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THE EFFECT OF PLATELET-RICH PLASMA IN THE TREATMENT OF TENDONITIS AND DESMITIS IN HORSES: REPORT OF EIGHT CLINICAL CASES

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ABSTRACT

This study aimed to evaluate the clinical effect of autologous PRP, obtained by manual method with double centrifugation, and applied by intralesional ultrasound-guided injection in horses's tendons and ligaments. Eight horses were included in this study: four horses with tendonitis and four horses with suspensory ligament desmitis. Tendinitis and desmitis were diagnosed following clinical and ultrasonographic evaluation. Three applications of PRP were performed every 15 days. Platelet count of the whole blood used for preparation of PRP from the horses with tendonitis ranged from 120.000 to 180.000/µl (144.250 ± 25.539/µl) and PRP count ranged from 406.000 to 720.000/µl (512.250 ± 144.965/µl). In horses with desmitis, the platelet count in the whole blood used for autologous PRP preparation ranged from 127.000 to 158.000/µl (140.250 ± 13.149/µl) and PRP count ranged from 513.000 to 713.000/µl (566.500 ± 97.722/µl). No correlation was found between platelet count in the PRP and the healing time of injuries. Clinical evaluation and ultrasonographic images used to monitor healing showed that intralesional PRP injections followed by a program of gradually increasing exercise resulted in horses returning to their previous athletic activities without recurrence of lesions.

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INTRODUCTION

The tendon is a thick band of fibrous connective tissue that acts as a link connecting muscle to bone that have a specific fiber arrangement, reflecting the mechanical demands of this tissue (McIlwraith, 2002). The suspensory ligament(SL) onsist of striated muscle, loose areolar connective tissue, onnective tissue fibers, blood vessels, and nerves (Hauser *et al.,* 1984).

The SL is typically described as consisting of three sections: the proximal region, body and branches. All three regions can be injured in any horse breed resulting in decreased performance and early retirement (Kasashima *et al.,* 2004; Dabareiner *et al.,* 2005). Suspensory ligament desmitis is a common injury in thletic horses. When affecting the proximal region, at the ligament insertion on the palmar/plantar aspect of the cannon bone, the inflammation of the SL predisposes to avulsion fractures in the proximal-palmar region of the third metacarpal bone (Hill *et al.,* 2001). The desmitis normal healing requires a long convalescent period, ranging from three to nine months (Dyson and Genovese, 2003), many times with a less to guarded prognosis.

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Platelet-rich plasma (PRP), derived from whole blood, is a promising therapy that has also been used for treatment of tendonitis (Maia *et al.,* 2009) and desmitis (Schnabel *et al.,* 2007; Waselau *et al.,* 2008; Romagnoli *et al.,* 2015) in horses. The increasing interest in PRP treatment resides on the fact that PRP contains various growth factors that are important for restoration of tissue architecture, because of its mitogenic, chemotactic and neovascular action and its higher deposition of extracellular matrix. The objectives of this paper is describe a series of cases of tendonitis in flexors tendons and desmitis in ligament suspensory in proximal and distal region in horses. Evaluate the possible beneficial effect and the clinical safety of autologous PRP, obtained using a manual method with double centrifugation. Additionally, evaluation the treatment success, recurrence rates, and possible return to activities.

MATERIALS AND METHODS

Committee on Animal Research and Ethics approval for this project was obtained from Federal University of Santa Maria (protocol 110/2013).

Case selection criteria

Eight adult horses were included in this study (table 1): two horses with injury of the superficial digital flexor tendon (SDFT), two in the deep digital flexor tendon (DDFT), one with forelimb proximal SL desmids, one with hindlimb SL desmids in the medial and lateral branches, and two horses with forelimb SL desmids of the medial branches. The tendonitis and desmids cases were diagnosed after clinical and ultrasonographic evaluation when referred to the Veterinary Teaching Hospital at the Federal University of Santa Maria (UFSM), Brazil. The inclusion criteria in this study were: lameness grade 1 to 4/5 (AAEP, 1991) in the forelimbs or hind limbs; ultrasound identification of echogenicity, architecture, size and shape alterations in the flexor tendons or SL; and successful diagnostic anesthesia of the palmar/plantar region of the third metacarpal / metatarsal bone, if necessary to diagnose these injuries. Animals with sub acute lesions with a maximum of 15 days of evolution.

Clinical and ultrasound evaluations

The clinical evaluation was performed by lameness grade the presence of swelling, increased local temperature and pain were assessed by palpation. Ultrasound scans (US) were made with a portable Pie Medical unit equipped with a 7.5-MHz linear probe to confirm tendonitis or desmitis. The hypoechogenicity and loss of collagen fiber parallelism are considered abnormalities of these structures (Genovese *et al.,* 1986). As described by (Romagnoli *et al.,* 2015), a complete examination was performed doing transverse and longitudinal scans after routine clipping, and careful comparison with the healthy contralateral limb was carried out. Lesions were measured by tracing the circumference of each core lesion on transverse scans. Core lesion cross-sectional area was calculated as a percentage of the total cross-sectional area of the flexors tendon (FT) or SL. Measurement of the damaged area was calculated by measurement of the total lesion area divided by the total area of the tendon/ligament multiplied by 100 (expressed in %). Lesions also were graded from 1 to 3 using the modified score proposed by (Genovese *et al.,* 1990), where 1 = core lesion of ≤ 25%, 2 = core lesion between 50 to 75%, and 3 = core lesion of ≥ 75%.

Criteria success of treatment

During the period of application with PRP, the veterinarian monitored the animals daily. After this period, the animals were evaluated every two months until the lesions were completely healed. The success of the treatment was evaluated by clinical examination, healing time evaluated by ultrasound imaging, rates of lesion recurrence and return to previous sports activities. Ultrasound evaluations were performed to determine the lesion score and progression of tendon and ligament injuries, establishing the time for healing to occur, which was characterized by a normal echogenecity and complete alignment/parallelism of collagen fibers.

Obtaining platelet-rich plasma

The method used to obtain PRP was previously described by (Pereira *et al.,* 2013). For this purpose, 450ml of blood were collected from each animal by puncturing the external jugular vein using a commercial blood-transfusion bag containing citrate-phosphate-dextrose solution with adenine as an anticoagulant (CPDA-1; 63mL for 450ml of blood). From each collection, PRP was obtained from 100ml of whole blood aspirated from the blood bag and distributed in three Falcon type polypropylene 50ml-tubes (30ml of blood in one tube and 35ml in the other two). Tubes were centrifuged the at 224g during 10 minutes to separate the plasma from the mist zone (phlogistic layer containing leukocytes) and red blood cells. The supernatant containing the platelet-rich plasma was transferred to a second Falcon tube and again centrifuged at 440g for 10 minutes. After the second centrifugation, the plasma supernatant was discarded, and only 10 ml of PRP were preserved, representing 10% of the initial total blood volume. The platelet count was performed by the manual method. For each blood sample and PRP was diluted and homogenized in 1% liquid with ammonium oxalate. The counting was performed in a Neuberger chamber, using a binocular optical microscope with a magnification of 400 x. The whole protocol of manually processing PRP was done in a laminar flow cabinet to prevent contamination.

Protocol for PRP treatments

The animals diagnosed with tendonitis/desmitis were treated with a series of three PRP applications, 15 days apart, as recommended by (Carmona, 2006), except horse 1 and 2 that received only one application (Table 2). Before each treatment, the area to be injected was clipped and aseptically prepared using iodine povidone and 70% alcohol. All horses were sedated with an association of 10% xylazine ($1mgkg^{-1}$) and buthorfanol tartarate ($0.02mgkg^{-1}$) intravenously. A 7.5 MHz linear ultrasound probe was covered with a sterile glove to perform the ultrasound-guided intralesional PRP injections using 21G x ¾" needles (Romagnoli *et al.,* 2015). The PRP volume distributed in several points of injection over the lesion was calculated in accordance with the extent of the lesions ranging from 1 to 2.5ml total volume injected (Maia *et al.,* 2009).

Exercise protocol

The animals were submitte to a gradually increasing exercise program that began five days after the first PRP application (Maia *et al.,* 2009). Exercise intensity or duration was increase

based on the healing progress assessed through ultrasound. The exercise protocol was performed according to previously described by (McIlwraith, 2002) at the farms where the horses came from.

Statistical analysis

Descriptive statistical analysis of the behavior of the variables was performed for: the lesion scores, platelet number of blood, platelet number of PRP and healing time. Platelet number of PRP and healing time were transformed in a log normal distribution (x + 1) and tested for normality using the Kolmogorov-Smirnov test (p = 0.15). Pearson correlation was used to evaluate PRP platelet count and healing time (P<0.05).

RESULTS

During period of the study the eight horses treated with PRP and monitored no showed increase in lameness, local reactions or infections at the application sites.

No correlation was observed between injury scores and healing time (P>0.05). None of horses treated with PRP showed any recurrence of lesions (tendinitis and desmitis). The platelet counts in the whole blood before preparation of PRP of the tendonitis group ranged from 120.000 to 180.000/µl (144.250 ± 25.539/µl) and PRP platelet count ranged from 406.000 to 720.000/µl (512.250 ± 144.965/µl) (Table 2).

In horses with desmitis, the platelet counts in the whole blood before PRP preparation ranged from 127.000 to 158.000/µl (140.250 ± 13.149/µl) and PRP platelet count ranged from 513.000 to 713.000/µl (566.500 ± 97.722/µl) (Table 2). There was no correlation between the number of platelets in the PRP and the healing time in both tendonitis and desmitis cases, with a 95% confidence interval (P>0.05). Healing time of tendonitis or suspensory ligament desmitis was not influenced by the platelet count in the manually processed PRP. Of the eight (100%) treated animals, seven (87.5%) returned their previous activities, one mare was destined to reproduction, at the option of the owner.

Table 1. Description of the animals included in the work.

Animals	Breed	Age	Modality	Injuries	Affected limb
01	Criollo	9 years	Reining	SDFT	LF
02	Brazilian Warmblood	9 years	Jumping	DDFT	RF
03	Thoroughbred	9 years	Jumping	SDFT	RF
04	Criollo	5 years	Reining	DDFT	RF
05	Criollo	5 years	Reining	PSLD	LF
06	Criollo	3 years	Reining	BSLD	RH
07	Thoroughbred	3 years	Racing	BSLD	LF
08	Thoroughbred	3 years	Racing	BSLD	LF

SDFT: superficial digital flexor tendonitis; DDFT: deep digital flexor tendonitis; PSLD: proximal suspensory ligament desmitis; BSLD: branch suspensory ligament desmitis; LF: left forelimb; RF: right forelimb; RH: right hindlimb.

Table 2. Descriptive statistics in clinical cases of tendonitis and desmitis treated with PRP

Horse	Lesion score	# PRP applications	Platelets/µl whole blood	Platelets/µl PRP	TRT (days)
Tendonitis					
01	1	1	180.000	720.000	360
02	3	1	120.000	420.000	210
03	2	3	142.000	503.000	300
04	2	3	135.000	406.000	180
Minimum value	2	-	120.000	406.000	180
Maximum value	3	-	180.000	720.000	360
Medium value	2.3	-	144.250	512.250	262
SD	0.57	-	25.539	144.965	82
Average + 1 SD	2.87	-	169.789	657.215	344
Average – 1 SD	1.73	-	118.711	367.285	180
Desmitis					
05	3	3	141.000	713.000	180
06	2	3	158.000	513.000	120
07	1	3	135.000	520.000	120
08	3	3	127.000	520.000	60
Minimum value	1	-	127.000	513.000	60
Maximum value	3	-	158.000	713.000	180
Medium value	2.6	-	140.250	566.500	120
SD	0.57	-	13.149	97.722	48
Average + 1 SD	3.17	-	153.399	664.222	168
Average – 1 SD	2	-	127.101	468.778	72

PRP: platelet-rich plasma; SD: standard deviation; TRT: treatment recovery time

There was no statistical difference when the lameness grade of the animals evaluated (P>0.05). Horses diagnosed with tendonitis in the first assessment at the clinic had an average injury score of 2.3 (± 0.57) and average healing time of 262 days (± 82). Horses diagnosed with desmitis had a mean 2.6 injury score (± 0.57) and average healing time of 120 days (± 42).

DISCUSSION

This paper described the diagnostic and clinical outcome of tendon and ligament disorders in eight clinical cases treated with local PRP injection, followed by a gradual exercise protocol. The slow healing capacity of tendons and, in most cases, the resulting formation of mechanically inferior

extracellular matrix occur, probably, due to the fact that the tendon is a minimally vascularized tissue, presents cells that exhibit reduced mitotic activity and fewer progenitor cells in the tissue (Schnabel *et al.,* 2007). It is reasonable to believe that important differences might exist when comparing clinical with experimentally induced tendonitis/desmitis. More recently, (Guercio *et al.,* 2015) in an experimental study, used PRP associated with stem cells to repair lesions in the SDFT of nine horses. These authors observed that horses returned to exercise 6 months after treatment. The authors believe that 180 days until complete healing is achieved seems more realistic in a clinical scenario. The Criollo horses of our study, with SL desmitis showed similar results as far as average time (120 ± 48 days) of complete lesion healing compared to Thoroughbreds according to the cases published by (Waselau *et al.,* 2008). There are studies about responce of intralesional injection of PRP in twenty sport horses with proximal SL desmitis and supports the PRP treatment, observing a good to excellent prognosis for returning to sport activity (12-24 weeks) (Romagnoli *et al.,* 2015). PRP is a promising therapy modality that has been widely used in veterinary medicine for the treatment of bone and soft tissue injuries (Carmona, 2006). It is considered the low cost and easiness to obtain the product through different centrifugation protocols (Vendruscolo *et al.,* 2012). The manual protocol to prepare the PRP used in the present study was previsouly evaluated by (Pereira *et al.,* 2013) and allowed the gaining of high platelet counts (539.375 ±118067/µl). According to (Anitua *et al.,* 2004), concentrations above 300.000 platelets/mL are enough for a true PRP preparation. An in vitro study on segments of the SDFT of horses was conducted by (Schnabel *et al.,* 2007). Those authors showed an anabolic PRP effect on the synthesis of the tendinous matrix using an average count of 395.000 platelets/µl.

PRP treatment is considered one of the cell therapy modalities because it promotes a decrease in tendonitis recurrence rates due to a stimulus mediated by growth factors that enhances collagen fiber extracellular matrix deposition (Maia *et al.,* 2009; Mishra *et al.,* 2009). This and an adequate execution of the exercise protocol post-aplication could be possible explanations for the absence of recurrence in our PRP-treated horses (Romagnoli *et al.,* 2015). Controlled exercise is paramount for tendon and ligament healing because it favors collagen type-I formation in a more organized and homogenous way, avoiding adhesion formation in the peritendineous or ligament tissues and recurrence of lesions. Recurrence of lesions is the most likely complication once tendon and ligament injuries have healed and they probably occur due to inadequate deposition of extracellular matrix or inadequate management of the exercise program (Sharma *et al.,* 2005; Smith, 2011). We believe that treatment with PRP associated with controlled exercise was beneficial for no rates of recidives and no adhesion formation in peritendineous or ligament tissues. A limitation of the present study was that the clinical cases presented were not matched by control horses and the recommended exercise protocol was monitored by owners and trainers.

Conclusion

Intralesional PRP injections followed by a program of gradually increasing exercise allowed horses to return to their previous athletic activities without recurrence of lesions, independent of injury score, in the flexors tendons and suspensory ligaments.

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