4. Discussion

Both B mode and elastography were efficient in evaluating pectineus muscle in healthy animals and patients with HD; and elastography demonstrated moderate sensitivity, specificity, and diagnostic accuracy.

Hip dysplasia is highly frequent in canine species, and it is diagnosed by radiographic evaluation [8]. In this study, the diagnosis and classification of the disease was made according to the Fédération Cynologique Internationale guidelines, using the extended ventrodorsal projection.

Some individual factors, such as patient's age, clinical signs, and radiographic findings, such as degree of arthritis and joint laxity, may be considered for diagnostic conclusion, and there may be considerable variation among observers [9]. It is possible that the absence of radiographic or clinical signs in young animals and the different interpretations lead to false diagnoses, often requiring other methods to confirm the condition, such as computed tomography or magnetic resonance imaging [9].

The use of ultrasound techniques proposed in this study to evaluate the pectineus muscles proved to be effective in differentiating between healthy and dysplastic animals, with moderate sensitivity, specificity, and diagnostic accuracy, increasing the imaging findings in patients with evident hip dysplasia, which may decrease the occurrence of false diagnoses.

In B mode scanning, pectineus muscle of the patients from the control group (CG) presented a hypoechoic pattern, with hyperechoic dogs distributed by the structure on a transverse view, corresponding to the arrangement of muscle fibers. The muscle was also surrounded by a hyperechoic structure, which represented the epimysium and muscular fascia [10–12]. Images obtained in the healthy dogs in this study were compatible with the findings considered normal for muscle tissue, confirming that this is a feasible and applicable technique, since ultrasonographic evaluation of the pectineus muscle has already been described in previous studies [13,14].

Sonographic changes in the pectineus muscle pattern were significant in the patients with hip dysplasia. Hyperechogenicity and heterogenicity observed in the muscle of DG animals corroborate findings described in the literature, which associated these findings with scar formation and the affected areas due to muscle fiber rupture [15].

Considering that the pectineus muscle is one of the hip adductor muscles and that dysplastic patients demonstrate limb abduction limitations [3], it is possible that the sonographic findings indicate muscle fiber lesion, leading to scar formation and chronic alterations with similar echogenicity changes, due to higher requirement of this structure under these conditions. In a study performed with dogs, changes in the echogenicity of the iliopsoas muscle were observed in agility athletes due to chronic injuries caused by the activity [12].

Due to poor joint conformation and consequent decrease in limb amplitude, it is known that there is tension of the abductor musculature, and dysplastic patients may develop contracture of the pectineus muscle according to the chronicity of the process, becoming rigid to palpation [3,7,16,17]. This stiffness was confirmed in qualitative and quantitative elastographic evaluations of this study, in
which dysplastic patients presented changes in elastogram colors and higher SWV values, respectively.

Although histopathological evaluations of the muscle tissue were not performed in this study, it is believed that the increase in muscle stiffness observed in dysplastic animals can be attributed to pectineus muscle fibrosis. According to the literature, histopathological evaluations of the pectineus muscle of dysplastic patients that underwent pectinectomy demonstrated positive correlation between muscle fibrosis/atrophy and difficulty in deambulation and limb abduction in dysplastic dogs [17].

The assessment of SWV was shown to be feasible and repeatable in the muscles of human patients [18]. In dogs, regarding the musculoskeletal system, findings regarding the stiffness of the patellar tendon and intra articular structures of the stifle joint of healthy animals have been described [19,20]. In the present study, the technique was shown to be easy to perform, fast, and noninvasive, and it did not require chemical containment of the patients since the pectineus muscle is a superficial structure and easy to palpate.

SWV did not show any correlation with the depth of assessment, and it is believed that this might be due to the superficial and identifiable nature of the pectineus muscle. The easy execution of the technique in this tissue is due to the high reliability of measurements of thicker and more superficial muscle structures [21,22].

Although body weight is an important factor in development of hip dysplasia, there was no correlation with muscle stiffness in the animals evaluated [23–26]. This lack of association might be due to lack of standardization of body weight between CG and DG animals, because healthy overweight and sick underweight patients were included. Still, excessive food intake does not interfere directly in the development of hip dysplasia. It only enhances manifestation in susceptible animals [27].

The age showed a direct and weak correlation with SWV of the pectineus muscle in the dogs observed in the present study. In humans, aging has been associated with increased brachial biceps muscle stiffness, since in elderly patients there is a decrease in the number of muscle fibers and reduction of the strength and functionality of the muscles, leading to atrophy or muscle contracture [28]. This decrease in the total number of muscle fibers has also been associated with age in healthy dogs [29], and it is known that hip dysplasia grading is positively related to age [30], which justifies the positive correlation between age and muscle stiffness observed in the present study.

It was found that there was no statistical difference between right and left limb SWV, and this result might be due to the fact that hip dysplasia is normally a bilateral condition, in which similar intensities of joint laxity and hip arthrosis in both limbs are expected [31,32].

Progression of hip dysplasia results in inflammatory and degenerative changes, with worsening of osteoarthritis in adult dogs [33]. This might justify the correlation found between elastography and the grade of the hip dysplasia, which is significantly higher in grades C, D, and E than in B (near normal hip joint) or A (normal joint).
The radiographic study and clinical examination still represent the gold standard for the diagnosis of osteoarthrosis secondary to hip dysplasia [34]. However, the results obtained in this study may aid in understanding the pathophysiology of the pectineus muscle in HP, so that a more adequate management in each situation might be established, since pectinectomy effectiveness has been questioned [3,35].

The results of the present study may be useful for selecting patients who are suitable for pectinal muscle surgical intervention, as well as for postoperative follow-up or for evaluating the effectiveness of rehabilitation techniques. Some studies have already demonstrated the use of shear wave elastography in the musculoskeletal biomechanical evaluation of surgical techniques to repair shoulder changes in humans, as well as its application to quantify the effectiveness of rehabilitation techniques [21,36–39].

It is concluded that B mode ultrasonography and ARFI elastography (qualitative and quantitative) have shown great applicability in evaluating the pectineus muscle of dogs, since it was possible to establish echogenicity, echotexture, and muscle stiffness characteristics as predictors of hip dysplasia.

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Conflict of interest
The authors declare no conflict of interest.

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