Arq. Bras. Med. Vet. Zootec., **v.56, n.6, p.701-708, 2004**

Intralesional polysulphated glycosaminoglycan as treatment of equine collagenase induced tendinitis: clinical, ultrasonographic and histopathologic evaluation

[*Polissulfato de glicosaminoglicanas no tratamento intralesional de tendinite induzida em eqüinos: avaliação clínica, ultra-sonográfica e histopatológica*]

S. *Marxen*[2], J.C. *Lacerda Neto*[1]*, J.C. *Canola*[1], J.R.E. *Moraes*[3], G. *Ribeiro*[1]

[1]Departamento de Clínica e Cirurgia Veterinária - FCAV - UNESP
Via de Acesso Prof. Paulo Donato Castellane, km5
14870-000 - Jaboticabal, SP
[2]Porto Alegre, RS
[3]Departamento de Patologia Animal – FCAV – UNESP

ABSTRACT

The effect of intratendineous injections of polysulphated glycosaminoglycan (PSGAG) as treatment of collagenase-induced tendonitis was studied. Two groups (GI and GII) of five Arabian horses each, males and females, two to six year-old, were submitted to experimental tendinitis of the superficial digital flexor tendon of the left thoracic limb by intratendineous injection of 1.0ml of collagenase (2.5mg/ml). Seven days after the induced-lesions were created, the horses of GI received five intralesional injections of 1.0ml (125mg) of PSGAG, every four days. Horses of GII received injections of saline in the same dose and rate. Clinical and ultrasonographic evaluations were performed periodically, during 150 days. All animals showed lameness, increased local pain, heat and swelling 24 hours after the injury was created. All signs, except to swelling, which remained visible by the end of the study, showed regression in all animals. Lesions of variable size, shape and position were evidenced by the ultrasonographic evaluation, reaching maximum severity between the seventh and the 23rd days. By the end of the study, the echogenicity grade ranged from 1 to 2, and the grade of fiber alignment from 0 to 2. The histopathologic analysis demonstrated repair areas with intense fibroplasia and neovascularization, collagen fibers poorly organized, and thickened hypercellular endotenon. The data of this study did not show significant differences between the treated and control groups, therefore leading to the conclusion that the intralesional injection of PSGAG did not have beneficial effects in the treatment of collagenase-induced tendinitis.

Keywords: polysulphated glycosaminoglycan, tendinitis, equine

RESUMO

Estudou-se o efeito de aplicações intratendíneas do polissulfato de glicosaminoglicanas (PSGAG) no tratamento de tendinite induzida pela colagenase. Dois grupos (GI e GII) de cinco eqüinos da raça Puro-Sangue Árabe, machos e fêmeas, com idades entre dois e seis anos, foram submetidos à tendinite do tendão flexor digital superficial do membro torácico esquerdo por aplicação intratendínea de 1,0ml de colagenase (2,5mg/ml). Decorridos sete dias da indução da lesão, os eqüinos do GI receberam cinco aplicações intralesionais de 1,0ml (125mg) de PSGAG, a intervalos de quatro dias, enquanto que os do GII receberam aplicações de solução fisiológica em igual volume e freqüência. Efetuaram-se avaliações

Recebido para publicação em 17 de fevereiro de 2003
Recebido para publicação, após modificações, em 4 de fevereiro de 2004
*Autor para correspondência
E-mail: jlacerda@fcav.unesp.br

clínicas e ultra-sonográficas, periodicamente, durante 150 dias. Todos os animais apresentaram claudicação e aumento local de sensibilidade, de temperatura e de volume 24 horas após a indução da lesão. Com exceção do aumento de volume, que permaneceu visível até o final do experimento, observou-se regressão de todos os sinais em todos os animais. A avaliação ultra-sonográfica evidenciou lesões de tamanho, forma e posição variados, de maior severidade entre o sétimo e 23º dia. Ao término do experimento, o grau de ecogenicidade encontrava-se entre 1 e 2, e o grau de paralelismo entre 0 e 2. A análise histopatológica evidenciou áreas cicatriciais com intensa fibroplasia e neovascularização, fibras colágenas pouco organizadas e endotendão hipercelular e espessado. Não se observou diferenças significativas entre os grupos quanto ao processo de reparação das lesões, concluindo-se que a aplicação intralesional de PSGAG não produziu efeito benéfico para tratar tendinite induzida por colagenase.

Palavras-chave: polissulfato de glicosaminoglicanas, tendinite, eqüinos

INTRODUCTION

During athletic activity repetitive forces on tendon structures predisposes them to injuries, which culminates in partial or total rupture of the tendon. Tendinitis represents a great financial loss in the equestrian competitive sports due to the long lay off period during tendon healing and rehabilitation, in addition to a great risk of recurrence, even in apparently well repaired tendons, leading to the end of the horse's athletic career.

Various methods were studied in the search for an experimental model to standardize tendon injuries. Because of the lack of success in producing a tendinitis by mechanical means, the possibility to induce a tendon damage enzymatically with the use of a bacteria-derived collagenase was explored, resulting in lesions with similar characteristics to those occurring naturally (Silver et al., 1983; Williams et al., 1984).

The main objectives in the treatment of acute tendinitis are to decrease the inflammatory process, minimize the adhesion formation and to restore tendon structure and function. The use of anti-inflammatory drugs for tendinitis has been recently broadened with the introduction of polysulfated glycosaminoglycans (PSGAG). This drug is largely used in degenerative joint diseases in many species. Their beneficial effects in tendon repair are also being investigated. However, only few researches evaluated the efficacy of the intralesional application of PSGAG concerning tendon repair in horses with collagenase-induced lesion (Smith, 1992).

An accurate diagnosis of tendon injury and careful monitoring during treatment are important factors in the rehabilitation of the horse. The ultrasonographic imaging provides us the size, shape, location and severity of soft-tissue lesions, based on the echogenicity and fiber alignment (Genovese et al., 1986). Small changes in ultrasonographic size, echogenicity and fiber pattern are associated with relatively large changes in biomechanical strength (Gillis, 1997). Close correlations have been also established between ultrasonographic observations of injured and normal tendons and their histopathologic appearance (Marr et al., 1993; Wood et al., 1994).

Considering that few experimental studies have been conducted addressing the treatment of tendinitis in horses, the purpose of this investigation was to evaluate histologically the efficacy of intratendineous injection of PSGAG to treat tendinitis induced with the use of collagenase.

MATERIAL AND METHODS

Ten Arabian horses, males and females, with ages between two and six years was used in this study. The horses were clinically evaluated for the absence of lameness, local pain, heat and swelling involving the superficial flexor tendon of the thoracic limbs. Ultrasonographic examination was performed to determine the normal echogenic aspects of the tendon tissue. The horses were randomly divided into two groups of five animals. In all horses, tendinitis

was induced in the superficial digital flexor tendon (SDFT) of the left thoracic limb, through injection of 1.0ml (2.5mg/ml) of collagenase[1] solution into of the tendon. The tendon of the right limb remained as negative control.

One week after induction the lesion, the horses in group I received 125mg of polysulphated glycosaminoglycan in 1.0ml injected at two sites along the lesion. A total of five injections were given at four-day intervals. The horses in group II (controls) received injections of saline under the same conditions.

The animals were evaluated for lameness, pain, heat and swelling at the injection site 24 and 48 hours after creation of the injury and at every day of the treatment. The evaluations were performed on days 30, 45, 60, 75, 90, 120 e 150 to monitor the repair process.

The ultrasonographic examinations were performed at intervals suggested before for the other parameters. A Pie Medical[2] ultrasound machine, a stand-off block and echolucent gel were used. The echogenicity was determined based on a scale of 1 to 4, suggested by Genovese et al. (1986) and Reef (1998). The evaluation of changes in fiber alignment, based on the linear arrangement of the echoes in the longitudinal images, were graduated between 0 and 3 (Reef, 1998).

On day 150, all horses were euthanized and the palmar metacarpal region of the left thoracic limb was carefully dissected to isolate the lesion area and determine correlated structures.

Samples were collected from tendon scar tissue of the left limb and from normal tendon of the contralateral limb for standard histologic procedures and preparation of frozen sections. The tendon segments were fixed in a solution with n-hexane (Labsynth) at –14ºC and sectioned longitudinally and transversally in 5-7µm slices with a cryostat. All samples were stained with hematoxylin-eosin (Luna, 1992).

Each segment was analyzed qualitatively using the following characteristics: a) vascularization, cellularity, alignment and retraction of collagen fibers, b) presence of inflammatory and fibrocartilaginous cells, and c) characterization of the interfascicular connective tissue.

The differences between the medians of treated and control groups for the clinical and ultrasonographic parameters analyzed in the various moments and for the macro and microscopic on day 150 were tested based on the non-parametric statistical model, Wilcoxon test, for two independent samples. The rate of significance adopted was $P<0.05$ (Steel and Torrie, 1960).

RESULTS

Lameness scores, ranging from 2 to 3, according a classification system recommended by the Association of Equine Practioners (Guide..., 1991), were observed in all horses 24 hours after induction of injury, decreasing gradually after this time. On day 30, only one horse of each group was lame (grade 1), which lasted until day 45. After 45 day, all horses were not lame. Local pain and heat at the affected limbs remained in three horses of each group for up to 90 days. Local swelling was evident in all horses during the experiment.

Transverse and longitudinal ultrasonographic images showed tendon lesions of variable size, shape and position, from hypo to anechoic, with loss of the linear pattern of fibers, being generally located in the core of the tendon (Fig. 1). The most severe lesions were identified between the seventh and the 23rd day after the injury was induced. At this moment, the echogenicity ranged from 2 to 4 and the fiber alignment from grades 1 to 3. At the end of the study, diffuse or uniform hypoechoic areas characterized the lesions, with echogenicity between grade 1 and 2 and fiber alignment between 0 and 2.

After removing the skin and subcutaneous tissue, the tendon affected area was easily identified by its hyperemic appearance (Fig. 2-A). Thickness and opacity were observed in the paratenon of six animals, with evident vascularization. Adhesion of the paratenon to the SDFT was

[1] Collagenase type 1:C 0130, Sigma.
[2] Mod. 200, 4 cm linear transducer, Pie Medical.

noticed in six animals, being severe in only one of them. The changes most frequently observed are shown in Fig. 2-B. Cross sections of the tendons showed a variable consistency from firm to gelatinous, and increased cross-sectional area in relation to a normal tendon (Fig. 2-C).

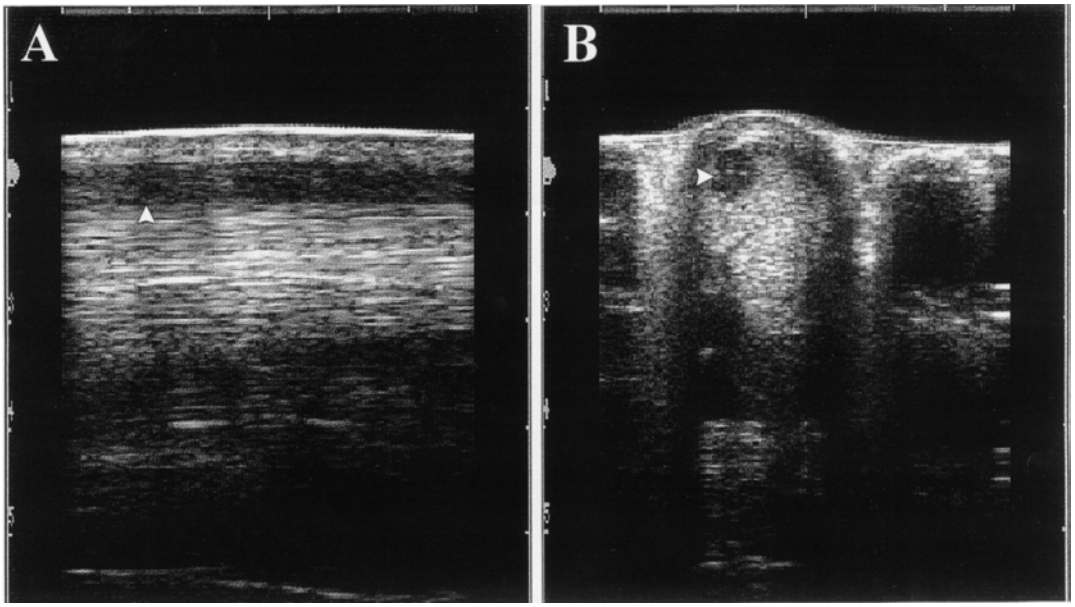

Figure 1. Longitudinal (A) and transverse (B) ultrasonographic images of instead aspects of an experimental lesion (arrows) observed in the superficial digital flexor tendon of a horse of control group (saline injection) 15 days after injury, with grades 4 in echogenicity and 3 in fiber alignment.

Independent of the treatment, the histopathologic examination revealed intense to very intense vascularization (grades 2 to 3), exhibiting a large number of residual, newly formed vessels in all the tendon fragments. Intense to very intense cellularity (grades 2 to 3), with numerous fibroblasts with round to slightly elongated nuclei, organized in a parallel fashion among the collagen fibers, were also observed.

Partial longitudinal and parallel disposition of the collagen fibers was seen in all the horses (grade 2). The retraction of the collagen fibers was completely absent in five horses, and was shown to be partial in the remaining animals.

The number of inflammatory cells was irrelevant to the study. Fibrocartilaginous metaplasia was occasionally observed at the lesion site, characterized by the presence of only isolated fibrocartilaginous cells. The results of this investigation demonstrated a hypercellular endotenon, with cells proliferating in a disorderly manner among collagen bundles and appearing thicker than normal with evident and abundant vascularization.

The various results analyzed during this study did not show any significant statistical difference in the healing process of tendon injury between treated and control limbs.

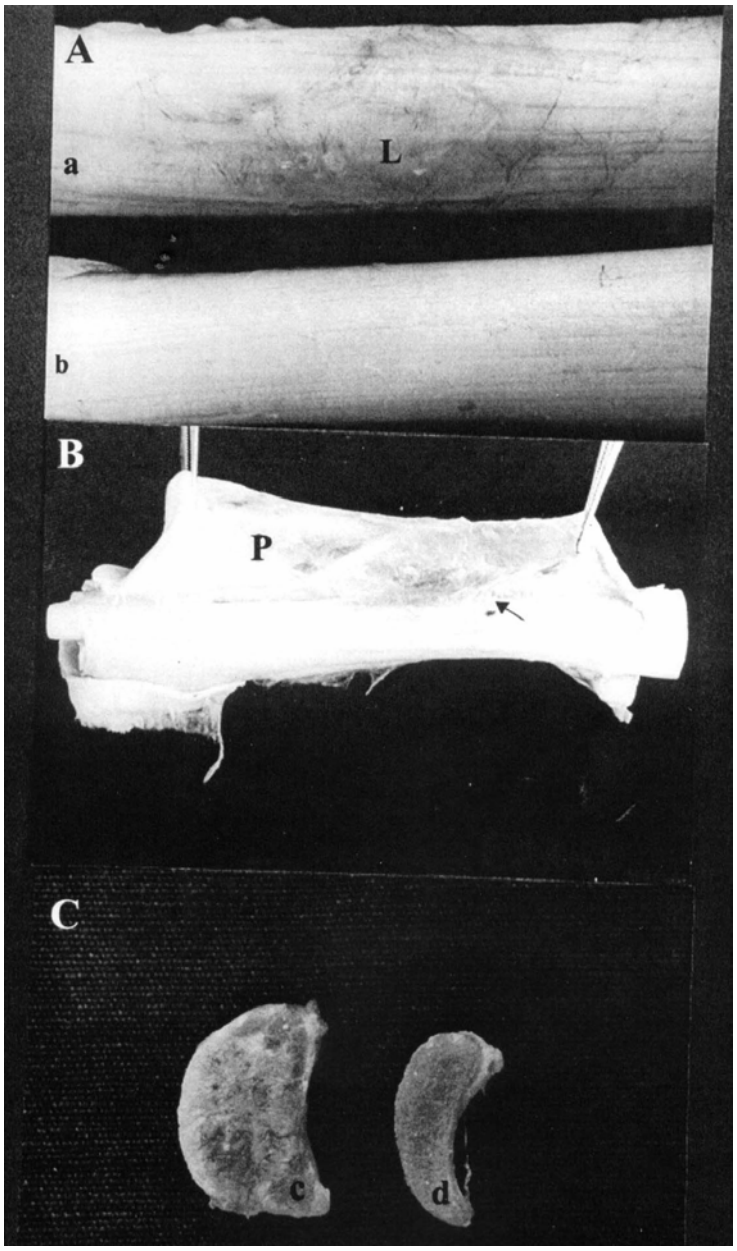

Figure 2. A) Macroscopic palmar aspect of superficial digital flexor tendon (SDFT) of the left limb (a) of a horse from treated group (GI), showing a hyperemic area indicative of the experimental lesion (L) 150 days after the lesion was induced; normal aspect of the contralateral limb (b). B) Macroscopic aspect of the SDFT of the left limb of a horse from GI, showing a moderately thick paratenon (P), slightly opaque, with very evident vascularization, and the presence of moderated adhesions (arrow). C) Macroscopic aspect of cross-section of the SDFT of the left limb (c) of a horse from GI, showing an increased cross-sectional area. Notice the pallid area with loss of fascicular pattern and hyperemic area indicating hemorrhagic foci; normal aspect of the SDFT of the contralateral limb (d).

DISCUSSION

The model of creating lesions by the use of collagenase showed to be effective in developing a model of tendinitis of the SDFT in horses in various studies evaluating tissue repair (Silver et al., 1983; Williams et al., 1984; Keg et al., 1992) and in the comparison of the most commonly used treatments for tendinitis (Foland et al., 1992; Alves, 1998; Redding et al., 1999).

All horses showed pronounced lameness 24 hours after collagenase injection. The remission of this clinical sign occurred rapidly, being absent in eight horses on day 30, remaining as grade 1 until 45 days in only two animals, which developed the more severe lesions. This findings are close to those described by Silver et al. (1983), Williams et al. (1984), Spurlock et al. (1989), Foland et al. (1992), Keg et al. (1992) and Alves (1998) who noticed lameness few hours after collagenase application, until a period of 60 days on average, in similar model.

A significant increase in pain and heat in the tendon were observed in the first 48 hours in all horses, noticeable by palpation until day 90. These observations are conflicting and disagree to those described previously by Silver et al. (1983), Williams et al. (1984) and Spurlock et al. (1989), who, although verifying an increase in pain and heat immediately after collagenase injection, observed the total remission of these signs in seven to 14 days. On the other hand, Alves (1998) observed the same clinical alterations on experimental tendinitis until 45 days in horses submitted to five intratendineous treatments with β-aminopropionitrile fumarate. The persistent increase in pain and heat in the present study could have resulted from the intralesional inoculation of PSGAG or of saline, resulting in mechanical disruption of immature collagen fibers with the development of a new acute inflammatory process at each new treatment.

The local swelling was attributed to the formation of hematoma and edema, as part of the inflammatory process, remaining visible in all horses of both groups until the end of the study. Reports of clinical observations presented by Keg et al. (1992) and Alves (1998) corroborate these findings. In a research performed by Williams et al. (1984) about the pathogenesis of experimental tendinitis, local swelling was still present 14 months after induction with collagenase.

In the most severe stages the echogenicity ranged from grade 2 to 4 and the fiber alignment from 1 to 3. The determination of the most severe lesions between 15 and 19 days agree with findings of Foland et al. (1992) and Palmer et al. (1994). This last group of investigators justify the aggravation in the acute stage until 10 to 20 days after injury because of a continuous enzymatic activity of proteases and collagenases released by the macrophages of the inflammatory exsudate. According to preliminary studies, the anechoic and hypoechoic lesion observed at this moment correspond to areas of hemorrhage, edema and fibril disruption (Genovese et al., 1986; Spurlock et al., 1989; Crass et al., 1992; Marr et al., 1993; Palmer et al., 1994; Reef, 1998).

Slight improvement in echogenicity and fiber alignment occurred during tendon repair, which, according to Crass et al. (1992) and Marr et al. (1993), reflects the presence of immature granulation tissue with active fibrogenesis.

The echogenicity and fiber alignment observed on day 150 correspond histologically to the most advanced stage of tendon repair, but still showing collagen production and tendon fibers reorganization.

Although Smith (1992) observed satisfactory ultrasonographic parameters in one horse 177 days after PSGAG intratendinous treatment and any signs of recurrence were observed by him when the horse returned to athletic activities, he also did not compare the efficacy of the drug against a conservative treatment. On the other hand, Redding et al. (1999) obtained significant beneficial effects with PSGAG intramuscular treatment, in experimental tendinitis induced by collagenase. The ultrasonographic evaluation has revealed an increased echogenicity and a decreased lesion size earlier in the treated group when compared to the control group, during the eight weeks of the study. At the present study, the intratendinous fluid injections was not effective. Probably the PSGAG injected in two sites of the tendon, with a period of four days between each aplication, spreaded in the tendon and increased the extension of the lesion, as

observed by van den Belt et al. (1992) when they injected higher volumes than 0.5ml of sodium hyaluronate in equine tendon. In order to observe the complete healing of the tendon lesions, more time and further evaluations will be necessary.

The thickness of the paratenon and evidence of adhesions to the palmar surface of the SDFT observed in this study were also shown to be present in subacute and chronic tendinitis investigated by Webbon (1977) and in related observations by Williams et al. (1984) and Alves (1998).

These observed histologic changes in tendon scar 150 days after induction of lesions in the present study are similar to findings reported by Silver et al. (1983), Williams et al. (1984), Marr et al. (1993) and Crevier et al. (1997) from studies on the pathophysiology of clinical and enzymatically induced tendinitis. Silver et al. (1983) even showed hypercellular scar tissue areas with little subdivision into fascicles, associated with abnormal histologic and biochemical characteristics 14 months after enzymatic induction of injury.

According to correlations made previously by Crass et al. (1992) and Marr et al. (1993), the differences observed between horses are probably related to the severity of injury defined initially by the clinical and ultrasonographic evaluations. Keg et al. (1992) and Palmer et al. (1994) emphasized the possibility of a variation in the individual response, becoming difficult to predict the behavior of a lesion repair.

From the results of the statistical analysis and the correlation of the macroscopic and histopathologic findings observed in this study and the reports from several authors about the pathogenesis of tendinitis, related to the results obtained in the clinical and ultrasonographic evaluations, it can be concluded that the intralesional treatments with PSGAG or saline in this tendinitis model did not induce any benefit in tendon healing.

ACKNOWLEDGEMENTS

This project was funded by a grant from the Fundação de Apoio à Pesquisa do Estado de São Paulo, process n. 99/02699-2.

REFERENCES

ALVES, A.G. *Influência da beta-aminopropionitrila associada à atividade física na reparação tendínea de eqüinos após agressão pela colagenase. Análise ultra-sonográfica e morfológica.* 1998. 92f. Dissertação (Doutorado em Patologia) - Universidade Estadual Paulista, Botucatu, SP.

CRASS, J.R.; GENOVESE, R.L.; RENDER, J.A. et al. Magnetic resonance, ultrasound and histopathologic correlation of acute and healing equine tendon injuries. *Vet. Rad. Ultrasound*, v.33, p.206-216, 1992.

CREVIER, N.; COLLOBERT, C.; POURCELOT, P. et al. Mechanical properties of pathological equine superficial digital flexor tendons. *Equine Vet. J.*, v.23, Suppl, p.23-26, 1997.

FOLAND, J.W.; TROTTER, G.W.; POWERS, B.E. et al. Effect of sodium hyaluronate in collagenase-induced superficial digital flexor tendinitis. *Am. J. Vet. Res.*, v.53, p.2371-2376, 1992.

GENOVESE, R.L.; RANTANEN, N.W.; HAUSER, M.L. et al. Diagnostic ultrasonography of equine limbs. *Vet. Clin. North Am.: Equine Pract.*, v.2, p.145-226, 1986.

GILLIS, C.L. Rehabilitation of tendon and ligament injuries. In: ANNUAL CONVENTION OF THE AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS, 43., 1997, Phoenix. *Proceedings...* Phoenix, American Association of Equine Practitioners, 1997. P.306-309.

GUIDE for veterinary service and judging of equestrian events. 4.ed. Lexington: American Association of Equine Practitioners, 1991. 18p.

KEG, P.R.; VAN DEN BELT, A.J.M.; MERKENS, H.W. et al. The effect of regional nerve blocks on the lameness caused by collagenase induced tendonitis in the midmetacarpal region of the horse: a study using gait analysis, and ultrasonography to determine tendon healing. *J. Vet. Med. Assoc.*, v.39, p.349-364, 1992.

LUNA, L.G. *Histopathologic methods and color atlas of special stains and tissue artifacts.* Downers Grove: Johnson, 1992. 767p.

MARR, C.M.; McMILLAN, I.; BOYD, J.S. et al. Ultrasonographic and histopathological findings in equine superficial digital flexor tendon injury. *Equine Vet. J*., v.25, p.23-29, 1993.

PALMER, S.E.; GENOVESE, R.; LONGO, K.L. et al. Practical management of superficial digital flexor tendinitis in the performance horse. *Vet. Clin. North Am.: Equine Pract.*, v.10, p.425-481, 1994.

REDDING, W.R.; BOOTH, L.C.; POOL, R.R. The effects of polysulphated glycosaminoglycan on the healing of collagenase induced tendinitis. *Vet. Comp. Orthop. Traumatol.*, v.12, p.48-55, 1999.

REEF, V.B. *Equine diagnostic ultrasound.* Philadelphia: Saunders, 1998. 560p.

SILVER, I.A.; BROWN, P.N.; GOODSHIP, A. et al. A clinical and experimental study of tendon injury, healing and treatment in the horse. *Equine Vet. J*., v.1, Suppl., p.5-22, 1983.

SMITH, R.K.W. A case of superficial digital flexor tendinitis: ultrasonographic examination and treatment with intralesional polysulphated glycosaminoglycans. *Equine Vet. Educ*., v.4, p.280-285, 1992.

SPURLOCK, G.H.; SPURLOCK, S.L.; PARKER, G.A. Ultrasonographic, gross, and histologic evaluation of a tendinitis disease in the horse. *Vet. Radiol.,* v.30, p.184-188, 1989.

STEEL, R.G.D.; TORRIE, J.H. *Principles and procedures of statistics*. New York: Mc Graw-Hill, 1960. 482p.

VAN DEN BELT, A.J.M.; KEG, P.R.; DIK, K.J. The correlation between the dose and distribution of intratendinous fluid injections in the flexor tendon of the horse*. Vet. Radiol. Ultrasound*, v.33, p.120, 1992.

WEBBON, P.M. A post mortem study of equine digital flexor tendons. *Equine Vet. J*., v.9, p.61-67, 1977.

WILLIAMS, I.F.; McCULLAGH, K.G.; GOODSHIP, A. et al. Studies of the pathogenesis of equine tendonitis following collagenase injury. *Res. Vet. Sci.*, v.36, p.326-338, 1984.

WOOD, A.K.W.; SEHGAL, C.M.; REEF, VB. Three dimensional sonographic imaging of the equine superficial digital flexor tendon. *Am. J. Vet. Res.*, v.55, p.1505-1508, 1994.