Najeb *et al. Irish Veterinary Journal* (2025) 78:13
https://doi.org/10.1186/s13620-025-00299-y

Irish Veterinary Journal

RESEARCH Open Access

Clinical benefits of early-stage autologous conditioned serum and injectable platelet-rich fibrin on healing superficial digital flexor tendonitis in donkeys

Mahmoud Najeb[1], Alaa Samy[1], Awad Rizk[1], Esam Mosbah[1] and Gamal Karrouf[1*]

Abstract

Injectable platelet-rich fibrin (I-PRF) has been explored for its regenerative potential, but its application in tendon healing remains unstudied. Autologous conditioned serum (ACS), commonly used in joint therapies, inhibits interleukin 1-mediated inflammation, though its role in tendon healing is not well established. This study aimed to evaluate the clinical benefits of I-PRF alone and in combination with ACS in tendon healing. Twenty-three donkeys with forelimb superficial digital flexor (SDF) tendonitis were examined at admission (T0) and randomly assigned to PRF/ACS treated group ($n=10$), PRF group ($n=7$), or included as a control group ($n=6$). Among clinical parameters, tendon shape and weight-bearing scores at T150 were significantly improved ($P<0.001$) in the PRF/ACS group compared to the control group. Regardless of the tendon cross-sectional area (T-CSA), all other ultrasonographic parameters showed significant improvement in the PRF/ACS group compared to the control group. T-CSA increased throughout the study in PRF group but significantly decreased at T150 in control and PRF/ACS groups. The combination therapy had a significant improvement in fiber alignment score (FAS) and T-CSA compared to PRF group. Early lesion resolution in PRF/ACS at T90 and later in PRF at T150 suggests superior healing in PRF/ACS group. Persistent increase in T-CSA in the PRF group indicates ongoing healing, while its reduction in the PRF/ACS groups reflects more progressed healing stages and superior outcomes. Conversely, the presence of hyperechoic dots and misaligned tendon fibers in the control group suggests inferior healing outcomes. Intralesional I-PRF combined with ACS significantly enhanced clinical and ultrasonographic healing characteristics in donkeys with SDF tendonitis.

Keywords Injectable platelet-rich fibrin, Autologous conditioned serum, Superficial digital flexor tendon, Interleukin-1 receptor antagonist protein

*Correspondence:
Gamal Karrouf
drgamalkarrouf1966@gmail.com; gamalkarrouf@mans.edu.eg
[1]Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

This month's Peer Review is the first of a two-parter. Part two will be published next month.

Introduction

Tendon injuries remain a major concern in equine and are often career-ending. The prevalence of SDF tendonitis is particularly high among performance and racehorses, with reported incidence rates ranging from 11 to 43% [1]. These injuries are challenging to treat, as tendons heal through fibrotic scar rather than true regeneration. Once severely damaged, they rarely regain their original elasticity and functional capacity, leading to increasing reinjury rates of up to 80% with conservative treatment [2, 3].

Regenerative therapies, such as platelet concentrates and stem cell therapy, present promising alternatives to traditional treatments for SDF tendonitis [4]. Platelet concentrates are more widely used for managing tendon disorders [5]. Among platelet concentrates, platelet-rich plasma (PRP) has shown beneficial effects in experimental models [6, 7]. However, its efficacy in equine tendon healing remains debated. A systemic review involved eight studies reported that PRP improved structural integrity, reduced lameness, enhanced tissue organization, and led to better performance outcomes [8]. In contrast, a recent meta-analysis of fifteen studies found no definitive evidence that PRP significantly enhances tendon healing in horses [9].

Platelet-rich fibrin (PRF), a second-generation platelet derivative, offers several advantages over PRP. It can be easily prepared stall-side from autologous blood without the need for complex equipment or prolonged processing. PRF contains higher levels of various growth factors with a sustained release of up to 14 days, compared to just four days in PRP [10, 11]. Additionally, its fibrin matrix supports cellular migration, and it is free from chemicals that could interfere with therapeutic activity [12]. While PRF has not been specifically evaluated for treating equine SDF tendonitis, studies have reported varied outcomes in its use for treatment of other tendinopathies [13–16]. Injectable PRF (I-PRF) demonstrated promising regenerative potential in human dentistry [17]. To the authors' knowledge, this study is the first to assess the clinical application of I-PRF for the treatment of equine SDF tendonitis.

Despite their regenerative potential in tendon healing, their efficacy in equine tendons remains uncertain [9]. This inconsistency may be attributed to excessive and uncontrolled inflammation in equine tendons, which could hinder the expected regenerative effects of platelet-rich derivatives [18]. Effective inflammation control is essential for promoting tendon regeneration and minimizing excessive scar formation [19]. The early tendon repair phase is marked by inflammation and tissue proliferation, which, if uncontrolled, can accelerate tendon fiber degradation. Simultaneously, dysregulated collagen synthesis favors the deposition of weaker type III collagen over type I, leading to disorganized scar formation [20].

Autologous conditioned serum (ACS) is a promising treatment for tendon healing which are crucial for counteracting inflammation and promoting tissue repair, it enhances the concentration of key cytokines and growth factors compared to regular blood, with interleukin 1 receptor antagonist (IL-1Ra) being a significant component [21–23]. ACS specifically inhibits IL-1-mediated inflammation, reducing matrix degradation and pain, and preserving tissue integrity [24]. Although ACS products are commonly used in joint therapies, their application in tendon injuries remains debated among equine clinicians [5]. Horses with SDF tendinopathy treated with ACS showed increased type I collagen content compared to saline-treated tendons, suggesting a potential improvement in tissue quality and mechanical properties [25].

Since tendonitis involves both inflammation and tissue degeneration, a combined intralesional specific anti-inflammatory and regenerative approach may yield superior therapeutic outcomes. This study aims to evaluate the therapeutic efficacy of PRF, both alone and in combination with ACS, in SDF tendonitis. The authors hypothesize that ACS treatment will help regulate excessive inflammation, creating a more favorable environment for PRF mediated regeneration potential.

Materials and methods

Animals

The inclusion criteria for this study included adult donkeys with a history of unilateral forelimb SDF tendonitis with intact skin. Cases were eligible if clinical signs of inflammation had been reported 6–14 days before presentation. Enrollment was further restricted to donkeys whose owners consented to the study design and who had not received systemic medical treatment for this specific SDFT injury, nor intralesional tendon injections at any point in their lives. The study was conducted between 2021 and 2024, including a total of 23 donkeys (Equus asinus). The study was approved by the Medical Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (Approval Code: MU.ACUC. Ph.D.25.04.54). Affected limbs were randomly assigned to one of three groups: the control group ($n = 6$), the PRF-treated group ($n = 7$), or the PRF/ACS-treated group ($n = 10$). Animals underwent a thorough clinical and ultrasonographical evaluations on the admission day (T0) and on 7, 14, 30, 60, 90, and 150 days after treatment.

Clinical examination

A comprehensive clinical evaluation was semi quantitatively scored to assess lameness, pain response to pressure, and localized heat upon palpation (Table 1). Tendon

Table 1 Clinical assessment scores for tendon healing modified according to Carlier et al. and Reix [26, 27]

Score	Description
Heat	
0	Normal findings
1	Mild increase
2	Moderate increase
3	Severe increase
Pain	
0	Normal findings
1	Mild reaction
2	Moderate reaction
3	Severe reaction
Modified clinical score assessment for lameness	
0	Normal gait
1	Mild, intermittent lameness or difficult to observe, regardless of surface
2	Mild lameness, intermittent at a walk but consistently present under certain conditions (e.g., hard surface, weight-bearing)
3	Mildly abnormal gait and/or stiff walk
4	Reluctance to move when motivated/ sever lameness at a walk
5	No movement or lying down; limb barely touches the ground (minimal weight-bearing or resting limb in flexion posture)
Tendon shape upon palpation	
0	Smooth uniform normal thickening
1	Minimal irregularity or slight thickening
2	Notable irregularity
3	severe irregularity with a soft consistency
4	Hard nodular area
Intensifying weight bearing (static examination)	
0	Even weight distribution
1	Slight reduction in weight-bearing, slight preference for the opposite limb
2	Notable reduction in weight-bearing, notable preference for the opposite limb
3	Notable offloading of the limb

shape and intensifying weight-bearing ability under static condition were assessed at T0 and T150. Tendon shape upon palpation was graded as follows: 0 for smooth, uniform tendons with normal thickness; 1 for minimal irregularity and slight thickening; 2 for notable irregularity and thickening; and 3 for hard nodular areas. Intensifying weight-bearing ability under static conditions was evaluated by applying a load equivalent to 30% of the animal's body weight, with scores ranging from 0 for even weight distribution; 1 for slight preference for the opposite limb; 2 for Notable reduction in weight-bearing, notable preference for the opposite limb; and 3 for notable offloading of the affected limb. To ensure objectivity, all clinical evaluations were conducted blindly by two independent observers, and the average scores used for analysis.

Ultrasonographic evaluation

On the scheduled times, all tendons underwent B-mode ultrasonographic examination using a 10 MHz linear transducer (CHISON Digital Ultrasound, iVis 60 EXPERT VET; CHISON Medical imaging Co., Ltd, China) in both transverse and longitudinal orientations. Images were digitally stored and analyzed to assess the degree and progression of structural changes over time. Both fiber echogenicity (FES) and fiber alignment scores (FAS) were evaluated using a scoring method previously described by Carlier et al. [26].

Both tendon cross sectional area (T-CSA) and lesion cross-sectional area (L-CSA) at the maximum injured zone were quantitatively measured (mm^2) on transverse ultrasound images. Ultrasonographic examination was performed by MN, while the analysis of ultrasonograms was conducted by another examiner (A.S.), who was blinded to the individual treatment modality. Measurements were made three times and the mean was used for statistical analysis. The percentage of the tendon lesion (Lesion %) was calculated by the following equation [lesion % = (L-CSA/T-CSA) × 100]. To address the statistically significant differences in lesion percentage and T-CSA at baseline (T0), proportional changes in T-CSA (P.T-CSA) and lesion percentage (PL%) over time were calculated by standardizing the T0 value to 1 within each group (by dividing T0 by itself), and then dividing each subsequent time point by the corresponding T0 value [28].

Autogenous injectable PRF (I-PRF) preparation

Briefly, 4 ml of whole blood was collected in a sterile plastic plain tube and immediately centrifuged at 700 rpm for 3 min (RCF = 22×g) at a 45° rotator angle with radius of 40 mm, separating it into an upper I-PRF layer and a lower RBC layer [29]. The I-PRF was injected within 5 min before clotting.

Autologous conditioned serum (ACS) preparation

Under aseptic conditions, 10 ml of autologous blood was collected into an orthokine®vet irap 10 ml syringe system (Orthogen, Düsseldorf, Germany) and incubated at 37 °C for 6 h. It was then centrifuged at 4000 rpm for 10 min (RCF = 716×g), separating into an upper yellowish conditioned serum (ACS) layer and a lower RBC layer. The conditioned serum was aspirated and filtered through a 0.22 µm syringe-driven filter before use [25].

Therapy

Donkeys were sedated intravenously with acepromazine (0.05 mg/kg, Castran, 15 mg/mL) and xylazine HCl (1.1 mg/kg, Xyla-Ject, 20 mg/mL), administered 20 min apart, followed by butorphanol (0.05 mg/kg, Torbugesic 10 mg/ml). The medial and lateral palmar nerves were

anesthetized 2 cm distal to the carpal joints using 2 ml of 2% lidocaine after skin preparation. Tendons were treated under aseptic conditions according to their respective groups, with a fixed 3 ml inoculum administered in all groups. This volume consisted of normal saline in the control group, 1.5 ml of I-PRF and 1.5 ml of saline in the PRF group, and a combination of 1.5 ml I-PRF and 1.5 ml ACS in the PRF/ACS group. Intratendinous injections were performed using 21-gauge needles under ultrasound guidance, inserted laterally and perpendicular to the tendon's long axis. The inoculum was distributed at the site of maximal lesion size, as well as 0.5 cm proximal and 0.5 cm distal to that point. All groups received a systemic Flunixin meglumine (Flamicure 5%, Pharma Swede, Egypt) at a dose rate of 1.1 mg/kg for 5 successive days.

Follow-up and controlled exercise

After treatment, a pressure bandage was applied to the metacarpus for 24 h, consisting of a sterile non-adherent gauze layer, followed by cotton padding, and secured with a cohesive elastic wrap. All animals followed a gradually increasing exercise program as described by Bosch et al. [30]. Briefly, following a 6-day stall rest, the exercise program commenced one week post-treatment, starting with a 10-minute walk, with the duration increasing by 10 min every 3 weeks until the study's conclusion.

Statistical analysis

Data were analyzed using the Statistical Package for Social Science IBM SPSS software package (Armonk, NY: IBM Corp), version 28.0. The normality of the quantitative variables was determined using the Shapiro–Wilk test. Normally distributed variables, including age, body weight (BW), L-CSA, and T-CSA, were expressed as mean ± SD and assessed for normality using probability plots. A pairwise comparison of quantitative variables between different groups at different times was carried out using two way ANOVA and the subsequent Tukey's post hoc correction. To compare the T-CSA of the affected limb at T150 with that of the contralateral limb within the same group, an unpaired Student's t-test was used, whereas comparisons with that of T0 values of the affected limb were performed using a paired Student's t-test. To enable pairwise comparisons among lesion percentage and T-CSA, the T0 value (the value before treatment) was standardized to 1 within each group by dividing it by itself. Subsequently, changes in lesion percentage and T-CSA over time were calculated by dividing each value at later time points by the corresponding T0 value. Non-parametric data (pain, heat, lameness, tendon shape, and intensifying weight-carrying scores, FES, FAS) were expressed as median (minimum–maximum). A pairwise comparison of these non-parametric variables between different groups at the same time was carried out using the Kruskal-Wallis non-parametric ANOVA. Concerning pain, heat, lameness, FES, and FAS the Friedman multiple comparison test was used to analyze the effects of different times within the same group, while the Wilcoxon test was used to identify the statistical difference between T0 and T150 among the tendon shape and intensifying weight-carrying scores within the same group. Results were considered statistically significant at $P < 0.05$. All graphs were performed using Graph Pad Prism version (8.4.3., Software Inc., La Jolla, CA).

Results

Description and history of donkeys

A total of 23 donkeys (Equus asinus), aged between 3 and 7 years (4.91 ± 1.31) and weighted 185.78 ± 12.91 kg, met the inclusion criteria and were randomly assigned to one of three treatment groups: the control group ($n = 6$), the PRF group ($n = 7$), and the PRF/ACS group ($n = 10$). The underlying cause of tendonitis was primarily attributed to strain due to heavy-drafting activity, affecting 18 of the 23 donkeys (78.3%). This was observed in 8 of 10 SDFTs (80%) in the PRF/ACS group, 5 of 7 SDFTs (71.4%) in the PRF group, and 5 of 6 SDFTs (83.3%) in the control group. Traumatic-induced tendonitis was historically diagnosed in five cases (21.7%), with one case assigned to the control group and two cases in each of the PRF and PRF/ACS groups (Table 2).

Clinical findings

On the day of admission (T0), pain and heat scores across all groups ranged from mild to normal [Score (S) = 0 (0–1)], with most animals ($n = 16/23$) within the normal range and a few ($n = 7/23$) exhibiting mild heat and pain responses to palpation [S = 1 (1–1)]. The lameness score showed a normal gait [(S = 0 (0–0)] in 17 donkeys while 6 animals displayed intermittent, difficult-to-detect lameness [S = 1 (1–1)]. Following treatment, all animals ($n = 23$) in the three treatment groups achieved full clinical normalization of these specific parameters [S = 0 (0–0)] by T7, with these values remaining stable within the normal range throughout the rest of the study period.

Regarding tendon shape (Fig. 1a), all donkeys enrolled in the study exhibited an irregular tendon shape upon palpation at T0 [S = 3 (3–3). By the end of the study (T150), donkeys in the control group developed firm, nodular areas within the SDFT [S = 4 (4–4)], with a non-significant improvement from their T0 values ($P = 0.083$), yet a significant deviation from their normal tendon morphology ($P = 0.014$). In contrast, the PRF group demonstrated a marked improvement, with a reduction in score to [S = 1 (0–1)], which was statistically significant compared to both T0 ($P = 0.020$) and the control group ($P = 0.046$). The PRF/ACS group demonstrated the most

Table 2 Description, clinical history, and diagnostic data of 23 donkeys with SDFT lesions

	Groups			
	Total	**Control**	**PRF**	**PRF/ACS**
Count	23	6	7	10
Age	4.91 ± 1.31	4.50 ± 1.05	4.86 ± 1.57	5.20 ± 1.32
Body weight (Kg)	185.78 ± 12.91	187.19 ± 9.51	176.742 ± 8.09	184.28 ± 13.83
Gender	Jk (*n* = 18)	5	5	8
	G (*n* = 3)	1	2	1
	Jn (*n* = 1)	--	--	1
Affected limb	RF (*n* = 9)	2	3	4
	LF (*n* = 14)	4	4	6
Cause	St (*n* = 18)	5	5	8
	Tr (*n* = 5)	1	2	2
Lesion type	Core (*n* = 14)	4	4	6
	Marginal (*n* = 9)	2	3	4
Maximal injury zone	1B (*n* = 3)	1	--	2
	2 A (*n* = 9)	2	3	4
	2B (*n* = 11)	3	4	4
Duration of tendonitis until initial admission (days)	8.22 ± 2.75	7.8 ± 3.12	8.14 ± 2.73	8.5 ± 2.8

Jk Jackass, *G* Gelding, *Jn* Jennet, *RF* right-Fore limb, *LF* left-Fore limb, *St* strain induced, *Tr* traumatic induced

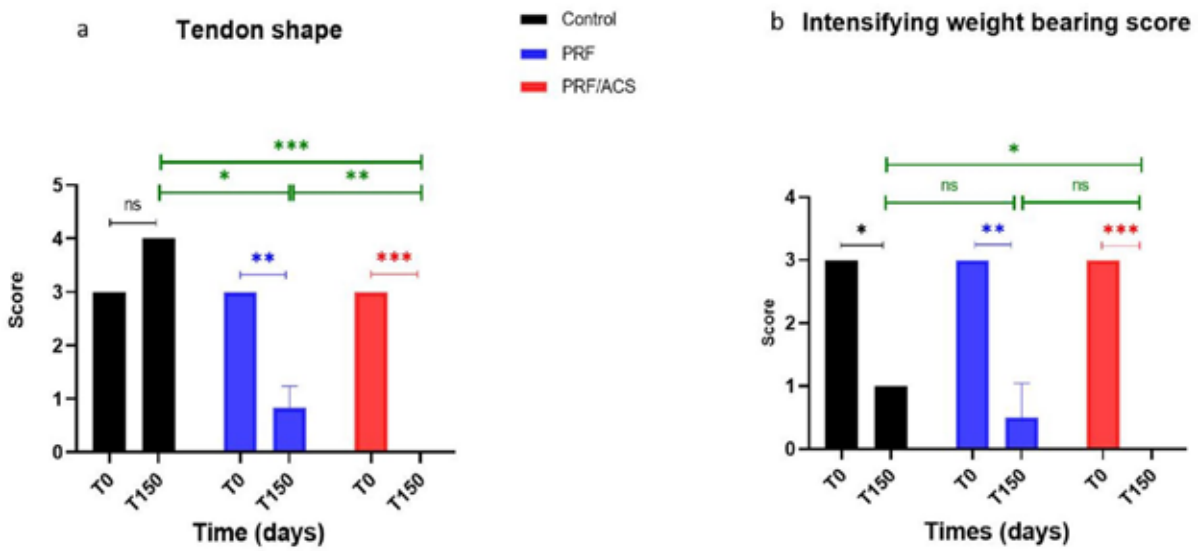


Fig. 1 Showing the clinical evaluation of palpable shape of the tendons (**a**), and intensifying weight carrying score (**b**) in both control and treated groups at T0 and T150

substantial improvement at T150, with near-complete restoration of normal tendon shape [S = 0 (0–0)]. This outcome was significantly improved compared to T0 (P = 0.014), the control group (P < 0.0001), and the PRF group (P = 0.046), and was not statistically different from normal tendons (P = 1.000).

At T0, all animals exhibited notable limb offloading during the intensifying weight-carrying test [score = 3 (3–3)]. By T150, the control group showed a partial improvement, characterized by a mild preferential weight shift toward the contralateral limb [S = 1 (1–1); P = 0.014 vs. T0], yet still significantly different from normal limb loading (P = 0.014). In contrast, donkeys treated with PRF or PRF/ACS achieved near-complete restoration of weight distribution in response to axial loading [S = 0 (0–1) and 0 (0–0), respectively] at T150, with significant improvements compared to T0 (P = 0.024 and P = 0.014, respectively). Compared to the control group at T150, both PRF and PRF/ACS groups showed superior outcomes (P = 0.08 and P < 0.0001, respectively) in intensifying weight-carrying score. While the PRF group showed a non-significant difference from normal values (P = 0.083) at T150, the PRF/ACS group achieved complete normalization with no statistical difference from normal scores (Fig. 1b).

Part two of this paper will appear in next month's Veterinary Ireland Journal.