Clinical evaluation of autologous platelet-rich plasma for the treatment of tendinous and ligamentous lesions in 7 horses

D. Argüelles,[§] J.U. Carmona, [§†] M. Prades[§] , F. Climent,[§] R. Soler,* L. Orozco,* F. Vidal*

[§]Equine Surgery Service, Veterinary Medical Teaching Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain. [†]Department of Animal Health, School of Veterinary Medicine. Universidad de Caldas, Manizales, Colombia *Instituto de Terapia Regenerativa Tisular, Barcelona, Spain.

Part of this study was presented as a poster in the 14th Annual Scientific Meeting of the European College of Veterinary Surgeons. Lyon, France. July 2005.

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Summary

The efficacy of platelet rich plasma (PRP) in 7 horses with soft tissue musculoskeletal injuries namely: superficial digital flexor (SDFT) tendinopathy and desmitis of the suspensory ligament (DSL) was evaluated. Initial degree of lameness (DL), flexion test reponse (FT), ultrasonic images, and long term clinical follow-up were documented. Three injections of PRP were performed at two week intervals. Horses were evaluated before each injection, two months after the last treatment and 1 year after. Platelet counts, WBC counts, and determination of TGF-β_1 levels per ml of PRP were performed. Two patients had acute SDFT tendinopathy, 1 horse had chronic SDFT tendinopathy, 1 horse had chronic bilateral forelimb proximal DSL (PDSL), 2 horses had chronic bilateral hindlimb PDSL, 1 horse had bilateral forelimb desmitis of the branches of the suspensory ligament (BDSL). All the horses in this report presented clinical and statistical ($P < 0.05$) decrease of DL and response to FT. Ultrasound appearance improved in the horses with SDFT lesions, but remained the same in the horses with DSL. The horses with acute SDFT tendinopathy returned successfully to competition level without reinjury. The horse with chronic SDFT tendinopathy relapsed. Horses with forelimb and hindlimb PDSL returned successfully to competition level without reinjury. A mean of 250 ± 71.8 x 10^6 platelets, 8.68 ± 3.78 leucocytes x 10^6, and 12515 ± 2443 pg of TGF-β_1 per 1 ml of PRP were obtained.

Keywords: horse, superficial flexor tendonitis, desmitis of the suspensory ligament, platelet-rich plasma, growth factors, TGF-β_1

Introduction

Injuries of tendons and ligaments heal slowly and inefficiently. Once a lesion is produced, these structures do not recover their original biomechanical properties. It has been hypothesized that factors like fatigue, hypoxemia, hyperthermia, and overstrain can unchain a degenerative process of the extracellular matrix (ECM) of these tissues (17), including changes in the collagen molecules ratio with a shift to collagen type III (Col-III) production in detriment of Col-I synthesis (11), and death of resident cells (20). However, the exact pathophysiologic mechanism that produces these changes remains not well understood (13, 27).

The presence of catabolic cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 1 alpha (IL-1α) and beta (IL-1β) in biopsies of equine tendons suffering from chronic tendinopathy has been reported (18). It has been shown that the exogenous administration of IL-1β to human tenocytes upregulates the expression of IL-1β and IL-6, cyclo-oxigenase 2 (COX-2), and matrix metalloproteinases (MMPs). It is known that all these substances have catabolic and inflammatory properties which have detrimental effects on ECM and resident cells of many tissues, including tendons and ligaments (32).

The negative effects of IL-1 or TNF-α on connective tissue could be down-regulated by some growth factors (GFs) (33). Studies *in vitro* have demonstrated the beneficial properties of transforming growth factor beta (TGF-β) (2, 23, 24,), insulin like growth factor I (IGF-I) (9), vascular endothelial growth factor (VEGF) (35), fibroblastic growth factor (FGF)(23, 34), epidermal growth factor (EGF) and

platelet derived growth factor (PDGF) (23, 34) on tendon and ligament metabolism of different animal species. Moreover, Dahlgren et al (10) described a positive action of IGF-I in a collagenase induced tendonitis model of the equine superficial digital flexor tendon (SDFT). The results from these studies suggest the possible benefit of using GFs as a complementary treatment of soft tissue musculoskeletal injuries, either using pure recombinant proteins or gene therapy (6, 9, 12).

Platelet-rich plasma (PRP) is a natural and autologous source of some GFs, especially TGF-β_1, TGF-β_2, PDGF, VEGF, EGF, FGF and of other molecules that modulate inflammation and the tissue repair process (2, 3, 30). Platelet rich plasma has been used successfully in human medicine, for several purposes, including alveolar-maxilar reconstruction (2, 7), plastic (5), and orthopedic surgery (2). Moreover, there are anecdotic reports about the use of PRP for the treatment of Achilles tendonitis in two athletes and for cartilaginous avulsion in a football player (26). Recently, Anitua et al (3) documented the positive effects of PRP on human tenocytes. In that study, PRP produced tenocyte proliferation and upregulation of two angiogenic peptides, VEGF and hepatocyte growth factor (HGF).

In the present study the results from the treatment with PRP in 7 horses with tendon and ligament injuries are reported. The objectives of this report were to describe the possible beneficial efficacy and clinical safety of PRP injection in horses with soft tissue musculoskeletal lesions.

Material and methods

This pilot, clinical, prospective study was approved by the Ethical Committee of our institution. The owners were informed about the nature of the experiment and the possible complications derived from the PRP injection. All the observations and determinations were performed by two separate clinicians.

Horses

Seven horses of different sex, age, breed and aptitude were included in this study. The average age of the horses was 9.14 years (range 3-14 years). A total of 11 limbs were treated as some of the horses had a bilateral lesion (table 1). All the patients were evaluated seven days before being treated. A complete lameness examination was done including perineural and intraarticular regional anesthesia when necessary in order to localize the lesion in the limb. A complete ultrasonographic and lameness examination was performed before every treatment, two months after the last injection and 1 year after.

Design of the study

Lameness examination. The lameness exam was scored from 0 to 5 following the AAEP criteria (1). Where 0= Horse presents a normal gait – 5= Horse is not able to bear weight on the affected limb. A one minute flexion test was done and the

results were classified with a qualitative scale from 0 to 3, where 0= negative result 1=slight increase in lameness 2= moderate increase in lameness 3= severe increase in lameness

Ultrasound evaluation. A modified Rantanen et al's ultrasound classification (24) for equine tendon and ligament injuries was used. Clinical signs (heat, inflammation, lameness, sensitivity, thickness of the anatomic structures) and clinical history of each patient was evaluated together with the results of the ultrasound exam. Ultrasonographic parameters used in this study included: cross sectional area (CSA), maximum injury zone (MIZ), MIZ percentage respect to the healthy portion of ligament or tendon, echogenic pattern of the lesion and orientation of the fibers in both longitudinal and transverse sections.

Clinical and ultrasonic examination of each horse were combined in order to create a qualitative scale of lesions in six categories from 0-V, where 0= tendon ultrasound image normal and no lameness or any clinical sign associated with tendonitis or desmitis is present – V= Presence of very severe anechoic lesions, large increase in the CSA-MZI, severe disorganization of the fibers in longitudinal and cross section, evident clinical signs with a moderate or severe degree of lameness (24).

Clinical follow-up. Clinical follow-up of each patient was conducted during 1 year. Four patients were evaluated personally by us; the other three were examined by the referring veterinarians and images of the ultrasound examinations were sent to us for evaluation. The treatment with PRP was considered successful when the

horse returned to the same pre-injury training or competition levels without relapse of the injury in the follow-up time.

Preparation and analysis of the PRP

Whole blood was aseptically drawn of the jugular vein of each patient via a 23G butterfly catheter (Terumo, Belgium), and deposited in 3.2% sodium citrate tubes with capacity for 5 ml. It was then centrifuged at 120 g during 5 minutes. The first supernatant plasma fraction (50%), adjacent to the buffy coat, was obtained under aseptic conditions in a laminar flow chamber. This fraction was centrifuged at 240 g during 5 minutes and the 25% from the first fraction was obtained. This last fraction was placed into sterile syringes and activated with calcium chloride (4.5 mEq/5 ml), using 50 µl per ml of PRP. An average of 5 ml of PRP was obtained from every 75 ml of whole blood. A fraction of PRP (2 ml) of each patient was analyzed for platelet and leukocyte count and TGF-β_1 concentration determination. Cell count was performed by flow cytometry (ADVIA 120, Bayer, USA), and TGF-β_1 levels were determined by a human commercial ELISA kit (R&D System, USA).

Schedule of the PRP treatment

The affected area of the limb of each horse was prepared aseptically for injection. The horses were sedated with an intravenous bolus of detomidine (Domosedan, Pfizer) and butorphanol tartrate (Torbugesic, Fort Dodge Laboratories Inc). PRP

was injected 3 consecutive times at two week intervals. The amount of PRP used in each patient was subjectively determined depending on the type and size of lesion, and weight of each patient (table 1). The horses were generally kept at a lower level of exercise during treatment and for 2 weeks following the last injection.

Statistical Analysis

The analgesic effect of the PRP was statistically analyzed. Only the patients with soft tissue lesions were evaluated (1-6). Data from degree of lameness and flexion test obtained before each injection with PRP and two months later were analyzed with a Kruskall-Wallis test. It was assumed that in the case of finding significant differences in time, a non parametric pairwise comparison Wilcoxon test would be done. A $p \leq 0.05$ value was established as a significant for both tests. Data from the ultrasound exam and the values obtained from the PRP were presented just in a descriptive way.

Results

Of the evaluated horses, 2 patients had acute SDFT tendinopathy, 1 horse had chronic SDFT tendinopathy, 1 horse had chronic forelimb proximal desmitis (PD) of the suspensory ligament (PDSL), 2 horses had chronic hindlimb PDSL, 1 of these patients also had desmitis of the medial branch of the suspensory ligament of the right hindlimb, and 1 horse had bilateral forelimb desmitis of the branches of the suspensory ligament (BDSL) accompanied by bilateral fetlock osteoarthritis (table 1). The animals with SDFT lesions presented initial ultrasound scores between IV-V (median V) (*see details in* Table 2). The horses with desmitis of the SL had initial ultrasound scores between I-IV (median II) (*see details in* Table 3). At the beginning of the study the horses presented a slight grade of lameness and a moderate response to flexion test (Table 4). PRP produced a statistically significant decrease of degree of lameness ($p=0.0053$) and of the response to flexion ($p=0.0062$) during the study. The improvement in both parameters was most evident at the end of the study. The cosmetic appearance of the injured area was excellent already after the first injection in the cases of SDFT.

Serial ultrasound examinations showed improvement in the score of the lesions, especially in the horses with SDFT tendonitis (Table 2). The horses with either forelimb or hindlimb bilateral PDSL did not change their ultrasound category. However, they improved the scores of both degree of lameness and response to flexion test. Horse number 7, with a severe bilateral BDSL showed both a marked ultrasonographical and clinical improvement (Table 3 and Fig. 1). The horses with

PDSL finished with a median ultrasound lesion score (median II), but with a decrease in the range of the classification (I-II) (Table 3).

The horses with acute DSFT tendinopathy returned to complete exercise and to competition level 4.5 months after last PRP injection without reinjury at 1 year of the last PRP treatment. Patient number 3 with chronic DSFT tendinopathy presented a relapse of the lesion 3 months after the last PPR treatment when galloping was started. This horse returned to work again 6 months after relapse and did not return to competition level. The patient is now used as a master school horse.

Horse number 4 with chronic forelimb PDSL returned to pre-injury level 2 months after the last PRP injection without reinjury at 1 year of follow-up. Horse number 5 with one year duration bilateral hindlimb DPLS and horse number 6 with the same pathology returned to competition level 6 months after the last PRP injection without reinjury at 1 year of follow-up. Patient number 7 with bilateral BDLS improved from its soft tissue lesions, but it is still lame because of the fetlock joint osteoarthritis.

A mean of 250 ± 71.8 x 10^6 platelets, 8.68 ± 3.78 leucocytes x 10^6, and 12515 ± 2443 pg of TGF-β_1 per 1 ml of PRP were obtained.

Discussion

This is the first report, to our knowledge, about the use of PRP for the treatment of musculoskeletal soft tissue injuries in horses although and although we are aware that some equine clinicians have used PRP before for soft tissue injuries, the results have not been published. In the study presented here, we describe an easy and inexpensive technique for obtaining PRP. This pilot clinical study was performed on clinical equine patients because there is scientific and anecdotic evidence about the positive effects of this substance on human soft tissues (2, 7, 5), including tendon and ligament injuries (3, 26).

Tendon and ligament pathologies are frequent in human and equine sports medicine. The pathologic process that affects these structures could be considered as degenerative in nature (11, 22). Traumatic events should be considered as a separated entity. However, many tendon or ligament acute (traumatic) lesions could be related to reinjury of a chronically active lesion or to failure of scar tissue within the structure (13, 17, 27). Maybe, the SDFT lesions presented in the horses number 1 and 2 could be included into this pathologic condition. It is known that horses with the same classification of SDFT tendon lesion (grades IV-V) to the horses of this study are very prone to reinjury, despite having followed a controlled exercise programme and conventional treatment. Normally, 9-12 months of rest are recommended as a part of the treatment of these patients (14,21). We found that the 2 horses with acute SDFT tendinopathy treated with PRP returned to competition level in half of the expected time and did not relapse in comparison

with literature documented controls treated with conventional treatments, such as polysulfated glycosaminoglicans, hyaluronan. or fumarate of beta aminopropiontrile (BAPN) intra or perilesional injection amongst others (14, 21).

Horses with SDFT tendinopathy are prone to develop reinjury of the initially affected tendon or to overstress and injury of the contralateral SDFT (14, 21). Horse number 3 reinjured the SDFT above the original area of lesion despite of PRP treatment. It is likely that this treatment is less useful in chronic lesions where scar tissue already exists and there has been permanent loss of tissue elasticity. Another explanation at a cellular level for this situation could be the exaggerated apoptosis that has been seen in this pathology. Recently, Hozaka et al (20) demonstrated that apoptosis plays an important role in the pathophysiology of this disease. It is possible to think that a if there is a reduction in the population of tenocytes and if these cells aditionally are metabolically impaired by the action of catabolic cytokines (18), mainly TNF-α (19), they will not react in a positive fashion to the addition of any purified GF or PRP. In this sense, the use of mesenchymal stromal cells accompanied with PRP (28) or other delivery systems of GFs could be an option for the treatment of this pathology in advanced chronic phases (27). In addition, it may be reasonable to perform microsplitting of the affected area with a needle to stimulate bleeding and a vascular accesss to the center of the lesion together with PRP injection. This was not performed in these horses but this procedure could be evaluated in a prospective clinical study. Peripheral blood contains mesenchymal stromal cells that have the potential capacity to transform into tenocytes if adequately stimulated (28). Platelet rich plasma contains many

peptides that could potentially stimulate the differentiation of these cells (3, 8, 30), in addition to an environment with linear tension, like in tendons or ligaments. Although, to our knowledge, molecular changes associated with catabolic cytokines and apoptosis have not been described in the SL, it could be possible to assume that the same occurs in ligamentous tissues.

The prognosis of SL injuries depends on the extent, the affected area and limb. PDLS has a better prognosis in forelimbs than in hindlimbs. Horses with forelimb PDSL can attain pre-injury training level after a three month programme of rest followed by controlled exercise (15, 16). Horse number 4 of the series presented here with forelimb PDLS, returned to pre-injury exercise level two months after the last PRP injection. In this horse the effect of time and rest could have influenced recovery. However, when considering clinical history and the positive follow-up of horses 5 and 6 with chronic severe bilateral hindlimb PDSL, we think that the treatment with PRP could be useful for the management of this frustrating pathology. It is important to note that PDSL of the hind limbs can be totally limiting for an athletic career and that some of the radical treatments that have been tried, fasciotomy with or without neurectomy of the lateral branch of the plantar nerve will fail since most of the horses with this pathology will continue to have a degenerative process of the SL that could even lead to its rupture (15, 16).

Desmitis of the branches of the SL is more often described in the forelimbs than in the hindlimbs and is normally associated to a chronic process (15). The ultrasonographic lesions observed from horse number 6 were very severe. This patient's ultrasonic scores improved dramatically after even the first PRP treatment. It is known that this type of lesion heals very slowly (more than 18

months) and patients can take more than 9 months to return to normal performance (15, 16). Unfortunately, this horse had also osteoarthritis in both forelimb fetlock joints, and despite its excellent ultrasonographic progress the horse remained lame (Fig 1).

Some of the most relevant clinical observations from the perilesional treatment of these soft tissue injuries were the following: even after the first injection of PRP the cosmetic appearance of the injured area was excellent, meaning there was less heat, pain, swelling and lameness; there seemed to be a better response in injuries that were presented within a short period after injury. The improvement of the ultrasonic scores was most evident and remarkable for acute suspensory ligament branch injuries. The chronic proximal hindlimb suspensory ligament desmitis cases showed no improvement in their ultrasonic appearance although it has to be pointed out that these horses did not show anechoic lesions, just fiber disorganization and thickening of the origin of the ligament these horses though did improve clinically and actually went back into work with no relapse of the injury. The results observed from the patients of this study, especially in horses number 5 and 6, could indicate that PRP has a powerful and long-term analgesic and antiinflammatory effect. PRP has not only an important amount of TGF-β_1, but it has important levels of other angiogenic (FGF, VEGF), proliferative (PDGF, IGF, FGF and EGF) and anabolic (IGF-I) GFs of paramount importance in the wound repair process (2, 3). Peptides like TGF-β_1 or IGF-I have potent anti-inflammatory effects, since they downregulate the expression of nuclear proinflammatory factors, such as nuclear factor kappa beta (NF$\kappa\beta$) (33). This factor is activated for IL-1 or TNF-α during equine tendinopathy

(18, 19), and is responsible of the upregulation of several secondary catabolic substances, like MMPs, prostaglandin E_2, and nitric oxide, amongst others (32, 33).

Up to date, two techniques for obtaining equine PRP have been described: apheresis and buffy coat methods (8, 30). An additional technique in order to improve the efficiency of collection of equine platelets after using a conventional method has also been reported (30). The aforementioned techniques are good, but they present technical and economical restrictions for many equine practitioners. For example, the apheresis method can only be performed in a specialized laboratory. On the other hand, the buffy coat method is very expensive. We think that the double centrifugation tube method presented in this study, represents a reliable, inexpensive and alternative method in comparison to the other ones previously described.

In this study a lower concentration of platelets (250 ± 71.8 x 10^6 platelets/ml) was obtained in comparison with the values reported for the apheresis method by Carter et al (8) (490 x 10^6 platelets/ml) or by Sutter et al (30) (855 x 10^6 platelets/ml) or for the buffy coat method (1472 x 10^6 platelets/ml) (30). However, we obtained higher TGF-β_1 levels (12515 ± 2443 pg/ml) in comparison with the levels obtained by the apheresis method by Carter et al (8) (7480 ± 1315 pg/ml), and lower TGF-β_1 levels in comparison with Sutter et al (30) (23600 pg/ml). Using the buffy coat method, Sutter et al (30) reported a total of 1472 x 10^6 platelets/ml and 15300 pg/ml of PRP. If we compare the results obtained with our technique, it is possible to note that the platelet number is high in that study (30) in relation with

our results. However, TGF-β_1 levels obtained with both techniques were very similar. It is important to note that many, not well defined factors can influence the final GF levels of platelet concentrates obtained by different techniques (8, 30).

The main limitation of this clinical report is the small number of cases documented and the different pathologies. Nevertheless, from our clinical observations we think that PRP injection could be a highly promising new instrument in the treatment of desmopathies and tendinopathies in the sport horse. However, this therapy can not be recommended until more clinical cases have been assessed and ideally a larger prospective randomized clinical study with a longer follow up would be necessary to validate these initial clinical observations.

References

1. American association of equine practitioners (AAEP). Guide for veterinary service and judging of equestrian events: definition and classification of lameness. Lexington: AAEP. 1991, p 19.
2. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004; 91:4-15.
3. Anitua E, Andia I, Sanchez M, Azofra J, Zaldueno MM, de la Fuente M, Nurden P, Nurden AT. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res 2005; 23:281-286.
4. Arai K, Kasashima Y, Kobayashi A, Kuwano A, Yoshihara T. TGF-beta alters collagen XII and XIV mRNA levels in cultured equine tenocytes. Matrix Biol 2002; 21:243-50.
5. Bhanot S, Alex J.C. Current applications of platelet gels in facial plastic surgery. Facial. Plast. Surg 2002; 18:27-33.
6. Beredjiklian PK. Biologic aspects of flexor tendon laceration and repair. J Bone Joint Surg Am 2003; 85:539-550.
7. Carlson NE, Roach RB. Platelet-rich plasma: clinical applications in dentistry. J Am Dent Assoc 2002; 133:1383-1386.
8. Carter CA, Jolly DG, Worden CE, Hendren DG, Kane CJ. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. Exp Mol Pathol 2003; 74:244-255.

9. Dahlgren LA, Nixon AJ, Brower-Toland BD. Effects of beta-aminopropionitrile on equine tendon metabolism in vitro and on effects of insulin-like growth factor-I on matrix production by equine tenocytes. Am J Vet Res 2001; 62:1557-62.
10. Dahlgren LA, van der Meulen MC, Bertram JE, Starrak GS, Nixon AJ. Insulin-like growth factor-I improves cellular and molecular aspects of healing in a collagenase-induced model of flexor tendinitis. J Orthop Res 2002; 20:910-9.
11. Dahlgren LA, Mohamed HO, Nixon AJ. Temporal expresión of growth factors and matrix molecules in healing tendon lesions. J Orthop Res 2005; 23:84-92.
12. Dai Q, Manfield L, Wang Y, Murrell GAC. Adenovirus-mediated gene transfer to healing tendon-enhanced efficiency using a gelatin sponge. J Orthop Res 2003; 21:604-609.
13. Dowling BA, Dart AJ, Hodgson DR, Smith RK. Superficial digital flexor tendonitis in the horse. Equine Vet J 2000; 32:369-78.
14. Dyson SJ. Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992-2000). Equine Vet J 2004; 36:415-19.
15. Dyson SJ, Arthur RM, Palmer SE, Richardson D. Suspensory ligament desmitis. Vet Clin North Am Equine Pract 1995; 11:177-215.
16. Dyson SJ, Genovese RL. The suspensory apparatus. In: Ross MW, Dyson SJ (Eds). Diagnosis and management of lameness in the horse. Saunders. Philadelphia 2003; pp 654-672.
17. Goodship AE, Birch HL, Wilson AM. The pathobiology and repair of tendon and ligament injury. Vet Clin North Am Equine Pract 1994; 10:323-49.

18. Hosaka Y, Kirisawa R, Yamamoto E, Ueda H, Iwai H, Takehana K. Localization of cytokines in tendinocytes of the superficial digital flexor tendon in the horse. J Vet Med Sci 2002; 64:945-7.

19. Hosaka Y, Sakamoto Y, Kirisawa R, Watanabe T, Ueda H, Takehana K, Yamaguchi M. Distribution of TNF receptor and TNF receptor-associated intracellular signaling factors on equine tendinocytes in vitro. Jpn J Vet Res 2004; 52:135-44.

20. Hosaka Y, Teroaka H, Yamamoto E, Ueda H, Takehana K. Mechanism of cell death in inflamed superficial digital flexor tendon in the horse. J Comp Path 2005; 132:51-8

21. Jorgensen JS, Genovese RL. Superficial digital flexor tendonitis in racehorses. In: Ross MW, Dyson SJ (Eds). Diagnosis and management of lameness in the horse. Saunders. Philadelphia 2003; pp 628-634.

22. Molloy T, Wang Y, Murrel G. The roles of growth factors in tendon and ligament healing. Sports Med 2003; 33:381-94

23. Murray MM, Rice K, Wrigth RJ, Spector M. The effect of selected growth factors on human anterior cruciate ligament cell interactions with a three-dimensional collagen-GAG scaffold. J Orthop Res 2003; 21:238-244.

24. Rantanen NW, Jorgensen JS, Genovese RL. Ultrasonographic evaluation of the equine limb: technique. In: Ross MW, Dyson SJ (Eds). Diagnosis and management of lameness in the horse. Saunders. Philadelphia 2003; pp 166-188.

25. Sakai T, Yasuda K, Tohyama H, Azuma H, Nagumo A, Majima T, Frank CB. Effects of combined administration of transforming growth factor-β1 and

epidermal growth factor on properties of the in situ frozen anterior cruciate ligament in rabbits. J Orthop Res 2002; 20:1345-1351.

26. Sanchez M, Azofra J, Anitua E, Andia I, Padilla S, Santisteban J. and Mujika I. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc 2003; 35:1648-52.
27. Smith R, Schramme M. Tendon injury in the horse: current theories and therapies. In Practice 2003; 25:529-539.
28. Smith RKW, Korda M, Bunn GW, Goodship AE. Isolation and implantation of equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. Equine Vet J 2003; 35:99-102.
29. Spindler KP, Murray MM, Detwiler KB, Tarter JT, Dawson JM, Nanney LB, Davidson JM. The biomechanical response to doses of TGF-β2 in the healing rabbit medial collateral ligament. J Orthop Res 2003; 21:245-249.
30. Sutter WW, Kaneps AJ, Bertone AL. Comparison of hematologic values and transforming growth factor-β and isulin-like growth factor concentrations in platelets concentrates obtained by use of buffy coat and apheresis methods from equine blood. Am J Vet Res 65:924-930, 2004.
31. Tang JB, Xu Y, Ding F, Wang XT. Tendon healing in vitro: promotion of collagen gene expression by bFGF with NF-kappaB gene activation. J Hand Surg [Am] 2003; 28:215-20.
32. Tsuzaki M, Guyton M, Garrett W, Archambault JM, Herzog W, Almekinders L, Bynum D, Yang X, Banes AJ. IL-1β induces COX2, MMP-1, -3 and ADAMTS-4, IL-1β and IL-6 in human tendon cells. J Orthop Res 2003; 21: 256-264.

33. Van Miert ASJ. Present concepts on the inflammatory modulators with special reference to cytokines. Vet Res Commun 2002; 26:111-126.
34. Wong MW, Tang YY, Lee SK, Fu BS, Chan BP, Chan CK. Effect of dexamethasone on cultured human tenocytes and its reversibility by platelet-derived growth factor. J Bone Joint Surg Am 2003; 85-A:1914-1920.
35. Zhang F, Liu H, Stile F, Lei MP, Pang Y, Oswald TM, Beck J, Dorsett-Martin W, Lineaweaver WC. Effect of vascular endothelial growth factor on rat Achilles tendon healing. Plast Reconstr Surg 2003; 112:1613-1619.

Table 1. Clinical history and sex, breed, age and aptitude of the patients of the study

Horse No	Breed	Sex	Age (years)	Aptitude	Affected Limb	Diagnosis	PRP (ml/lesion)
1	Crossbreed	Female	10	Jumper	LF	Acute SDFT tendinopathy	5
2	Crossbreed	Castrated male	9	Pleasure	RF	Chronic SDFT tendinopathy	7
3	Crossbreed	Male	11	Jumper	RF	Acute SDFT tendinopathy	7
4	Warmblood	Male	11	Dressage	L&RF	Chronic PDSL	10
5	Angloarabian	Castrated male	6	Jumper	L&RH	Chronic PDSL	10
6	Trotter	Male	5	Trotter	L&RH	Chronic PDSL. BDLS of the medial branch of the RH	10-4
7	Árabian	Male	14	Pleasure	L&RF	Chronic bilateral BDLS. Bilateral Fetlock OA	4

RF: Right forelimb. LF: Left forelimb. RH: Right hindlimb. LH: Left hindlimb. SDFT: superficial digital flexor tendon. PDSL: Proximal desmitis of the suspensory ligament, BDSL: branch desmitis of the suspensory ligament. OA: osteoarthritis.

Table 2. Ultrasound evaluation and classification of the horses´ superficial digital flexor tendon lesions, before, during and at the end of the treatments.

	Ultrasound lesion evaluation								
Horse No	**Before 1st treatment**	**C**	**Before 2nd treatment**	**C**	**Before 3rd treatment**	**C**	**Two months after last treatment**	**C**	**1 year after last treatment**	**C**
1	Circular and lateral lesion at 2A region of 2 cm in length. Represents 10% of the CSA of the tendon.	IV	The lesion is smaller and less extended (1cm). Fibers are more organizad.	III	There is no objective ultrasound difference between the lesion and the rest of the tendon.	II	Similar to the last evaluation.	II	Similar to the last evaluation	II
2	Several small, hypoechoic lesions and a small anechoic lesion lateral aspect of 2A region, extending about 4 cm in length.	V	There is a moderate change in the increasing echogenicity of the lesions.	III	There is just a small hypoechoic lesion in 2A region.	III	There is no evidence of ultrasonic lesion in the tendon. Slight thickening was evident	I	There is no evidence of ultrasonic lesion in the tendon.	I
3	Hypoechoic lesion placed medially in region 2A . It represents 20% of the CSA. Lesion perimeter 17,3 mm^2.	IV	There is an increase of the echogenicity of the lesion and a decrease of its size. Lesion perimeter 14,8 mm^2.	III	There is an increase of the echogenicity of the lesion and good alignment of the fibers at the site of the lesion.	III	Close to normal echogenicity pattern and normal fiber orientation at the lesion site.	II	There is no evidence of ultrasonic lesion in the tendon.	I

C: Lesion category classification. CSA: Cross sectional area

Table 3. Ultrasound evaluation and classification of the horses´ suspensory ligament injuries, before; during and after the treatments.

Horse No	Ultrasound lesion evaluation									
	Before the 1st treatment	**C**	**Before 2nd treatment**	**C**	**Before 3rd treatment**	**C**	**Two months after last treatment**	**C**	**1 year after last treatment**	**C**
4	LF: Thickening of the SL origin in 1A region (10.8 mm in longitudinal section) and periostitis of 2nd and 4th MTT in the 2B region. RF: No changes in the SL are observed (8.3 mm in longitudinal section).	II I	LF: Decrease of the longitudinal section of the origin of the SL (10.5 mm) RF: no changes.	II I	LF: no changes RF: no changes	II I	LF: no changes. RF: no changes	II I	LF: no changes. RF: no changes	II I
5	Thickening of both SL origin in both hindlimbs.	II	No changes in any limb.	II	No changes in any limb.	II	No changes in any limb.	II	No changes in any limb.	II
6	Thickening of both SL origin in both hindlimbs and hypoechogenic lesion in the medial SL branch of the RHL	IV	No changes in SL origins. Increasing echogenicity of the medial branch the SL	III	No changes in the SL origins. No lesions observed in the SL branch	II	No changes from the last evaluation	II	No changes from the last evaluation	II
7	Heterogenicity and severe hypoechogenicity of both branches of the SL of both FL and periligamentous inflammation.	V	There is an increase of the homogenecity of the SL branches. The Sl lateral branch of RF presents a very small central lesion still.	III	No lesions observed.	I	Ultrasound normal	I	Ultrasound normal	I

For key see Tables 1 and 2.

Table 4. Scores of the degree of lameness and flexion test of the patients of the study.*

Variable	Value			
	At the beginning	**before 2nd PRP injection**	**Before 3rd PRP injection**	**Last evaluation (2 months later)**
Degree of lameness	0.9a Range 0-2	0.64a Range 0-1.5	0.44a,b Range 0-1.5	0. b Range 0-1
Flexion test	1.85 a Range 0-3	1.14 b Range 0-2	0.71 b Range 0-2	0. b Range 0

* The horse number 7 was not included. a-c: Values with different Letters in a same row are statistically significant (P<0.05).

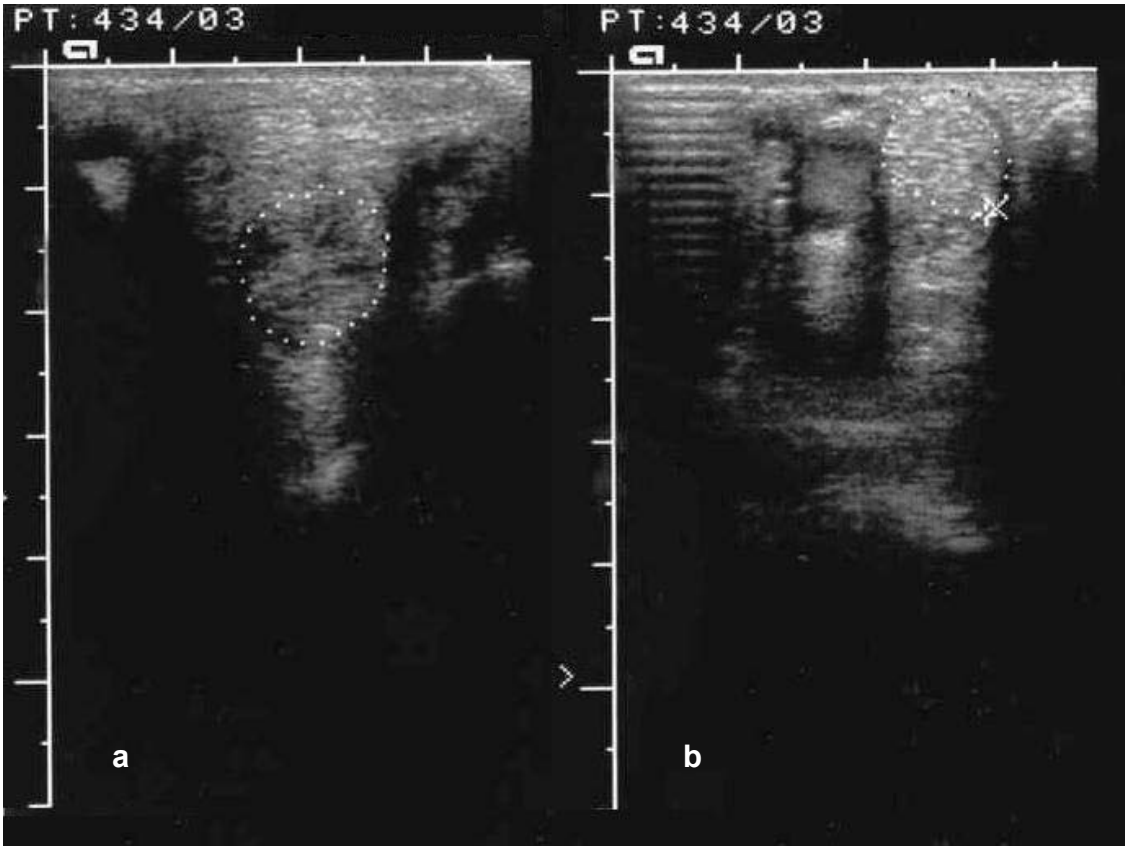

Figure 1. Ultrasound image of the lateral branch of the suspensory ligament of the right forelimb of an Arabian 14 year old male horse (patient Number 7), with chronic bilateral forelimb desmitis of the branches of the suspensory ligament. Note the diffuse hypoechogenecity, and the loss of the fiber pattern orientation before PRP treatment **(a).** The same structure three weeks after of the first PRP injection **(b).**

Correspondence to:

Marta Prades, DVM, PhD, Dip ACVS, Dip ECVS

Departament de Medicina i Cirurgia Animals

Universitat Autonoma de Barcelona.

Edifici V. 08193 Cerdanyola, Barcelona, Spain.

Phone: +34935812006

Fax: +34935813269

e-mail: Marta.Prades@uab.es