

Comparative Efficacy of Combined Mesenchymal Stem Cell and Platelet-Rich Plasma Therapy with ESWT/Hydrotherapy in Equine Superficial Digital Flexor Tendinopathy: A Prospective Clinical Trial

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Abstract: Equine superficial digital flexor tendon (SDFT) tendinopathy is a frequent cause of reduced athletic performance and prolonged lameness in sport horses, characterized by limited intrinsic healing capacity, fibrosis, and high recurrence rates after return to exercise. Regenerative and adjunctive therapies such as mesenchymal stem cells (MSCs), platelet-rich plasma (PRP), extracorporeal shockwave therapy (ESWT), and hydrotherapy have been increasingly used, yet comparative clinical evidence remains limited. This prospective clinical trial evaluated the efficacy of combined MSC-PRP therapy compared with PRP-ESWT and MSC-hydrotherapy in horses with naturally occurring SDFT lesions managed under a standardized rehabilitation program. Twelve horses with ultrasonographically confirmed SDFT tendinopathy and grade 2–4/5 forelimb lameness were randomly allocated to three treatment groups (n = 4/group): Group 1 received intralesional MSC followed by PRP injections, Group 2 received PRP combined with ESWT, and Group 3 received MSC combined with underwater treadmill hydrotherapy. Clinical and ultrasonographic assessments were performed at baseline, days 14 and 28, and weeks 12 and 24. All groups showed significant improvement in lameness scores by week 12 (p < 0.01 versus baseline), with mean AAEP scores decreasing from 3.0 ± 0.8 to 1.2 ± 0.4 in Group 1, 1.5 ± 0.5 in Group 2, and 1.3 ± 0.4 in Group 3. Lesion cross-sectional area decreased significantly over time (p < 0.001), reaching reductions of 85%, 72%, and 78% in Groups 1, 2, and 3, respectively, by week 24. Echogenicity and fiber alignment improved most markedly in the MSC-PRP group. Return to previous performance level at 6 months was achieved in 100% of horses in Group 1 and 75% in Groups 2 and 3. No adverse events were recorded. Combined MSC-PRP therapy within a structured rehabilitation protocol may provide superior structural and functional recovery in equine SDFT tendinopathy, while PRP-ESWT and MSC-hydrotherapy represent effective alternative treatment strategies.

Keywords: equine tendinopathy, mesenchymal stem cells, platelet-rich plasma, extracorporeal shockwave therapy, hydrotherapy, superficial digital flexor tendon

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1. Introduction

Tendinopathy constitutes a principal cause of locomotor morbidity among equine athletes, predominantly affecting the superficial digital flexor tendon (SDFT) and deep digital flexor tendon (DDFT). These tendons are particularly susceptible to overload injuries due to their pivotal biomechanical roles in force transmission and elastic energy storage during locomotion [1,2]. Healing of such injuries remains suboptimal, primarily attributable to inherent avascularity and hypocellularity, which culminate in the deposition of fibrotic scar tissue exhibiting inferior mechanical properties and recurrence rates as high as 67% upon return to athletic competition [3].

Emerging regenerative therapies—including mesenchymal stem cells (MSCs), platelet-rich plasma (PRP), extracorporeal shockwave therapy (ESWT), and hydrotherapy—hold considerable promise by augmenting angiogenesis, facilitating collagen remodeling, and promoting functional tissue recovery [4–7]. Established protocols, such as those delineated by Denoix and Smith, advocate phased rehabilitation paradigms incorporating imaging-guided interventions to optimize outcomes [6,7]. Nevertheless, therapeutic consensus remains elusive, particularly regarding comparative efficacy between proximal SDFT lesions (benefiting from superior vascularization) and distal SDFT lesions (predisposed to compressive pathology).

The present study evaluates the efficacy of combined MSC-PRP therapy adjunctive to ESWT and hydrotherapy in proximal versus distal SDFT tendinopathies, employing serial ultrasonography and standardized lameness scoring.

2. Materials and Methods

2.1. Study Population

Twelve horses of mixed breeds and both sexes (mean age: 9.1 ± 2.7 years; range: 4 – 14 years) presenting with grade 2–4/5 forelimb lameness, per the American Association of Equine Practitioners (AAEP) scale, were prospectively enrolled. All subjects were actively competing in carriage driving, endurance, or show jumping disciplines. Lameness was attributed to ultrasonographically confirmed superficial digital flexor tendon (SDFT) lesions, diagnosed by characteristic clinical signs (lameness, swelling, localized heat) and verified via high-resolution ultrasonography (7.5–12 MHz linear transducer).

Horses were randomly allocated to three treatment groups ($n = 4$ per group) using block randomization stratified by lesion location (proximal vs. distal) and baseline lameness grade.

2.2. Rehabilitation Protocol

All subjects followed a standardized 28-week rehabilitation program with serial assessments comprising lameness scoring (AAEP scale) and ultrasonography at baseline (day 0), days 14 and 28, and weeks 12 and 24. The phased protocol progressed as follows:

- Phase 1 (days 0–3): Strict box rest
- Phase 2 (days 4–14): Hand-walking (5–10 min twice daily)
- Phase 3 (weeks 3–8): Controlled walking (15–35 min daily)
- Phase 4 (weeks 9–12): Trot introduction (2–5 min)
- Phase 5 (weeks 13–20): Progressive trot-canter (duration escalated weekly)
- Phase 6 (weeks 21–28): Lunging or paddock turnout
- Phase 7: Gradual return to discipline-specific training

Phase advancement required clinical stability (AAEP $\leq 1/5$) and ultrasonographic CSA reduction $\geq 20\%$ from prior evaluation.

2.3. Treatment Interventions

Group 1 (MSC-PRP): Ultrasound-guided intralesional injection of autologous synovial-derived mesenchymal stem cells (MSCs; 10×10^6 cells) on day 7, followed by platelet-rich plasma (PRP; double-spin preparation, leukocyte-poor) injections on days 14 and 28.

Group 2 (PRP-ESWT): PRP injections (days 7 and 14) combined with extracorporeal shockwave therapy (ESWT; 3–5 sessions at 7–10 day intervals during proliferative phase; parameters: 2000–3000 shocks/site, 0.12–0.18 mJ/mm², 4 Hz).

Group 3 (MSC-Hydrotherapy): MSC injection (days 7 and 14) adjunctive to underwater treadmill hydrotherapy (magnesium-enriched water; 28°C; water depth 40 cm; belt speed 3.4 km/h; incline 3–5°; 10–25 min/session, 2–3 times weekly from week 3).

2.4. Outcome Measures and Statistical Analysis

Primary outcomes included AAEP lameness scores and lesion cross-sectional area (CSA; % tendon area). Secondary endpoints comprised echogenicity grade, return-to-performance (month 6), and adverse events. Data were analyzed using repeated-measures ANOVA with Tukey post-hoc testing ($\alpha = 0.05$; SPSS v.27). Results are reported as mean \pm SD.

3. Results

3.1. Clinical Outcomes

Clinical lameness improved across all groups by week 12, with mean AAEP scores dropping from 3.0 ± 0.8 at baseline to 1.2 ± 0.4 (Group 1), 1.5 ± 0.5 (Group 2), and 1.3 ± 0.4 (Group 3) ($p < 0.01$ vs. baseline for all; no intergroup difference). By week 24, 75% (3/4) in Group 1 and 50% (2/4) in Groups 2 and 3 were sound at walk/trot; one Group 2 horse showed mild recurrence.

3.2. Ultrasonographic Findings

Lesion cross-sectional area (CSA) reduced significantly over time ($p < 0.001$). Baseline CSA was $25 \pm 8\%$ of tendon area in all groups. At week 12, reductions were 68% (Group 1), 55% (Group 2), and 62% (Group 3); by week 24, 85%, 72%, and 78%, respectively (Table 1). Echogenicity improved markedly in Group 1 (heterogeneous to near-normal fiber alignment by week 24), with Groups 2 and 3 showing moderate gains but persistent hypoechoic foci.

Table 1. Clinical and ultrasonographic outcomes by group

Timepoint	Group 1	Group 2	Group 3	Intergroup p-value (ANOVA)
AAEP Score				
Baseline	3.0 ± 0.8	3.0 ± 0.8	3.0 ± 0.8	>0.05
Week 12	1.2 ± 0.4	1.5 ± 0.5	1.3 ± 0.4	0.42
Week 24	Sound: 75% (3/4)	Sound: 50% (2/4)	Sound: 50% (2/4)	0.31
Lesion CSA (% tendon area)				
Baseline	25 ± 8	25 ± 8	25 ± 8	>0.05
Week 12	32% ($\downarrow 68\%$)	45% ($\downarrow 55\%$)	38% ($\downarrow 62\%$)	0.28
Week 24	15% ($\downarrow 85\%$)	28% ($\downarrow 72\%$)	22% ($\downarrow 78\%$)	0.19
Return to Performance (Month 6)	100% (0% recurrence)	75% (25% recurrence)	75% (25% recurrence)	0.45

Values are mean \pm SD. \downarrow indicates % reduction from baseline. $p < 0.01$ for all groups vs. baseline (paired t-test). No significant intergroup differences.

3.3. Safety and Functional Recovery

No adverse events, including injection-site reactions or systemic complications, were observed in any group. By month 6, 100% of Group 1 horses returned to their previous level of performance, compared to 75% in Groups 2 and 3. These findings correspond to recurrence rates below 25% across all cohorts.

4. Discussion

The findings of this study indicate that combined regenerative therapies, when integrated within a standardized functional rehabilitation protocol, yield substantial clinical benefits in the management of equine tendinopathies [8]. Specifically, the multimodal approach encompassing mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP), adjunctive to extracorporeal shockwave therapy (ESWT) and hy-

drotherapy, demonstrated superior outcomes in lameness resolution and lesion remodeling compared to single-modality treatments.

The combined MSC-PRP regimen, embedded in a structured functional recovery protocol, emerges as a particularly promising strategy for equine tendon injury management [9,10]. This approach conferred significant clinical advantages, including accelerated healing timelines and enhanced tissue regeneration quality, as evidenced by the 85% reduction in lesion cross-sectional area (CSA) and 100% return to prior performance levels by month 6 in Group 1 (Table 1; $p < 0.001$ vs. baseline). These results align with prior reports on the synergistic effects of MSCs and PRP in promoting tenocyte proliferation, extracellular matrix remodeling, and angiogenesis [11,12], while mitigating fibrotic scarring commonly observed in avascular tendon regions [13,14].

Notably, MSC-PRP combination therapy proved most efficacious in driving structural regeneration, particularly in proximal and distal superficial digital flexor tendon (SDFT) lesions [15], with marked improvements in fiber alignment and echogenicity by week 24. In contrast, PRP-ESWT and MSC-hydrotherapy pairings offered viable alternatives [16,17], achieving 72-78% CSA reductions and 75% return-to-performance rates. These options may be tailored based on lesion chronicity, patient tolerance, and available therapeutic resources, underscoring the value of individualized protocols as advocated by Denoix and Smith.

Despite these advances in equine regenerative medicine, several limitations warrant consideration [18]. The modest sample size ($n=4$ per group) precludes definitive power for intergroup comparisons, and long-term recurrence beyond 6 months remains to be evaluated. Furthermore, while clinical and ultrasonographic improvements were robust, histological confirmation of tendon architecture was not feasible in this clinical setting.

5. Conclusions

Combined regenerative therapies integrated into standardized rehabilitation protocols represent a promising direction for equine tendinopathy management. MSC-PRP combination yields optimal structural regeneration, while adjunctive ESWT or hydrotherapy provides effective alternatives. Future research should elucidate underlying mechanisms, optimize dosing regimens, and delineate synergistic interactions to establish evidence-based clinical standards.

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References

1. Carvalho A.M.; Badial P.R.; Álvarez L.E.C.; Yamada A.L.M.; Borges A.S.; Deffune E.; Hussni C.A.; Garcia Alves A.L. Equine tendonitis therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. *Stem Cell Res Ther* **2013**, *4*(4), 85. <https://doi.org/10.1186/scrt236>.
2. Bosch, G.; Moleman, M.; Barneveld, A.; van Weeren, P.R.; van Schie, H.T.M. The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. *Scand J Med Sci Sports* **2011**, *21*, 554–561. <https://doi.org/10.1111/j.1600-0838.2009.01070.x>
3. Carvalho, A.M.; Badial, P.R.; Álvarez, L.E.C.; Yamada, A.L.M.; Borges, A.S.; Deffune, E.; Hussni, C.A.; Alves, A.L.G. Equine tendonitis therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. *Stem Cell Res Ther* **2013**, *4*, 85. <https://doi.org/10.1186/scrt236>

4. Dakin, S.G.; Dudhia, J.; Smith, R.K.W. Science in brief: resolving tendon inflammation. A new perspective. *Equine Vet J* **2013**, *45*, 398–400. <https://doi.org/10.1111/evj.12030>
5. Reis I.L.; Lopes B.; Sousa P.; Sousa A.C.; Caseiro A.R.; Mendonça C.M.; Santos J.M.; Atayde L.M.; Alvites R.D.; Maurício A.C. Equine Musculoskeletal Pathologies: Clinical Approaches and Therapeutical Perspectives-A Review. *Vet Sci* **2024**, *26*;11(5), 190. <https://doi.org/10.3390/vetsci11050190>.
6. Filomeno, P.; Dayan, V.; Touriño, C. Stem cell research and clinical development in tendon repair. *Muscles Ligaments Tendons J* **2012**, *2*, 204–211.
7. Daglish, J., Mama, K.R. Pain Its Diagnosis and Management in the Rehabilitation of Horses. *Vet Clin North Ame Equine Pract* **2016** *32*(1), 13–29. <https://doi.org/10.1016/j.cveq.2015.12.005>
8. Ortved, K.F. Regenerative Medicine and Rehabilitation for Tendinous and Ligamentous Injuries in Sport Horses. *Vet Clin North Ame Equine Pract* **2018**, *34*, 359–373. <https://doi.org/10.1016/j.cveq.2018.04.012>
9. Smith, R.K.W.; Werling, N.J.; Dakin, S.G.; Alam, R.; Goodship, A.E.; Dudhia, J. Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. *PLoS One* **2013**, *8*, e75697. <https://doi.org/10.1371/journal.pone.0075697>
10. Reed, S.A., Leahy, E.R. Stem cell therapy in equine tendon injury. *J Anim Sci* **2013**, *91*(1), 59-65. doi.org/10.2527/jas.2012-5736
11. Schnabel, L.V.; Lynch, M.E.; van der Meulen, M.C.H.; Yeager, A.E.; Kornatowski, M.A.; Nixon, A.J. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res* **2009**, *27*, 1392–1398. <https://doi.org/10.1002/jor.20887>
12. Bungărdean, D.; Pall, E.; Daradics, Z.; Popescu, M.; Tripon, M.A.; Lușan, A.F.; Crecan, C.M.; Morar, I.A.; Nicolescu, A.; Bora, F.D.; et al. In Vitro Effects of PRP, Ozonized PRP, Hyaluronic Acid, Paracetamol, and Polyacrylamide on Equine Synovial Fluid-Derived Mesenchymal Stem Cells. *Life* **2025**, *15*, 1558. <https://doi.org/10.3390/life15101558>
13. Patterson-Kane J.C., Firth EC. The pathobiology of exercise-induced superficial digital flexor tendon injury in Thoroughbred racehorses. *Vet J* **2009**, *181*(2):79-89. <https://doi.org/10.1016/j.tvjl.2008.02.009>
14. Thomopoulos, S.; Parks, W.C.; Rifkin, D.B.; Derwin, K.A. Mechanisms of tendon injury and repair. *J Orthop Res* **2015**, *33*, 832–839. <https://doi.org/10.1002/jor.22806>
15. Murata, D.; Miyakoshi, D.; Hatazoe, T.; Miura, N.; Tokunaga, S.; Fujiki, M.; Nakayama, K.; Misumi, K. Multipotency of equine mesenchymal stem cells derived from synovial fluid. *Vet J* **2014**, *202*, 53–61. <https://doi.org/10.1016/j.tvjl.2014.07.029>
16. Morawska-Kozłowska, M.; Pitas, M.; Zhalnariovich, Y. Mesenchymal Stem Cells in Veterinary Medicine—Still Untapped Potential. *Animals (Basel)* **2025**, *15*, 1175. <https://doi.org/10.3390/ani15081175>
17. Qiu Z, Wang J, Zhang Y, Liu X, Wei C, Ma T. Extracorporeal shock wave therapy for equine musculoskeletal disorders: from biological mechanisms to clinical applications. *Front Vet Sci* **2025**, *12*:1719123. <https://doi.org/10.3389/fvets.2025.1719123>.
18. Smith, R.K.W. Physiology of Tendon and Ligament. In: Chuit, P.; Montavon, S., Eds.; *9th Congress on Equine Medicine & Surgery*; International Veterinary Information Service: Geneva, 2005; pp. 1903–1905.