Research in Veterinary Science 126 (2019) 38–44

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Prospective randomized comparison of platelet rich plasma to extracorporeal shockwave therapy for treatment of proximal suspensory pain in western performance horses

Kelly Giunta[a], Josh R. Donnell[b], Alan D. Donnell[b], David D. Frisbie[c,*,1]

[a] Blue Ridge Equine Clinic, 4510 Mockernut Lane, Earlysville, VA 22936, USA
[b] La Mesa Equine Lameness Center, 8386 FM 455E, Pilot Point, TX 76258, USA
[c] Colorado State University, Equine Orthopaedic Research Center, 300 W Drake Road, Fort Collins, CO 80523, USA

ARTICLE INFO

Keywords:
Proximal suspensory desmitis
Platelet rich plasma
Autologous conditioned plasma
Extracorporeal shockwave therapy
Western performance horse

ABSTRACT

Proximal suspensory desmitis (PSD) is a frequent cause of lameness in the performance horse. Extracorporeal shockwave therapy (EST) and platelet rich plasma (PRP) have become common treatments for PSD yet clinical data on efficacy is limited. The main objective of this randomized, prospective clinical study was to compare long-term effectiveness of EST and PRP in Western performance horses. One hundred horses with lameness localized to the proximal suspensory ligament received treatment with either PRP or EST following baseline ultrasonographic evaluation. A veterinarian and agent evaluated the horses for lameness independently four days following the first treatment and long-term follow up was obtained from the agent at six and twelve months. Four days post treatment, horses treated with EST had significantly greater lameness improvement compared to PRP. At one-year horses with less severe baseline ultrasound changes (grades 0–1) appeared to respond better (degree of lameness) with EST treatment whereas horses with more severe ultrasound changes (grade 2) responded better to PRP. Horses with baseline lameness graded 1 or 2 were 5.1 times more likely to be back in work at 1 year compared to those presenting with grade 3 or 4. EST treatment was associated with going back to work 3.8 times more at one year compared to PRP independent of baseline ultrasound score. Both PRP and EST can be expected to yield favorable therapeutic responses in Western performance horses with lameness localized to the proximal suspensory region. Baseline ultrasound may guide treatment selection.

1. Introduction

Proximal suspensory desmitis (PSD) is a common cause of lameness in the horse, often resulting in poor performance and even decreased value of the affected animal (Dyson, 1994, 2007). PSD is typically diagnosed through clinical signs of pain including sensitivity to direct palpation, positive response to flexion, and lameness, which improves post regional anesthesia (Dyson and Genovese, 2011). Changes in size, shape, and/or echogenicity of the ligament upon ultrasonographic examination may confirm the diagnosis, but ultrasonographic changes may not always correlate with histologic findings, making the syndrome challenging to diagnose beyond lameness localization (Dyson et al., 2017; Bischofberger et al., 2006).

Proximal suspensory desmitis has a guarded prognosis due to a high recurrence of lameness after returning to work, particularly when a hindlimb is involved (Dyson and Genovese, 2011). Previous studies looked at English sport horses and found those diagnosed with acute forelimb PSD had a 90% return to soundness after conservative treatment (three months stall rest and controlled exercise); however, chronic forelimb suspensory desmitis of three months duration has a less favorable prognosis, with only 53% of 20 horses returning to full work at six months in one study (Dyson, 2007). A compartment-like syndrome and subsequent neuritis is thought to play a role in chronic hindlimb PSD pain, but much of the exact pathogenesis of the disease remains unknown (Dyson, 1994; Dyson, 2007; Dyson et al., 2017). In a study of English sport horses with hindlimb PSD that presented with lameness for greater than three weeks duration, only 4 out of 42 horses returned to work without recurrent lameness following conservative treatment (Dyson, 1994). Extracorporeal shockwave therapy (EST) has been shown to improve the outcome of hindlimb PSD in several studies

* Corresponding author at: Colorado State University, Translational Medicine Institute, 2350 Gillette Drive, Fort Collins, CO 80523, USA.
E-mail addresses: giunta@blueridgeequine.com (K. Giunta), josh@equine-sportsmedicine.com (J.R. Donnell), alan@equine-sportsmedicine.com (A.D. Donnell), David.frisbie@colostate.edu (D.D. Frisbie).
[1] Current address: 2350 Gillette Drive, Fort Collins, CO 80523, USA.

https://doi.org/10.1016/j.rvsc.2019.07.020
Received 2 February 2019; Received in revised form 17 July 2019; Accepted 31 July 2019
0034-5288/ Published by Elsevier Ltd.

(Crowe et al., 2004; Lischer et al., 2006). Out of 52 horses treated with focused electrohydraulic shockwave therapy, 40.9% of hindlimb PSD cases were reported to be back in work at six months (Lischer et al., 2006). These numbers declined to 55.9% and 18.2%, respectively, at one year after diagnosis due to recurrent lameness (Lischer et al., 2006). Proximal suspensory desmitis may also present as poor performance in some cases where obvious lameness is not detectable (Dyson, 2007).

Information regarding the prevalence and prognosis of PSD specifically in the Western performance horse is sparse. Proximal suspensory desmitis was reported to be more prevalent in hunter, jumper, dressage, and racehorses according to a survey of equine practitioners. Western horses were included in the survey but ranked PSD less common than other lameness problems (Cowles, 2000). In a recent study of 2500 Western performance horses, the proximal metatarsus/distal tarsus was the second most common anatomic region of pain, compromising 16% of cases investigated with diagnostic analgesia (Johnson et al., 2017). Similar to other disciplines, it remains a frustrating source of lameness and poor performance.

While a frequently acknowledged region of pain, there is limited evidence supporting treatment modalities. Many treatments have been prescribed for PSD, including box stall rest with controlled exercise, intralesional injections of various therapeutics and EST (Cowles, 2000; Crowe et al., 2004; Dyson, 2007; Lischer et al., 2006). More recently EST and intralesional regenerative therapies, such as platelet rich plasma (PRP) and stem cell therapy, have emerged as the treatments of choice by many practitioners with limited published supporting evidence. Extracorporeal shockwave therapy (EST) is generally accepted as a safe, noninvasive treatment which may be performed stall side. There are several studies that have shown improvement in outcome of horses with PSD (Boening et al., 2000; Crowe et al., 2004; Lischer et al., 2006). It should be noted that there are other studies on tendon and ligaments that have not found any effect of EST (Waguespack et al., 2011; Kersch et al., 2006). EST works by producing acoustic pressure waves that can be focally directed at an area of pain or injury. Although the definitive mechanisms of action are not fully known, several studies have shown EST can promote pro-inflammatory and catabolic processes, which aid in healing (Maier et al., 2003; McClure and Weinberger, 2003).

Platelet rich plasma is a preparation commonly made from autologous whole blood that concentrates platelets and growth factors. Several studies utilizing various PRP products have shown promising results in tendon and ligament repair (Bosch et al., 2009; Rindermann et al., 2010; Romagnoli et al., 2015; Waselau et al., 2008). There are many commercially available kits (50 different kits based on the authors' last count) that enable equine veterinarians to easily produce autologous PRP. The aim of this study is to compare the use of EST versus PRP for clinical improvement in lameness previously localized to the proximal suspensory region.

2. Materials and methods

2.1. Study design

The study was designed as a two center, randomized, prospective clinical trial.

2.2. Case selection and enrollment

Horses were presented to Equine Sports Medicine, LLC or La Mesa Equine Lameness Center, PC (8386 FM 455 East, Pilot Point TX 76258) for lameness. Degree of baseline lameness was assigned using the American Associate of Equine Practitioner (AAEP) scale (0 to 5). In parallel, but independently, at initial presentation the agent for the horse was asked about the chronicity of the lameness and completed an evaluation form using a visual analog scale (VAS) (0 − 10) to assign a lameness score where a higher numeric response represented a greater degree of pain. Chronic lameness was characterized at clinical signs persisting for more than three months in comparison to acute lameness, where the lameness was noted for less than three months. Inclusion criteria required that clinical lameness be localized to the proximal metacarpal/distal tarsal region. Diagnostic regional analgesia of the proximal metacarpal/metatarsal region was performed in standard fashion by direct infiltration of the proximal suspensory ligament with 5 to 8 mLs of 2% mepivicaine (Carbocaine®, Pfizer Inc., New York, NY 10017). Horses were evaluated for improvement of equal to or > 70% at ten minutes following injection. Horses returned the following day for a baseline ultrasound exam of the proximal suspensory, which was graded using an ordinal scale (Table 1).

Table 1
Grading scale used to describe ultrasound lesions of the proximal suspensory ligament.

Grade	Description of ultrasound findings
0	No detectable lesion
1	Slightly more hypoechoic than normal, mild fiber pattern disruption
2	Approximately half echogenic and half anechoic, moderate fiber disruption
3	Mostly anechoic, moderate to severe fiber disruption
4	Totally anechoic, complete fiber disruption

2.3. Treatment assignment and application

Horses were randomly assigned to receive either EST or PRP, with treatment designations determined by random assignment. A veterinarian different than the one who completed the initial evaluation administered the treatment. Extracorporeal shockwave therapy consisted of 800 pulses with a high energy, focused shockwave unit (VersaTron®, Pulse Veterinary Technologies, Alpharetta, GA 30009) at energy level E6 with a 20 mm probe. A double-syringe technique was used to prepare an autologous PRP using a standardized protocol supplied by the manufacturer (Arthrex, Inc. Naples FL, 34108). Administration of PRP was performed using sterile technique with a 20–22 gauge 1.5-inch needle and 6 mL syringe. If a discrete lesion was defined with ultrasound PRP was administered using ultrasound guidance into the lesion and if a discrete lesion was not identified on ultrasound administered by direct infiltration of proximal suspensory ligament region, at a volume deemed appropriate by the clinician (3 to 6 mL), was performed.

2.4. Assessment of improvement – short term outcome

Short term outcome was assessed at four days. The same veterinarian that performed the initial evaluation (unaware of treatment administered) on the horse reported an AAEP lameness score and an estimated percentage of improvement from baseline (0–100%). The agent, who was also blinded to the initial treatment, completed an evaluation form identifying improvement using the VAS scale and a percentage of improvement from the baseline lameness. Horses were recommended to receive two additional treatments, at the owner's expense, at one-week intervals for a total of three treatments.

2.5. Assessment of improvement – long term outcome

Moderate (six month) and long (one year) term outcomes were collected by telephone or email interview with the agent. At both these time intervals the agent was again asked to grade lameness using the same described VAS scale. For the purpose of data analysis horses were considered sound if they were given a score of less than or equal to two. These numbers were chosen based on the authors' experience that some degree of a symmetry or ‘unsoundness’ is routinely encountered in performance horses. Agents were also asked to score the level of work

using a VAS scale where one equaled stall rest and ten equaled maximum training level. Horses were considered back to work for purposes of analysis if they were given a score greater than or equal to eight. Horses were considered "rested" if they had been stall rested for a period greater than one month without training exercise. Agents were also queried about concurrent therapies the horse received including bandaging, topical medications (DMSO, sweat, poultice or diclofenac), nonsteroidal anti-inflammatory medications, steroid injections into the proximal suspensory ligament or any additional treatments throughout the follow up period. These concurrent therapies were recorded and analyzed as concurrent therapies but there were not sufficient numbers of each therapy to warrant individual comparisons. Horses were withdrawn from further analysis if they were assigned to a specific treatment group but were later given the opposite treatment; i.e., if a horse in the PRP group received EST.

2.6. Statistical methods

Improvement was calculated by subtracting the lameness score at each time point by the baseline lameness score. Statistical analysis was performed using SAS version 9.4. Proc Glimmix. Fixed effects considered were treatment (PRP or EST), limb treated (fore or hind), ultrasound grade and study period as well as their interactions. When supported by a significant F-test (type III fixed effects) individual comparisons were made using a least square mean comparison. The Least Square Mean (LS Mean) and standard error of the means (SEM) are reported. Statistical significance was considered alpha at < 0.05. A chi square test was used to ensure proportions were not significantly different in each of the treatment groups using breed, sex, and age outcomes. Improvement in lameness was correlated between veterinarian and agent assessments at the four-day follow-up interval using a Pearson's co-efficient. This provided insight on the strength of correlation at later time points when only the agent's assessment was available.

Predictive statistics were performed using Proc Logistic with a backwards model selection method and the inclusion alpha set at $P < .10$. The horse being back in work (either six months or one year) was the response variable considered. As previously noted, horses that were rated as greater than or equal to eight by the agent as being back to work were considered an event (i.e., back in work) all other horses (score of less than eight) were considered not in work. The baseline lameness; using the AAEP scale was also considered as a binomial outcome with horses being collapsed into one of two groups, in order to utilize a more robust analysis of the predictive statistics. The first group was horses with baseline lameness grade one or two (mild to moderate) and the second group was horses with lameness grade three or four (severe). Concurrent therapies and chronicity of lameness were analyzed using Fisher's exact test.

3. Results

3.1. Study demographics

Cases were collected over a four-year period (2010 − 2013). One hundred horses were enrolled in the study. Four horses were excluded from the short-term data analysis because two failed to complete the four-day follow-up and two failed to meet other study criteria. There were 96 horses used in the statistical analysis. The mean age was six years (range 2 to 22 years), which included 89 Quarter horses (42 PRP, 47 EST), 5 Arabians (3 PRP, 2 EST), 2 other breeds (2 PRP, 0 EST). There were 31 mares (17 PRP, 14 EST), 55 geldings (26 PRP, 29 EST), and 10 stallions (4 PRP, 6 EST). The PRP group included 47 horses, 19 with affected forelimbs and 28 with affected hindlimbs. The EST group included 49 horses, 14 with affected forelimbs and 35 with affected hindlimbs. There were no significant differences in the proportion of horses in the treatment groups based on a Chi squared test comparing breed, age, and sex.

3.2. Veterinary and agent correlation

Statistical analysis showed a significant ($P < .0001$) moderate to strong correlation (correlation coefficient = 0.72) between the agent and veterinary improvement scores at four days.

3.3. Short term follow-up

Both treatments were generally well tolerated by the horses. In the first three months of the clinical study, there were two out of ten horses in the PRP treatment group developed heat, swelling and increased lameness by 24 h post injection. These signs resolved after treatment with anti-inflammatories. The authors' found that eliminating the anticoagulant from the protocol decreased the incidence of this side effect, which was done for all subsequent PRP treatments.

Based on veterinary assessment four days post treatment, horses receiving EST showed a higher percentage of improvement in lameness 47.23 ± 5.61 over baseline compared to the percentage of improvement in horses treated with PRP, 32.48 ± 5.26($p = .06$). Agent percentage of improvement at four days mirrored the clinician response with horses treated with EST showing a 45.80 ± 8.98% improvement over baseline lameness compared to those treated with PRP, 34.77 ± 5.90. There appeared to be an association with a higher ultrasound grade (more severe changes) and less percentage of improvement in lameness at the four-day time interval, with horses graded 0 or 1 having the highest percentage of improvement over baseline, with a mean of 43.67 ± 5.21 and 52.14 ± 5.62 respectively (Table 2). There were seven horses with a grade 3 ultrasound score (3 EST, 4 PRP). One of those treated with EST showed marked improvement (80%) in lameness at the four day follow up while the others did not improve. There was not an association between limb (fore or hind) or degree of presenting lameness in the short-term outcome.

3.4. Long term follow up

There were 66 horses for which six-month follow up data was available. There were 17 horses that were selectively withdrawn from statistical analysis of the long-term follow up. Five horses were removed from the EST group for receiving PRP treatments and ten horses were removed from the PRP group for receiving EST treatments. One horse was euthanized due to colic at four months post treatment and one mare was retired for use as a broodmare. Two horses were treated

Table 2
Veterinarian scores for mean percent improvement in lameness at four days post treatment (Mean ± SEM). Data analyzed with ultrasound score as co-variant. See Table 1 for ultrasound grading scale. P value comparing improvement between horses that were treated with EST vs PRP. N = 95 horses.[a]

Ultrasound Score	All horses	EST	PRP	% Difference	N =	P value
0	43.67 ± 5.21	50.33 ± 7.87	37.0 ± 6.82	13.33 ± 10.41	36	0.20
1	52.14 ± 5.62	52.35 ± 7.39	51.92 ± 8.46	0.43 ± 11.23	30	0.97
2	41.13 ± 6.53	46.25 ± 8.80	36.0 ± 9.64	10.25 ± 13.05	22	0.43
3	22.50 ± 11.65	40.0 ± 17.60	5.0 ± 15.24	35.0 ± 23.28	7	0.69

[a] N = 95, one horse did not have an ultrasound score recorded.

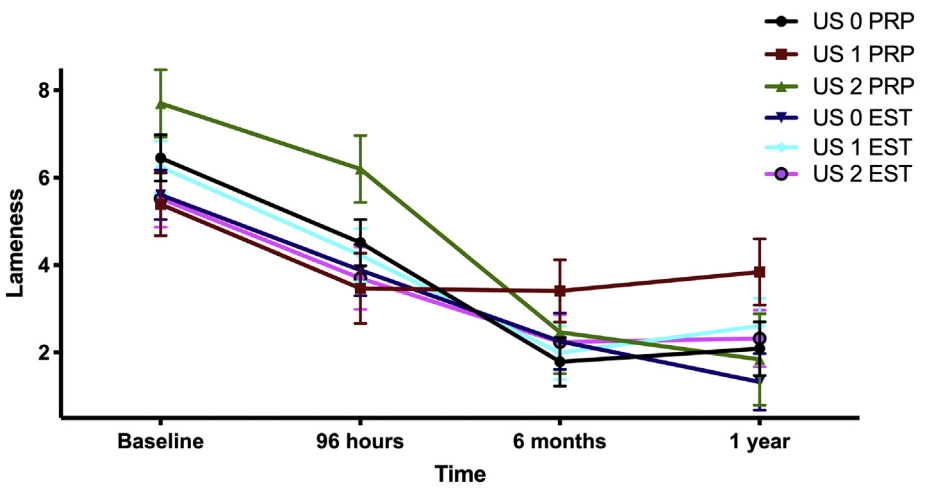

Fig. 1. Mean lameness scores by the study period. Each color represents a different ultrasound score (US) and treatment group (PRP or EST) with mean lameness scores at different time points in the study. Regardless of treatment group, the mean lameness score decreased significantly from the short term to long term followup time points.

with stem cells and one horse had surgery. Ten horses were lost to follow-up; either sold or their agents could not be reached. At the one-year time point, 58 agent responses were obtained.

There were 18 PRP horses and 21 EST horses that received all three treatments (39 horses total) and 1 PRP horse received an additional treatment. In the PRP treatment group, 15 horses only participated in the first treatment and 8 only received two treatments. In the EST group, 13 horses in the EST treatment group received only the first treatment and 13 received two treatments.

When the cohort group was analyzed as a whole, regardless of treatment, there was a significant level of mean improvement in agent lameness scores from the short term to long term follow up time points (Fig. 1). There were no significant changes in work level at six months between the two treatment groups using a similar analysis.

At one year follow up, horses demonstrating a grade 1 ultrasound lesion and treated with EST showed significantly greater improvement in lameness ($p = .01$) than those treated with PRP (Fig. 2). Amongst the EST group, there was not a significant difference in improvement based on ultrasound grade. Horses with an ultrasound score of 0 had similar responses to both treatments ($p = .90$). Horses with ultrasound score of 2 showed a trend for more improvement with PRP treatment when compared to those treated with EST ($p = .07$). When comparing the PRP treatment group, horses with an ultrasound grade 1 treated with PRP had significantly less improvement at the one-year time point than horses with an ultrasound grade 2 ($p = .001$) or ultrasound grade 0 ($p = .002$).

Chronic lameness was reported in 19 horses (12 PRP, 7 EST). Chronicity and level of work in a backward elimination procedure were not found to have a significant effect at six months ($p = .19$). In the same analysis at one year, horses reported to have lameness for greater than three months were significantly less likely to be back in work at one year ($p = .04$).

3.5. Predictive statistics

Two variables were retained in the model when considering if horses were back to work at six months, baseline lameness ($p = .05$) and number of treatments the horse received ($p = .02$). Horses that were graded 1 or 2 at baseline lameness evaluation were 3.3 times more likely to be back in work at six months when compared to those that presented with a grade 3 or 4. Further, horses that required or received one fewer treatment were 2.3 times more likely to be back in work at six months. Baseline lameness was a significant predictor ($p = .02$) of being back in work at one year, as was the number of treatments the horse received ($p = .02$), as well as the specific treatment ($p = .05$) the horse received. At one year, a horse that presented with a grade 1 or 2 lameness was 5.1 times more likely to be in work than a horse presenting with a grade 3 or 4 lameness. For each additional treatment received after the initial treatment, the horse was 2.5 times less likely to be back in work at one year. Horses that were randomized and received EST were 3.8 times more likely to be in work at one year compared to those receiving PRP. There was a trend for horses with forelimb injuries to be in work 3.3 times more often than horses suffering hind limb injuries ($p = .09$).

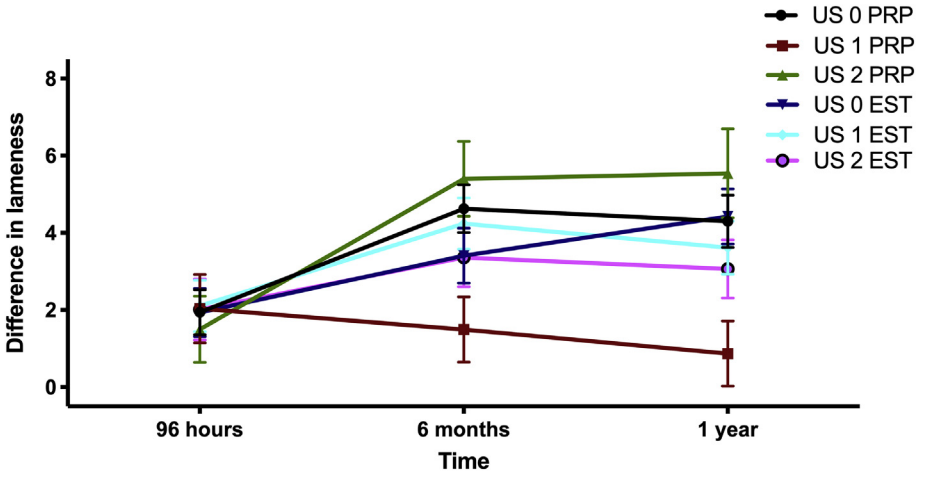

Fig. 2. Mean improvement by study period considering treatment (PRP or EST) and ultrasound (US) score. Graph represents the difference between agent lameness scores from baseline to four-day, six month and one year. Horses treated with PRP with an ultrasound grade of 1 showed significantly less improvement over time than horses with Grade 0 ($p = .002$) or grade 2 ($p = .001$) ultrasounds that were also treated with PRP.

Table 3
Percentage of horses that were considered in full work (defined by VAS score ≥ 8) and sound (defined by VAS score ≤ 2) at six months and one year. 79.49% (21/39) horses with hindlimb PSD and 78.95% (15/19) with forelimb PSD were sound at one year.

	PRP	EST	All Horses
6 month in work	59%	68%	64%
	17/29	25/37	42/66
6 month sound	72%	78%	75%
	21/29	28/36	49/65
1 year in work	67%	76%	72%
	16/24	26/34	42/58
1 year sound	75%	82%	79%
	18/24	28/34	46/58

4. Discussion

In the authors' hands, both EST and PRP were safe treatments. Only 2 out of 47 horses had mild transient reactions post PRP injection, where most received multiple treatments. The authors believe the reactions were caused by anti-coagulant (ACD). All subsequent PRP was made without anticoagulant and no other reactions occurred. There were no reported adverse reactions in the EST treatment group. Both treatments could potentially be used in an ambulatory setting, as they are easy to process and administer.

The results of this study suggest that both EST and PRP are appropriate treatment choices for Western performance horses with lameness localized to the proximal suspensory ligament. Both treatments produced a high percentage of successful long-term outcomes with 79% of all horses in the study reported to be sound and 72% reported to be in full work after one year (Table 3). Horses with a lower baseline ultrasound score or treated with EST showed more improvement in lameness at the short-term evaluation (Table 2). When the data was assessed based on ultrasound scores, some interesting observations were made. At the short term follow up interval, horses with higher ultrasound scores showed greater improvement in lameness following EST treatment when compared to the ACP group. At the six month and one year follow up intervals, there was significantly less difference in lameness in the PRP treatment group than the EST group for horses given an ultrasound score of one. However, horses with grade two ultrasound scores when treated with PRP showed a significant difference in lameness, indicating the degree of lameness had decreased from baseline (Fig. 2). Statistical analysis identified several positive predictors, which include lower baseline lameness scores, requiring fewer treatments, receiving EST and having an affected forelimb versus hindlimb.

Four days was chosen as the initial evaluation period to prevent horses being lost to veterinary follow up due to their traveling show schedule. Extracorporeal shockwave therapy has been reported to have a transient analgesic effect that occurs 24 h after treatment and may last up to three days (McClure and Weinberger, 2003). In a study where EST was used on horses with chronic forelimb lameness, force platform analysis showed average improvement in lameness lasted for two days post treatment (Dahlberg et al., 2006). In another study, horses with proximal suspensory disease that were treated with EST showed no significant improvement until seventy-two hours post treatment (Imboden et al., 2009). Given both of the tested therapies provided a significant improvement at 96 h and this time is longer than reported analgesia with EST, the authors believe other mechanisms are responsible for the symptom modifying effects. Other mechanisms that may result in improvement in lameness following EST could include anti-inflammatory cytokines and structural changes within the ligament. In a collagenase induced model of suspensory desmitis, EST has been shown to increase the pro-inflammatory cytokine transforming growth factor B-1 (TGFB-1) and enhance fibroblast synthesis of collagen, fibronectin, and glycosaminoglycan (Caminoto et al., 2005). Structural changes in the ligament may happen quickly, as it has been shown that EST produces a stimulating effect on metabolism (higher GAG and total protein) in as little as 3 h post treatment (Bosch et al., 2007). Thus, the authors attribute short term improvement in lameness following EST to factors other than analgesia.

In the present study, horses treated with EST showed more improvement overall when compared to the PRP treatment group. This trend was found in the short term and long term data, as well as the predictive statistics. Extracorporeal shockwave therapy has been shown to improve the outcome of hindlimb PSD in several studies (Crowe et al., 2004; Lischer et al., 2006). In a previous study, 52 horses with PSD used for English disciplines were treated with EST and 61.8% with affected forelimbs and 40.9% with affected hindlimbs were back in work at six months (Lischer et al., 2006). These numbers declined to 55.9% and 18.2%, respectively, at one year due to recurrent lameness (Lischer et al., 2006). In the present study 68% of horses (both fore and hindlimbs) treated with EST were back in work at six months and 76% at one year. Horses treated with EST were more likely to be in work at one year compared to those treated with PRP. When comparing the results of this study to what has previously been reported in English sport horses, Western performance horses may have a better prognosis for returning to work following treatment for PSD (Dyson, 2007).

Ultrasound grade at initial presentation may assist with treatment choice, as there was a significant change in lameness scores amongst the ultrasound grades and treatment groups (Fig. 2). Other studies have also shown that long term prognosis of PSD may be influenced by ultrasound findings (Crowe et al., 2004; Dyson, 2007, 1994; Dyson et al., 2017). In one study where horses with PSD were treated with radial pressure wave therapy, horses with severe ultrasound changes in the proximal suspensory ligament were less likely to be in work at six months than those with mild to moderate ultrasound changes, particularly in the forelimb where only 17% returned to full work (Crowe et al., 2004). Interestingly, Lischer et al. did not find a significant correlation with baseline ultrasound score in their study mentioned above. The results of the present study also suggest that baseline ultrasound grade may influence the long-term prognosis (failure to return to work), thus the authors have adapted their treatment protocols to recommend treating mild PSD with EST while reserving PRP for horses with more severe ultrasound findings. There may also be benefit to combining therapies as recent research has shown that EST applied to PRP in vitro can enhance the release of growth factors from platelets (Seabaugh et al., 2017).

A lower baseline lameness and receiving fewer treatments were identified as positive predictors of being back to work at six months and one year. The authors hypothesize that less severe injuries require fewer treatments to return to soundness. The second and third treatments were not financially covered by the study, thus there was little incentive to pursue additional treatments if the horse was not lame. While it was the number of treatments and not the specific treatment that was statistically significant, the authors must acknowledge that a protocol for dose and frequency of injection of PRP has not been established. In another study where PRP was injected twice in a two-week interval until the ultrasonographic lesion improved, 16 out of 20 (80%) horses were back in work without lameness in 12–24 weeks (Romagnoli et al., 2015). In the present study, 21/29 (72%) horses treated with PRP were reported sound and 17/29 (59%) were back in work by 6 months (24 weeks). In the present study, PRP was injected at one-week intervals for one to three treatments in order to be given at the same frequency as the EST group. More research is needed to determine the exact number and frequency of PRP treatments that produces optimal healing. Based on the authors experience, injections (two to three) at a one to two-week interval are currently recommended for PSD in the Western performance horse.

PRP may be variable in composition, as upwards of 50 different PRP kits are currently available, and biologic constituents can vary widely between kits, with a different profile of platelets, red blood cells, white

blood cells and growth factors. PRP has been shown to increase metabolic activity, neovascularization and advance maturation of repair tissue in surgically created tendon lesions (Bosch et al., 2010, 2011). In a comparative study of commercially available PRP products including Angel, ACP, EPET and GPS, ACP was found to have lower concentrations of TGF beta and PDGF and significantly fewer neutrophils than the other products. ACP has been shown to increase plates 1.5 times and result in a low white blood cell concentration (Hessel et al., 2015; Arthrex white paper). In vitro studies have shown promotion of normal collagen matrix and less inflammatory cytokines than other PRP products (Hessel et al., 2015). The PRP used in the present study was not characterized for each individual horse. A previous study that characterized the same product in seven horses found that platelet counts were on average 1.3-fold higher in ACP compared to whole blood, ranging from 160×10^9 platelets/L to 197×10^9 platelets/L and white blood cell count was 10.92-fold lower in ACP compared to whole blood, ranging from 0.44×10^9 cells/L to 1.58×10^9 cells/L (Rindermann et al., 2010). The authors are unaware if addition of the anti-coagulant affects the concentration of platelets in the study but hypothesize that if the blood is promptly spun down and injected there would be minimal differences. In our study, the majority of horses treated with ACP had successful long-term outcomes with 66.67% reported to be in full work at one year. Further research with direct comparisons of various PRP products is certainly needed.

Previous reports of horses in other disciplines have demonstrated PSD a forelimb has a more favorable prognosis than a hindlimb. While conservative treatment of acute forelimb PSD has been reported to have up to a 90% returned to soundness, while the prognosis for conservative treatment of hindlimb PSD is poor (Dyson, 1994, 2007; Dyson and Genovese, 2011). In one study, only 6 out of 42 horses were able to resume full work for one year without a reoccurrence of lameness (Dyson, 1994). Chronic forelimb PSD of three months duration has been shown to have a less favorable prognosis, with only 53% of 20 horses returning to full work at six months in one study (Dyson, 2007). In the current population of Western performance horses those with forelimb injuries are 3.3 times more likely to be back in work at one year compared to horses with hindlimb injuries. Horses reported to have chronic lameness were significantly less likely to be in work at one year ($p = .04$). Western performance horses show patterns similar to previous research on English sport horses, with forelimb suspensory injuries being more likely to return to work and chronic suspensory injuries less likely to return to work.

There are several limitations the authors wish to acknowledge in this study. The first is a lack of serial evaluations performed by a veterinarian; however, the strength of correlation between veterinary and agent improvement scores at four days provides the agent evaluations could act as a surrogate at later evaluations periods. Due to the nature of conducting a large clinical study with horses from all over the country, it was impossible to keep the agents blinded for the duration. The agents and veterinarians were both unaware of the initial treatment at the short term follow up. However, follow up care was often performed by another veterinarian so the agents became aware of the treatment performed and recommendations going forward. The authors are aware this may have resulted in some bias of the long term follow up data. Diagnostic blocking and incidence of concurrent proximal metatarsal and distal tarsal disease can occur. Thus, without advanced imaging such as MRI (Barrett et al., 2018) it is very likely in our population that some of the PSD horses had concurrent tarsal disease. This study does, however, represent standard field conditions and provide guidance to practitioners without access to advanced imaging. Ultrasound alone is not the most sensitive imaging method to detect pathology in the proximal suspensory ligament. Many changes are diagnosed with MRI that are not detected on ultrasound (Barrett et al., 2018). Also, the lack of serial ultrasounds is another limitation of this study. Despite these limitations, the authors found useful information about the prognosis of PSD in the Western performance horse and the success of both EST and PRP treatment.

5. Conclusions

To the authors' knowledge, this is the first and largest published study comparing EST to PRP for treatment of PSD in Western performance horses for which long term follow-up has been obtained. Degree of presenting lameness, number of treatments administered, having a forelimb vs hindlimb affected, and receiving EST were found to be significant predictors of being back in work by one year. Baseline ultrasound findings may be helpful to help establish a prognosis and treatment plan for Western performance horses with PSD. The authors conclude in their practice they use EST for cases demonstrating mild ultrasonographic change in comparison to those with severe lesions for which PRP therapy are used based on the results of this study.

Acknowledgements

This work was supported by Colorado State UniversityOrthopaedic Research Center Foundation Funds, Arthrex Inc., and Sanuwave Inc.

Declaration of Competing Interest

The industry sponsorship had no influence on data collection, analysis, interpretation or manuscript preparation. The authors' declare no conflicts of interest.

References

Barrett, M.F., Selberg, K.T., Johnson, S.A., Hersman, J., Frisbie, D.D., 2018. High field magnetic resonance imaging contributes to diagnosis of equine distal tarsus and proximal metatarsus lesions: 103 horses. Vet. Radiol. Ultrasound 59, 587–596.

Bischofberger, A.S., Konar, M., Ohlerth, S., Geyer, H., Lang, J., Ueltschi, G., Lischer, C.J., 2006. Magnetic resonance imaging, ultrasound and histology of the suspensory ligament origin: a comparative study of normal anatomy of Warmblood horses. Equine Vet. J. 38, 508–516.

Boening, K.J., Loffeld, S., Weitkamp, K., Matuschek, S., 2000. Radial extracorporeal shock wave therapy for chronic insertion desmopathy of the proximal suspensory ligament. Proc. Am. Assoc. Equine Pract. 46, 206–207.

Bosch, G., Lin, Y.L., van Schie, H.T., van De Lest, C.H., Barneveld, A., van Weeren, P.R., 2007. Effect of extracorporeal shock wave therapy on the biochemical composition and metabolic activity of tenocytes in normal tendinous structures in ponies. Equine Vet. J. 39 (3), 226–231.

Bosch, G, de Mos, M., van Binsbergen, R., van Schie, H.T., van de Lest, C.H., van Weeren, P.R., 2009 Apr. The effect of focused extracorporeal shock wave therapy on collagen matrix and gene expression in normal tendons and ligaments. Equine Vet J 41 (4), 335–341.

Bosch, G., van Schie, H.T., De Groot, M.W., Cadby, J.A., van de Lest, C.H., Barneveld, A., van Weeren, P.R., 2010. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: a placebo-controlled experimental study. J. Orthop. Res. 28, 211–217.

Bosch, G., Moleman, M., Barneveld, A., van Weeren, PR., van Schie, H.T., 2011. The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. Scand J Med Sci Sports 21 (4), 554–561. Epub 2010 Mar 10. 10.1111/j.1600-0838.2009.01070.x.

Caminoto, E., Alves, A., Amorim, R., Thomassian, A., Hussni, C., Nicoletti, J., 2005. Ultrastructural and immunocytochemical evaluation of the effects of extracorporeal shock wave treatment in the hind limbs of horses with experimentally induced suspensory ligament desmitis. Am. J. Vet. Res. 66, 892–896.

Cowles, R.R., 2000. Proximal suspensory desmitis a qualitative survey. Proc. Am. Assoc. Equine Pract. 46.

Crowe, OM, Dyson, S.J., Wright, I.M., Schramme, M.C., Smith, R.K., 2004 May. Treatment of chronic or recurrent proximal suspensory desmitis using radial pressure wave therapy in the horse. Equine Vet J 36 (4), 313–316.

Dahlberg, J.A., McClure, S.R., Evans, R.B., Reinertson, E.L., 2006. Force platform evaluation of lameness severity following extracorporeal shockwave therapy in horses with unilateral forelimb lameness. J. Am. Vet. Med. Assoc. 229, 100–103.

Dyson, S., 1994. Proximal suspensory desmitis in the hindlimb: 42 cases. Br. Vet. J. 150, 279–290.

Dyson, S., 2007. Diagnosis and management of common suspensory lesions in the forelimbs and hindlimbs of sport horses. Clin. Tech. Equine Pract. 6, 179–188.

Dyson, S.J., Genovese, R.L., 2011. The suspensory apparatus. In: Ross, M.W., Dyson, S.J. (Eds.), Diagnosis and Management of Lameness in the Horse, 2nd ed. Elsevier, St. Louis, pp. 738–760.

Dyson, S., Murray, R., Pinilla, M.J., 2017. Proximal suspensory desmopathy in hindlimbs: a correlative clinical, ultrasonographic, gross post mortem and histological study. Equine Vet. J. 49, 65–72.

Hessel, L.N., Bosch, G., Van Weeren, P.R., Ionita, J.C., 2015. Equine autologous platelet concentrates: a comparative study between different available systems. Equine Vet. J. 47, 319–325.
Imboden, I., Waldern, N.M., Wiestner, T., Lischer, C.J., Ueltschi, G., Weishaupt, M.A., 2009. Short term analgesic effect of extracorporeal shock wave therapy in horses with proximal palmar metacarpal/plantar metatarsal pain. Vet. J. 179, 50–59.
Johnson, S.A., Donnell, J.R., Donnell, A.D., Frisbie, D.D., 2017. Retrospective analysis of lameness exam in the Western performance horse. Proc. Am. Assoc. Equine Pract. 63, 213–214.
Kersch, K.D., McClure, S.R., VanSickle, D., Evans, R.B., 2006. The evaluation of extracorporeal shock wave therapy on collagenase induced superficial digital flexor tendonitis. Vet. Comp. Orthop.Traumatol. 19 (2), 99–105.
Lischer, C.J., Ringer, S.K., Schnewlin, M., Imboden, I., Furst, A., Stockli, M., Auer, J., 2006. Treatment of chronic proximal suspensory desmitis in horses using focused electrohydraulic shockwave therapy. Schweizer Archiv. Fur. Tierheilkunde 148, 561–568.
Maier, M., Averbeck, B., Milz, S., Refior, H.J., Schmitz, C., 2003. Substance P and prostaglandin E2 release after shockwave application to the rabbit femur. Clin. Orthop. Relat. Res. 406, 237–245.
McClure, S., Weinberger, T., 2003. Extracorporeal shockwave therapy: clinical applications and regulation. Clin. Tech. Equine Pract. 2, 358–367.
Rindermann, G., Cislakova, M., Arndt, G., Carstanien, B., 2010. Autologous conditioned plasma as therapy of tendon and ligament lesions in seven horses. J. Vet. Sci. 11 (2), 173–175 Jun.
Romagnoli, N., Rinnovati, R., Ricciardi, G., Lambertini, C., Spinella, G., Spadari, A., 2015. Clinical evaluation of intralesional injection of platelet-rich plasma for the treatment of proximal suspensory ligament desmitis in horses. J. Equine Vet. Sci. 35, 141–146.
Seabaugh, K.A., Thoresen, M., Giguere, S., 2017. Extracorporeal shockwave therapy increases growth factor release from equine platelet-rich plasma in vitro. Front. Vet. Sci. 4.
Waguespack, R.W., Burba, D.J., Hubert, J.D., Vidal, M.A., Lomax, L.G., Chirgwin, S.R., Lopez, M.J., 2011. Effects of extracorporeal shock wave therapy on desmitis of the accessory ligament of the deep digital flexor tendon in the horse. Vet. Surg. 40 (4), 450–456.
Waselau, M., Sutter, W.W., Genovese, R.L., Bertone, A.L., 2008. Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in standardbred racehorses. J. Am. Vet. Med. Assoc. 232, 1515–1520 (In Vitro Comparison of Autologous Conditioned Plasma (ACP) to a Buffy Coat-Based Platelet-Rich Plasma (PRP) Product. Arthrex White Paper).