DOI: 10.5433/1679-0359.2017v38n2p765

Needle muscle biopsy: technique validation and histological and histochemical methods for evaluating canine skeletal muscles

Biópsia muscular por agulha: validação da técnica e dos métodos histológicos e histoquímicos na avaliação do músculo esquelético de cães

Sérgio de Almeida Braga[1]; Felipe Gomes Ferreira Padilha[2]; Ana Maria Reis Ferreira[3*]

Abstract

This study evaluated the needle muscle biopsy technique using a 6G Bergström percutaneous needle combined with histological and histochemical methods to analyze the skeletal muscle of dogs. There are few studies about canine skeletal muscles and a lack of reports in the literature about tissue collection and analysis for canine species. Evaluation of 32 German Shepherd samples collected from the gluteus medius, at a depth of 3 cm, was performed. The choice of gluteus medius and the 3-cm depth provided good quantity fragments with sufficient sizes (3–5 mm), which permitted optimal visualization of muscle fibers. Myosin ATPase, at pH 9.4, 4.6, and 4.3, and SDH reactions revealed that all muscle samples analyzed had fibers in the classic mosaic arrangement, enabling counting and typification. The mean percentages of fibers were 29.95% for type I and 70.05% for type II. On the basis of these results, we concluded that the percutaneous needle biopsy technique for canine skeletal muscles is a safe and easy procedure that obtains fragments of proper sizes, thereby enabling the study of muscle fibers. Standardization of the muscle of choice and the depth of muscle sample collection significantly contributed to this success. This is an important method to evaluate muscle fiber types of dogs and diagnose important diseases affecting the skeletal muscles.
Key words: Muscle. German shepherd. Diagnosis

Resumo

O objetivo deste trabalho foi avaliar a técnica de biopsia muscular por agulha de biópsia percutânea tipo Bergström nº seis, e os métodos histológicos e histoquímicos utilizados na análise do músculo esquelético de cães, uma vez que estudos sobre a musculatura esquelética em cães são poucos o que acarreta um número pequeno de relatos na literatura sobre métodos de coleta e análise desse tecido nessa determinada espécie. Foram avaliadas amostras de 32 animais da raça Pastor Alemão coletadas do músculo glúteo médio a uma profundidade de três centímetros. A escolha da profundidade de três centímetros e do músculo Glúteo Médio em cães da raça Pastor Alemão denotou fragmentos colhidos com suficiente viabilidade, bons padrões de tamanho (entre três e cinco milímetros) e boa quantidade e qualidade, permitindo ótima visualização das fibras musculares dos animais da amostra. Nas reações enzimo-histoquímicas da miosina ATPase no pH 9.4, 4.6 e 4.3 e SDH todos os músculos examinados apresentavam a distribuição das fibras musculares com o clássico padrão de mosaico, possibilitando

[1] Discente, Programa de Pós-Graduação em Medicina Veterinária, Clínica e Reprodução Animal, Universidade Federal Fluminense, UFF, Niterói, Brasil. E-mail: pastoralemao00@gmail.com
[2] Pesquisador, Programa de Pós-Graduação em Medicina Veterinária, Clínica e Reprodução Animal, UFF, Niterói, Brasil. E-mail: felipe_padilha@yahoo.com.br
[3] Prof., Programa de Pós-Graduação em Medicina Veterinária, Clínica e Reprodução Animal, Departamento de Patologia e Clínica Veterinária, MCV, Niterói, Brasil. E-mail: ana_ferreira@id.uff.br
[*] Author for correspondence

Received: June 13, 2016 – Approved: Nov. 21, 2016

a contagem e a diferenciação dos diferentes tipos. O valor médio de fibras tipo I foi de 29,95% e o valor médio de fibras tipo II, 70,05%. Com base nesses resultados, pode-se concluir que a técnica de biopsia com agulha percutânea utilizada em músculo esquelético de caninos, demonstrou ser um procedimento seguro e de fácil recuperação, obtendo fragmentos de tamanho adequado e, portanto, viabilizando o estudo. A padronização do músculo e da profundidade de coleta da amostra muscular teve contribuição importante. Sendo um importante meio para avaliar os tipos de fibras musculares em cães e no diagnóstico de importantes enfermidades que acometem a musculatura esquelética.
Palavras-chave: Músculo. Pastor Alemão. Diagnóstico.

Introduction

Few studies have discussed canine skeletal muscles. Therefore, the literature lacks reports of methods for collecting and analyzing this type of tissue within the canine species.

Significant muscle disorders may affect dogs, such as X-linked muscular dystrophy (Duchenne type), congenital myotonia, and myasthenia gravis, in addition to endocrine myopathy associated with hypothyroidism and hypercortisolism, and immune-mediated and parasitic myopathies (VALENTINE; McGAVIN, 2009). Studies about the different aspects of X-linked muscular dystrophy in dogs and its evolution are crucial, since they are appropriate animal models to study similar alterations in humans, as indicated by Grando et al. (2009).

Muscle biopsy was first performed by Duchenne in 1861, but its application was limited until Bergström introduced the percutaneous needle technique with similar characteristics, which is mainly used to study physiological variations in normal muscles (DUBOWITZ et al., 2013a).

Needle muscle biopsy is now widely used, and the technique is accepted in human medicine for the diagnosis and monitoring of neuromuscular diseases and to investigate muscular metabolism in healthy individuals and in diseases such as glycogenoses, lipid-related disorders, and lysosomal and mitochondrial myopathies (MITCHELL et al., 1989). Muscular biopsy has been widely used in human and equine medicine, and needle muscle biopsy has been an outstanding method to obtain canine muscle samples for evaluation (REYNOLDS et al., 1995).

Currently, the majority of canine muscle samples are obtained from open biopsies (ROSENBLATT et al., 1988), which requires general anesthesia and may lead to significant risk to animals with severe neuromuscular weakness, malignant hyperthermia, or cardiovascular, pulmonary, and liver or kidney diseases (DUBOWITZ et al., 1985). Furthermore, general anesthesia may affect the metabolic flow and the open biopsy procedure, which may become inappropriate for investigating metabolic energy (REYNOLDS et al., 1995).

Needle muscle biopsy causes little discomfort and is virtually inoffensive to dogs, and thus repeated collection procedures in a single animal are possible. The technique is performed rapidly, allows for effective and safe biopsies of muscle tissue in conscious and intact dogs, in addition to the fact that dogs are able to exercise normally one day after the procedure. The obtained fragment is appropriate for most morphological and histochemical evaluations (TREVINO et al., 1973, REYNOLDS et al., 1995). It is also a practical, rapid, and minimally invasive technique for equine examination (LEDWITH; MCGOWAN, 2004; PADILHA et al., 2008). These attributes of the technique provide significant potential for the diagnosis, clinical management, and research of nutrition and metabolism and neuromuscular diseases (GREIG et al., 1985).

The muscle fragments of dogs to evaluate fiber types and metabolism were obtained *post-mortem* in several studies (HARRIS et al., 1990; ACEVEDO; RIVERO, 2006). Percutaneous needle muscle biopsy is useful and effective for monitoring diseases, leading to significant results in studies of canine muscular disorders (SAMPAOLESI et al., 2006).

The gluteus medius is a superficial muscle of the pelvic that is easily visualized limb. This muscle, which is located on the gluteal surface of the ilium, begins at the iliac crest and the iliac tuberosity, and ends as a small and strong tendon attached to the greater trochanter (EVANS; CHRISTENSEN, 1979).

Histological techniques reveal the overall muscle fragment structure; enzyme histochemical reactions, however, permit differentiation of the biochemical properties of specific muscle fiber types (DUBOWITZ et al., 2013b), leading to a more thorough study of distinct fiber types.

This study evaluated percutaneous needle muscle biopsy technique using a 6G Bergström needle, and histological and histochemical methods of canine skeletal muscle analysis., since studies on the skeletal muscles in dogs are scarce leading to a small number of reports in the literature on methods of collection and analysis of this tissue in this particular species.

Materials and Methods

This study was analyzed and approved by the Animal Ethics Committee of the Federal Fluminense University (protocol no. 355/2013).

Muscle samples were collected from 32 asymptomatic, male and female German Shepherd dogs, aged from one to six years old, who were maintained under standardized management. The site of muscle fragment collection was the gluteus medius, selected due to its superficial location and importance for animal locomotion. It is therefore an important muscle to study athletic activities, and is unaffected by canine-hip dysplasia of German Shepherd dogs (CARDINET et al., 1997).

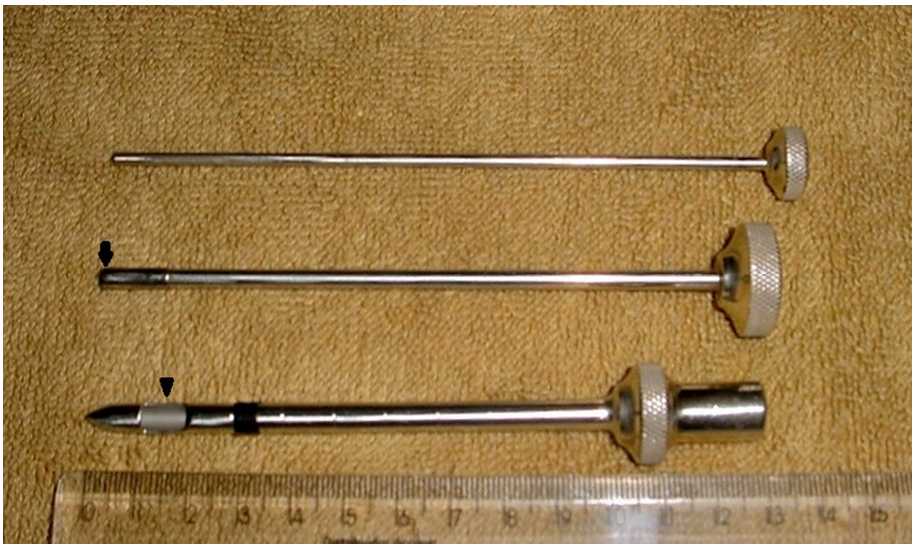
The dogs were grown under standardized management, in semi-confined system, within individual 4.5 m × 2 m cemented floor pens that were partially covered and with access to sunlight. They were fed twice a day with the same *super premium* dog food containing guaranteed levels of the following nutrients: crude protein, 240 g/kg; ether extract, 150 g/kg; minerals, 69 g/kg; fiber, 25 g/kg; water, 95 g/kg; phosphorus, 5,800 mg/kg; vitamin A, 12,900 IU; vitamin C, 200 mg; vitamin E, 500 IU; taurine, 1,530 mg/kg; eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA), 4,100 mg/kg; metabolizable energy, 4,014 kcal/kg. In addition, all dogs were had unrestricted access to water.

The needle muscle biopsy technique was performed after the animals were chemically restrained for the tranquilization protocol, which involved 1 mg/kg 2% xylazine hydrochloride injected intramuscularly through the pelvic limb opposite to the biopsied gluteus medius (left pelvic limb) using a 25 × 7 mm needle. All dogs were submitted to 12-hour time feed restriction prior to each procedure.

Trichotomy and sterilization were performed in the right gluteus medius area, and local anesthesia with lidocaine hydrochloride 2% (3mL per dog) was administered with the animal in the lateral recumbent position. The muscle biopsy was then performed using a 6G Bergström percutaneous needle 6 mm in length (Stille, Stockholm, Sweden) (Figure 1), and a skin incision was performed using a scalpel. A standardized 2-cm incision was performed in the muscle fascia, and then muscle fiber divulsion was performed to a depth of 3 cm, where the biopsy needle was inserted. The needle was centrally inserted at a 45° angle into the gluteus medius, 3 cm below the iliac crest. Three-point suture ("U") was performed using 2.0 nylon monofilament sutures after sample collection.

Subsequently, 1% topical polyvinylpyrrolidone was applied, in addition to a ketanserin tartrate healing ointment and prophylactic 5 mg/kg enrofloxacin antibiotic therapy for five days, administered subcutaneously on a daily basis. Stitches were removed seven days after the procedure.

Figure 1. Three components of the G6 Bergström percutaneous biopsy needle. A hollow puncture cylinder with a sharp edge, used for muscle fiber divulsion, and a compartment for sample storage (arrow head); another hollow cylinder that slides inside the needle, with a sharp edge (arrow), and a stylet used for extracting the sample from the needle's interior.



Muscle fragments were extracted and wrapped in aluminum foil, which has cryoprotective functions, thereby avoiding damage during freezing. The samples were then immediately frozen using liquid nitrogen at −160ºC by direct immersion until "bubbling" ceased; samples were then stored in an appropriate canister until processing. Transverse 10-µm histological sections were made, as per the method of Dubowitz et al. (2013a), using a cryostat at −20ºC, and samples were collected with glass slides.

The feasibility of visualization of the different structures and substrates using each sample, and differentiation of muscle fiber types, were evaluated using the following enzyme-histochemical and histological techniques: myosin adenosine triphosphatase (ATPase), at pH 9.4, 4.6, and 4.3; succinic dehydrogenase (SDH); modified Gomori trichrome (MGT); periodic acid-Schiff (PAS); as per the methods of Dubowitz et al. (1985) adapted by Ferreira (2000); and hematoxylin and eosin (H&E); in accordance with Ferreira (1994).

Exploratory data analysis was performed using descriptive statistics to determine the mean values of different muscle fiber types.

Results and Discussion

Needle muscle biopsy enabled safe and effective biopsies of animals in this study. It was a minimally invasive, safe, and easily performed technique with a fast recovery for dogs, corroborating the results obtained by Ledwith and McGowan (2004) and Padilha et al. (2008) in horses. The low cost of the procedure is an important point to consider.

Histological analysis of samples permitted evaluation of the overall muscle architecture and observation of individual morphological variations of the muscle fibers, as reported by Sundaram (2011). Hematoxylin and eosin (H&E) staining, a routinely used technique, clearly reveals tissue structure: fiber, nucleus, adipose and fiber tissue, presence of inflammatory cells, and vascular and neural components, as reported by Dubowitz et al. (2013b). This technique facilitated the

accurate histological evaluation of canine muscle. Histological evaluation revealed that the muscle fragments were composed of typical polygonal-shaped fibers with significant variations in size, eosinophilic sarcoplasm, and elongated nuclei with subsarcolemmal arrangement. Muscle fibers were separated from each other by a delicate layer of connective tissue, referred to as endomysium, with capillaries in some samples.

The modified Gomori trichrome (MGT) technique permitted examination of the endomysial connective tissue, as described by Dubowitz et al. (2013b), which was present as a thin layer between muscle fibers. All samples had similar morphological aspects, independently of gender or age of the dog, after application of both conventional and enzyme-histochemical techniques. The Periodic acid-Schiff (PAS) reaction permits the quantification of muscle glycogen, as demonstrated by Acevedo and Rivero (2006); pathological glycogen accumulation in canine muscle fibers was not observed during this study. PAS technique is especially useful for the diagnosis of different glycogenosis types that affect various animal species, including dogs (VALENTINE; McGAVIN, 2009).

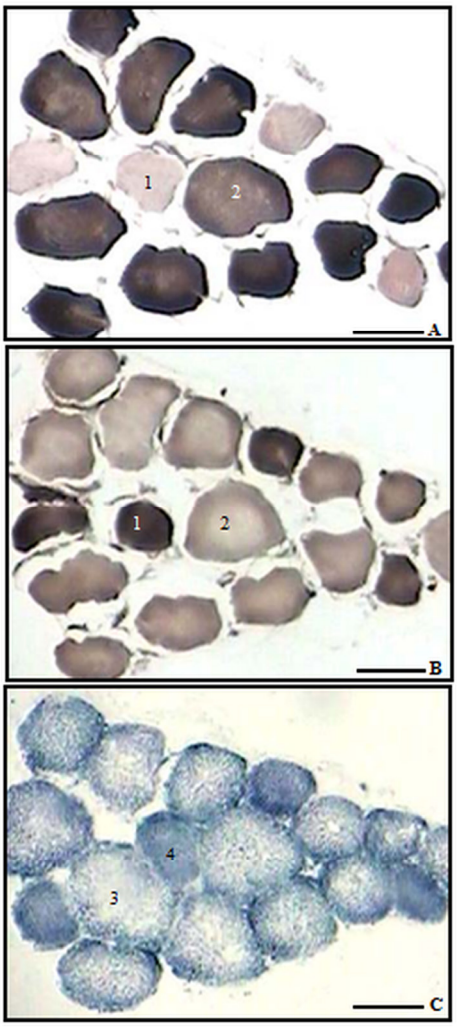
Myosin ATPase at pH 9.4, 4.6, and 4.3, and SDH reactions revealed that the muscle fibers of all samples were arranged in the classic mosaic pattern, which permitted the counting and differentiation of distinct fiber types, as previously described (VAN VLEET; VALENTINE, 2007; DUBOWITZ et al., 2013b), and resulted in different biochemical properties of specific muscle fiber types.

The reaction of myosin ATPase at pH 9.4 revealed light-colored, type I fibers and dark-colored, type II fibers; the reaction at pH 4.3 showed dark-colored type I fibers and light-colored type II fibers, corroborating with previous studies (LATORRE et al., 1993; ACEVEDO; RIVERO, 2006). This color arrangement is evidence of the characteristic mosaic pattern of this reaction. The mean value of type I fibers was 29.95% and that of fiber II was 70.05%. Evaluation of the SDH enzyme activity revealed that dark blue-colored fibers had prominent oxidative metabolism, while fibers with glycolytic metabolism are light blue-colored (Figure 2), similarly were described in dogs (LATORRE et al., 1993) and horses (PADILHA et al., 2011) with NADH-TR enzyme activity-another oxidative enzyme.

The gluteus medius is the muscle of choice and standardized needle insertion depth (three centimeters) permitted a satisfactory biopsy procedure, with minimal bleeding throughout the surgical incision, as previously described (TREVINO et al., 1973); these authors reported that this technique could be rapidly performed in animals, and dogs are able to exercise normally one day after biopsy. Oblique needle insertion into the gluteus medius enables the acquisition of sample sections with a satisfactory size for histological and histochemical evaluation (BRAGA; FERREIRA, 2002).

Dogs that were subjected to the muscle biopsy experiment using a 6G Bergström percutaneous needle (six millimeters) did not demonstrate immediate complications. Signs of discomfort were observed in approximately 50% of the procedures when the needle was inserted through the skin and fascia, which disappeared as the needle reached the muscle interior. Immediately after the biopsy, the animals did not show signs of claudication or walking problems, and there was no effect on exercise performance 15 to 30 minutes after biopsy collection. The incision wound healed rapidly without complications or suture dehiscence. The quality of the collected samples was appropriate for the identification of muscle fiber by enzyme histochemical analysis; similar results were described (REYNOLDS et al., 1995).

Figure 2. Transverse sections of muscle samples from German Shepherd dogs. (**A**) Identification of muscle fiber types by enzyme histochemical examination through the reactions for myosin ATPase at pH 9.4 and (**B**) myosin ATPase at pH 4.3; and (**C**) oxidative capacity of muscle fibers by succinic dehydrogenase enzyme histochemical reaction: muscle fibers classified as: type I (1); type II (2); low oxidative capacity (3); high oxidative capacity (4). Scale bar = 100 µm.



Although this muscle biopsy method is not a complete substitute for an open biopsy, it may be used in certain cases to obtain sufficient information regarding muscle fiber types and in the assessment of neuromuscular disorders, and poses a lower risk for the patient if it was submitted to general anesthesia. These observations in the dogs of this study are consistent with those described (REYNOLDS et al., 1995).

In contrast to the studies conducted by Braund et al. (1982), and Acevedo and Rivero (2006), who performed dog euthanasia with posterior muscle dissection, this technique considers animal welfare and enables studies that require fragments of canine muscle tissues in live animals. Greig et al. (1985) have indicated that this procedure has the potential for use in the diagnosis, clinical management, and research activities relative to nutrition, metabolism, and neuromuscular diseases. Biopsies repeated within a seven-week interval permitted evaluation of equine muscle tissue (LINDNER et al., 2002); thus, evaluation of training programs, age effects

and/or pathology evolution was possible, without injuries to the animals.

The standardized use of the gluteus medius muscle and a 3-cm depth in this study produced good quantity fragments with sufficient sizes (3–5 mm), which permitted optimal visualization of muscle fibers. Furthermore, the standardized use of the gluteus medius muscle and a 3-cm depth contributed to producing representative results for the ratio of the type of fibers and to standardizing animal samples; this was consistent with the findings of Acosta Junior and Roy (1987), who studied muscle samples from primates and observed that the ratio of the type of fibers is not the same along the muscle length and depth and depends on the muscle functional activity. The findings from this study corroborated with those of Ferreira (2000) and Reynolds et al. (1995) who obtained muscle samples with the appropriate size and quality for use in histological and biochemical analyses in horses and dogs, respectively. Mercado et al. (2001) observed that samples from percutaneous needle biopsies of canine vastus lateralis muscles were not sufficient in size for examination.

The orientation of transverse sections from the frozen muscle fragments and the 10-µm thickness, as described by Dubowitz et al. (2013a), guaranteed success of analyses and permitted the visualization of structures and the differentiation of muscle fiber types; these are consistent with those of another study (ACEVEDO; RIVERO, 2006).

Until nowadays, the needle muscle biopsy technique is widely used in sport horses for equine training evaluation, as reported by Lindner et al. (2013), and it is essential not only for obtaining fragments for histological and enzyme histochemical analyzes but also to understanding gene expression (MURPHY et al., 2014) and identify biomarkers related to skeletal muscles (TE PAS et al., 2013). Therefore, the needle muscle biopsy is a technique that can not only be used to evaluate the training protocol of canine athlete and performance dogs, but also to assess muscle tissues of working dogs and for the diagnosis of several canine neuromuscular diseases.

In horses, the use of microbiopsies is useful to analyze the mitochondrial respiratory capacity (VOTION et al., 2010, 2012), being an alternative method to evaluate muscle samples. However, the use of microbiopsies has no practical application in the evaluation of muscle fiber architecture and typification, since the size of samples collected by this technique is not sufficient, and thus, muscle fragments obtained by needle biopsy are preferred.

It is important to emphasize that studies using muscle biopsy as a method of diagnosis are very valuable for veterinary medicine, as reports of diseases such as canine muscle dystrophies have increased (ARAÚJO et al., 2009). Although an increase in the number of enzymes indicative of muscle disease, for example, creatine kinase in female dogs carrying the muscle dystrophy gene (MORINI et al., 2011), muscle biopsy is still the only method to diagnose these progressive lesions in these dogs.

The histological techniques permitted an effective evaluation of muscle fragments by enabling the observation of fiber structure. Enzyme histochemical methods permitted the observation of the mosaic pattern obtained by reactions and the different fiber types were highlighted, which enabled counting. Such success was also observed in studies of other species, for example, in equines (D´ANGELIS et al., 2006, 2008; REVOLD et al., 2011; LINDNER et al., 2013).

On the basis of these results, we conclude that percutaneous needle biopsy of canine skeletal muscles is a safe and convenient procedure, which obtains fragments with a proper size, thus enabling study of these muscles. Standardization of the muscle of choice and collection depth was essential. This technique represents an important method to evaluate canine muscle fiber types and facilitates the diagnosis of important skeletal muscular diseases.

References

ACOSTA JUNIOR. I.; ROY, R. R. Fiber-type composition of selected hindlimb muscles of a primate (*Cynomolgus monkey*). *The Anatomical Record*, Nova Iorque, v. 218, n. 2, p. 136-141, 1987.

ARAÚJO, K. P. C.; HAGIWARA, M. K.; LARSSON, M. H. M. A.; MIGLINO, M. A.; AMBRÓSIO, C. E. Aspectos clínicos das distrofias musculares em cães. *Ciência Animal*, Fortaleza, v. 19, n. 2, p. 89-102, 2009.

ACEVEDO, L. M.; RIVERO, J. L. L. New insights into skeletal muscle fibre types in the dog with particular focus towards hybrid myosin phenotypes. *Cell and Tissue Research*, Londres, v. 323, n. 2, p. 283-303, 2006.

BRAGA, S. A.; FERREIRA, A. M. R. Avaliação da técnica de biopsia com agulha percutânea em músculo esquelético de cães. *Revista Brasileira de Ciência Veterinária,* Niterói, v. 9, p. 167-169, 2002. Suplemento.

BRAUND, K. G.; MCGUIRE, A.; LINCON, C. E. Observations on normal skeletal muscle of mature dogs: a cytochemical, histochemical, and morphometric study. *Veterinary Pathology*, Thousand Oaks, v. 19, n. 6, p. 577-595, 1982.

CARDINET, G. H.; KASS, P. H.; WALLACE, L. J.; GUFFY, M. M. Association between pelvic muscle mass and canine hip dysplasia. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 210, n. 10, p. 1466-1473, 1997.

D´ANGELIS, F. H. F.; MARTINS, C. B.; SILVA, M. A. G.; OLIVEIRA, J. V.; BOLELI, I. C.; LACERDA-NETO, J. C.; QUEIROZ- NETO, A. Composição de fibras musculares esqueléticas de equinos jovens da raça Brasileiro de Hipismo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Belo Horizonte, v. 58, n. 4, p. 672-674, 2006.

D'ANGELIS, F. H. F.; SILVA, M. A. G.; MARTINS, C. B.; FERRAZ, G. C.; OLIVEIRA, J. A.; BOLELI, I. C.; LACERDA-NETO, J. C.; QUEIROZ-NETO, A. Light exercise causes adaptive changes in muscles of young Brasileiro de Hipismo horses. *Ciência Rural*, Santa Maria, v. 38, n. 5, p. 1313-1318, 2008.

DUBOWITZ, V.; SEWRY, C. A.; FITZSIMMONS, R. B. *Muscle biopsy:* a practical approach. 2. ed. London: Baillere Tindall, 1985. 720 p.

DUBOWITZ, V.; SEWRY, C. A.; OLDFORS, A. The procedure of muscle biopsy. In: _____. *Muscle biopsy:* a practical approach. 4. ed. United Kingdom: Saunders Elsevier, 2013a. p. 2-15.

_____. Histological and histochemical stains and reactions. In: _____. *Muscle biopsy:* a practical approach. 4. ed. United Kingdom: Saunders Elsevier, 2013b. p. 16-27.

EVANS, H. E.; CHRISTENSEN, G. C.The Muscular System. In: EVANS, H. E.; CHRISTENSEN, G. C. *Miller´s anatomy of the dog*. 2. ed. Philadelphia: W.B. Saunders, 1979. p. 381-384.

FERREIRA, A. M. R. *Análise histoquímica e imuno-histoquímica de macromoléculas da matriz extracelular dos tipos de fibras do músculo glúteo médio equino*. 2000. Tese (Doutorado em Patologia Experimental) – Faculdade de Medicina. Universidade Federal Fluminense, Niterói.

_____. *Estudo enzimo-histoquímico e histomorfométrico nos músculos tibial anterior e gastrocnêmio e cardíaco no camundongo distrófico mdx*. 1994. Dissertação (Mestrado em Patologia Experimental) – Faculdade de Medicina. Universidade Federal Fluminense, Niterói.

GRANDO, A. P.; MARIANA, A. N. B.; MIGLINO, M. A.; STERMAN, F. A.; ZATZ, M.; KANAYAMA, L. M.; FEITOSA, M. L. T.; MARTINS, D. S.; MORINI, A. C.; SANTOS, J. P. A. dos; FADEL, L.; ALVES, F. R.; AMBRÓSIO, C. E. Ultra-sonografia abdominal e pélvica em cães da raça golden retriever sadios, portadores e afetados pela distrofia muscular progressiva. *Ciência Rural*, Santa Maria, v. 39, n. 1, p. 123-128, 2009.

GREIG, P. D.; ASKANAZI J.; KINNEY, J. M. Needle biopsy of skeletal muscle using suction. *The Journal of Surgery, Gynecology and Obstetrics*, Chicago, v. 160, n. 5, p. 466-468, 1985.

HARRIS, R. C.; MARLIN, D. J.; DUNNETT, M.; SNOW, D. H., HULTMAN, D. H. E. Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. *Comparative Biochemistry and Physiology Part A: Physiology*, Amsterdã, v. 97A, n. 2, p. 249-251, 1990.

LATORRE, R.; GIL, F.; VAZQUEZ, J. M.; MORENO, F.; MASCARELLO, F.; RAMIREZ, G. Skeletal muscle fibre types in the dog. *Journal of Anatomy*, Chichester, v. 182, n. 3, p. 329-337, 1993.

LEDWITH, A.; MCGOWAN, C. M. Muscle biopsy: a routine diagnostic procedure. *Equine Veterinary Education*, Fordham, v. 16, n. 2, p. 62-67, 2004.

LINDNER, A.; DAG, S.; MARTI-KORFF, S.; QUIROZ-ROTHE, E. E.; LÓPEZ RIVERO, J.L.; DROMMER, W. Effects of repeated biopsying on muscle tissue in horses. *Equine Veterinary Journal*, Fordham, v. 34, n. 6, p. 619-624, 2002.

LINDNER, A.; ERGINSOY, S. D.; KISSENBECK, S.; MOSEN, H.; HETZEL, U.; DROMMER, W.; CHAMIZO, V. E.; RIVERO, J. L. Effect of different blood-guided conditioning programmes on skeletal

muscles ultrastructure and histochemistry of sport horses. *Journal of Animal Physiology and Animal Nutrition*, Berlin, v. 97, n. 2, p. 374-386, 2013.

MERCADO, M.; ALVAREZ, A.; TOLEDO, M.; DUCHENE, A.; LIGHTOWLER, C. *Evaluación de três técnicas de biopsia muscular em caninos*. Buenos Aires: Universidad de Buenos Aires, 2001. Disponível em: <http://www.fvet.uba.ar/biblioteca/resumenes/137.htm>. Acesso em: 7 fev. 2014.

MITCHELL, J. B.; COSTILL, D. L.; HOUMARD, J. A.; FINK, W. J.; PASCOE, D. D.; PEARSON, D. R. Influence of carbohydrate dosage on exercise performance and glycogen metabolism. *Journal of Applied Physiology*, Bethesda, v. 67, n. 5, p. 1843-1849, 1989.

MORINI, A. C.; BROLIO, M. P.; MILLANO, A. M. O. G.; BRAGGIO, L. Z.; MARTINS, D. S.; PERECIN, F.; AMBRÓSIO, C. E.; MIGLINO, M. A. Existem diferenças nos parâmetros hematológicos e bioquímicos séricos entre fêmeas normais e portadoras do modelo experimental GRMD (Golden Retriever Muscular Dystrophy)? *Pesquisa Veterinária Brasileira*, Rio de Janeiro, v. 31, n. 1, p. 94-98, 2011.

MURPHY, B. A.; WAGNER, A. L.; MCGLYNN, O. F.; KHARAZYAN, F.; BROWNE, J. A.; ELLIOTT, J. A. Exercises influences circadian gene expression in equine skeletal muscle. *The Veterinary Journal*, Amsterdã, v. 01, n. 1, p. 39-45, 2014.

PADILHA, F. G. F.; AMORIM, R. M.; SUMMA, R. Avaliação prática da biópsia muscular percutânea por agulha em eqüinos. A *Hora Veterinária*, Porto Alegre, v. 28, n. 164, p. 29-32, 2008.

_____. Comparação dos tipos de fibras musculares (Equus caballus) da raça Puro Sangue Árabe treinados e não treinados para enduro. *A Hora Veterinária*, Porto Alegre, v. 31, n. 184, p. 36-38, 2011.

REVOLD, T.; IHLER, C. F.; KARLSTRÖM, K.; LARSEN, S.; ESSÉN-GUSTAVSSON, B. Muscle characteristics in young Norwegian-Swedish Coldblooded Trotters and associations with breeding index, body size and early training. *Equine Veterinary Journal*, Fordham, v. 43, n. 6, p. 701-707, 2011.

REYNOLDS, A. J.; FUHRER, L.; VALENTINE, B. A.; KALLFELZ, F. A. New approach to percutaneous muscle biopsy in dogs. *American Journal of Veterinary Research*, Schaumburg, v. 56, n. 8, p. 982-985, 1995.

ROSENBLATT, J. D.; KUZON JUNIOR.; W. M.; PYNN, B. R.; PLYLEY, M. J.; MCKEE, N. H. Fiber type, fiber size and capillary geometric features of the semitendinosus muscle in three types of dogs. *American Journal of Veterinary Research*, Schaumburg, v. 49, n. 9, p. 1573-1576, 1988.

SAMPAOLESI, M.; BLOT, S.; D'ANTONA, G.; GRANGER, N.; TONLORENZI, R.; INNOCENZI, A.; MOGNOL, P.; THIBAUD, J. L.; GALVEZ, B. G.; BARTHÉLÉMY, I.; PERANI, L.; MANTERO, S.; GUTTINGER, M.; PANSARASA, O.; RINALDI, C.; ANGELIS, M. G. C. de; TORRENTE, Y.; BORDIGNON, C.; BOTTINELLI, R.; COSSU, G. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*, Londres, v. 444, n. 7119, p. 574-579, 2006.

SUNDARAM, C.; UPPIN, M. S. Approach to the interpretation of muscle biopsy. In: SUNDARAM, C. *Muscle biopsy.* Croácia: InTech, 2011. p. 15-32.

TE PAS, M. F. W.; WIJNBERG, I. D.; HOEKMAN, A. J. W.; GRAAF-ROELFSEMA, E. de; KEIZER, H. A.; VAN BREDA, E.; DUCRO, B.; VAN DER KOLK, J. H. Skeletal muscle transcriptome profiles related to different training intensities and detraining in Standardbred horses: A search for overtraining biomarkers. *The Veterinary Journal*, Amsterdã, v. 197, n. 3, p. 717-723, 2013.

TREVINO, G. S.; DEMAREE, R. S.; SANDERS, B. V.; O´DONNELL, T. A. Needle biopsy of skeletal muscle in dogs: light and electron microscopy of resting muscle. *American Journal of Veterinary Research*, Schaumburg, v. 34, n. 4, p. 507-514, 1973.

VALENTINE, B. A.; McGAVIN, M. D. Músculo esquelético. In: McGAVIN, M. D.; ZACHARY, J. F. *Bases da patologia veterinária*. 4. ed. Rio de Janeiro: Elsevier, 2009. p. 973-1040.

VAN VLEET, J. F.; VALENTINE, B. A. Muscle and tendon. In: MAXIE, M. G. *Jubb, Kennedy & Palmer's Pathology of domestic animals*. 5. ed. United States: Saunders, 2007. p. 185-280. v. 1.

VOTION, D. M.; FRAIPONT, A.; GOACHET, A. G.; ROBERT, C.; VAN ERCK, E.; AMORY, H.; CEUSTERS, J.; DE LA REBIÈRE DE POUYADE, G.; FRANCK, T.; MOUITHYS-MICKALAD, A.; NIESTEN, A.; SERTEYN, D. Alterations in mitochondrial respiratory function in response to endurance training and endurance racing. *Equine Veterinary Journal*, Fordham, v. 42, n. s38, p. 268-274, 2010.

VOTION, D. M.; GNAIGER, E.; LEMIEUX, H.; MOUITHYS-MICKALAD, A.; SERTEYN, D. Physical fitness and mitochondrial respiratory capacity in horse skeletal muscle. *PLoS One*, São Francisco, v. 7, n. 8, p. 1-12, 2012.