Evidence Supporting Intralesional Stem Cell Therapy to Improve Equine Flexor Tendon Healing

A Knowledge Summary by

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KNOWLEDGE SUMMARY

Clinical bottom line

Current experimental evidence suggests that intralesional stem cell administration improves the histological characteristics and matrix organisation of healing equine superficial digital flexor tendons (SDFT); however, the clinical relevance of these findings are not clear. Current case-based evidence suggests that cell-based therapies improve the quality of tendon healing and reduce the recurrence rates of SDFT injuries but the lack of any randomised, controlled prospective studies with function-based outcomes is still concerning, give the widespread advocacy for and use of 'stem cell' therapies for the treatment of equine tendon injuries.

Question

Does intralesional stem cell therapy improve healing of equine superficial digital flexor tendons?

The evidence

To date, equine experimental and clinical case studies of tendon healing demonstrate improved histological and ultrasonographic features of matrix architecture, respectively with intralesional stem cell therapy. These findings encourage clinical use of stem cell-based therapies to enhance equine tendon healing. A few clinical studies indicate that re-injury rates are lower following stem cell treatment, than without. However, these studies do not provide any firm conclusions regarding the ideal cell source for therapy or optimal treatment protocol. Collaborative research and long-term follow up among the veterinary community is needed to establish the optimal applications for cell-based therapies.

Summary of the evidence

Del Bue (2008)	
Population:	Clinical cases of superficial digital flexor tendon (SDFT) tendinitis with a distinct core lesion diagnosed via ultrasonography.
Sample size:	n = 16
Intervention details:	Intralesional administration of allogeneic adipose-derived mesenchymal stem cells (Ad-MSCs) suspended in autologous platelet rich plasma (PRP).
Study design:	Case series – (no controls, not blinded)
Outcome studied:	Serial ultrasonographic evaluations Return to exercise/activity
Main findings: (relevant to PICO question):	• 14 out of 16 horses returned to activity.
Limitations:	• Small/limited case numbers • No objective measurements of outcome • No control groups

	• Inconsistent follow-up • Uncertainties regarding the relative contributions of Ad-MSCs and PRP to outcome

Lacitignola (2008) Experimental study component	
Population:	Clinically healthy Standardbred horses. Pre-existing tendon injury was ruled out with baseline lameness and ultrasonographic evaluation.
Sample size:	Experimental study: n = 6 horses (24 SDFTs)
Intervention details:	Collagenase-induced tendinitis was created in both forelimb and both hindlimb SDFTs of four horses, and both forelimb and one hindlimb SDFTs of two horses. At the time of collagenase injection, bone marrow aspirates were collected from tuber coxae to isolate bone marrow-derived MSCs (BMMSC). Bone marrow-derived mononuclear cells (BMMNC) were isolated from a second bone marrow aspirate just prior to treatments. Three weeks after collagenase injections, the SDFT lesions were treated with autologous BMMSCs (5.5 x 10^6 cells) or BMMNCs (1.22 x 10^8 cells) re-suspended in fibrin, fibrin alone or saline. The two injured hindlimb SDFTs served as sham controls.
Study design:	Randomised, partially controlled, experimental study; not blinded
Outcome studied:	Semi-objective data: Type lesion score (TLS), fiber pattern score (FPS) and percentage cross-sectional area of the lesion (% CSA) at the maximal injury zone were derived from ultrasonographic evaluations every 2-3 weeks, up to 21 weeks after cell administration. Collagen architecture was assessed in histological sections at 21 wks. Collagen types I and III, COMP and CD34 distribution was assessed by immunohistochemistry
Main findings: (relevant to PICO question):	• At 8 weeks, cell-treated SDFTs had a decreased lesion size (% CSA) compared to controls; however, there was no difference between BMMSC- and BMMNC-treated tendons. • At 16 weeks, % CSA, FPS, TLS were significantly better in the cell-treated SDFTs compared to control SDFTs. • Both cell treatments improved collagen alignment compared to controls.
Limitations:	• Small sample size and low power • Two of six hindlimb SDFTs were used as the collagenase control • Uncertainties about 'stem cell' characteristics of primary culture-expanded BMMSC cell population • Quantitative data were not presented; only summary statements • Outcome measures were predominantly qualitative • Nominal outcome data were misrepresented and improperly analysed. Significant outcomes were not designated • Biomechanical or other functional testing were not carried out

Lacitignola (2008) Clinical study component	
Population:	Standardbreds and Showjumpers presented for evaluation and treatment of SDFT or suspensory ligament injuries.
Sample size:	8 STBs, 12 showjumpers,14 SDFT lesions, 3 suspensory ligament (SL) body lesions, 3 SL branch lesions.
Intervention details:	1.23 x 10^8 BMMNCs in fibrin were injected into the lesions under U-S guidance. Horses were stall-rested for 8 weeks, followed by gradual return to exercise and competition over the following 20-30 weeks.
Study design:	Clinical case series; no control group, not blinded.
Outcomes studied:	Ultrasonographic assessment 3 and 8 weeks after injections. Changes in Type lesion score (TLS), fiber pattern score (FPS) and percentage cross sectional area of the lesion (% CSA) were recorded. Time to return to training/racing, and re-injury rates
Main findings: (relevant to PICO question):	• 3 weeks after treatment (T3), the injury sites were pain-free. • After 6 weeks, clinical lameness was resolved in all cases • %CSA, FPS and TLS improved in all patients by week 3, and this improvement continued throughout the study • 12 of 20 patients (60%) had returned to work. The re-injury rate was assessed at 35%.
Limitations:	• Small case numbers • No control group • Variable lesion severities and locations • Variable follow-up intervals and functional outcome expectations • No quantitative outcomes were presented; only summary statements. No statistical analyses

Nixon (2008)	
Population:	Clinically healthy horses of various breeds. Pre-existing tendon injury was ruled out with baseline lameness and ultrasonographic evaluation.
Sample size:	n = 8
Intervention details:	Collagenase-induced tendinitis was created in 1 randomly chosen forelimb SDFT of all horses. Five days after collagenase injection, adipose tissue was obtained from gluteal region for isolation of autologous adipose-derived nucleated cells (ADNCs). Four randomly chosen horses received intralesional ADNCs in saline and remaining 4 horses received an equal volume of saline, 7 days after collagenase injection. All horses were euthanised 6 weeks after treatment.
Study design:	Randomised, controlled, experimental study; unclear whether analyses were blinded

Outcome studied:	Semi-objective data – serial ultrasonographic evaluations to determine the CSA and fiber alignment; histological scores for fiber alignment, inflammatory cell infiltration and vascularity. Objective data – Biochemical (total DNA, proteoglycan and collagen contents) and molecular characteristics (collagen type I and III, COMP gene expression) were measured in ADNC- and saline-treated SDFTs 6 weeks following treatment.
Main findings: (relevant to PICO question):	• ADNC improved collagen organisation, in both ultrasonographic and histological analyses. • ADNCs decreased inflammatory cell infiltration, suggesting immunomodulatory effects. • The biochemical and molecular characteristics of ADNC- and saline-treated SDFTs were largely similar, except that COMP mRNA levels were higher in ADNC-injected SDFTs.
Limitations:	• Small sample size (although satisfactory power in results were reported) • Short time frame, relative to clinical disease • No biomechanical or other functional outcomes were performed.

Schnabel (2009)	
Population:	Clinically healthy horses of various breeds.
Sample size:	n = 12
Intervention details:	Collagenase-induced tendinitis was created in both forelimb SDFTs of all horses. Five days after collagenase injection, 1 SDFT from 6 randomly chosen horses received 1 x 10^7 autologous BMMSCs and the other 6 horses received 1 x 10^7 autologous adenoviral IGF-I infected BMMSCs (adIGF-BMMSCs). The contralateral SDFT in all 12 horses was injected with saline. All horses were euthanised 8 weeks after treatment.
Study design:	Randomised, controlled, experimental study
Outcome studied:	Semi-objective data – serial ultrasonographic evaluations; histological scores (evaluated by 2 blinded observers). Objective data – Biochemical and molecular characteristics, biomechanical properties (elastic modulus, stiffness) were measured in patient-matched cell-treated and saline-treated SDFTs, 8 weeks following treatment.
Main findings: (relevant to PICO question):	• Ultrasonographic evaluations did not demonstrate any difference between MSC-treated and control SDFTs. • Histologic scores of both cell treatments were significantly improved over saline control; however, BMMSC and AdIGF-BMMSC SDFTs were not significantly different from each other. • Biochemical and molecular characteristics were statistically similar among the cell-treated and saline SDFTs.

	• Biomechanical properties – stiffness of both cell-treated SDFTs was higher than saline SDFT; however, this was not significant.
Limitations:	• Short time frame, relative to clinical disease • Improved healing demonstrated only in histological scores which was determined via semi-objective data. • No functional outcomes (apart from biomechanical testing) were performed.

Watts (2011)	
Population:	Clinically healthy horses of various breeds.
Sample size:	n = 8
Intervention details:	Collagenase-induced tendinitis was created in 1 randomly chosen forelimb SDFT of all horses. Seven days after collagenase injection, allogeneic fetal-derived embryonic stem cells (ESCs) were injected intralesionally. Four randomly chosen horses received 3 x 10^6 ESCs suspended in culture medium and remaining 4 horses received an equal volume of culture medium. All horses were euthanised 8 weeks after treatment.
Study design:	Randomised, controlled, experimental study
Outcome studied:	Semi-objective data – Serial ultrasonographic evaluations; Magnetic resonance imaging (MRI) immediately after euthanasia; histological scores (evaluated by 2 blinded observers). Objective data – Biochemical and molecular characteristics were measured in treated and control SDFT samples, 8 weeks following treatment.
Main findings: (relevant to PICO question):	• Ultrasonographic evaluations and histology demonstrated improved fiber pattern and decreased lesion size in ESC-treated SDFTs compared to control SDFT lesions. • MRI did not demonstrate any difference in lesion size or signal intensity between the treated and control SDFTs. • Biochemical and molecular characteristics of both groups were similar.
Limitations:	• Improved healing demonstrated only in histological scores which was determined via semi-objective data. • Short time frame, relative to clinical disease • No biomechanical or other functional outcome data.

Caniglia (2012)	
Population:	Clinically healthy horses of various breeds.
Sample size:	n = 6
Intervention details:	Core lesions were created in both forelimb SDFTs with a synovial resector. One randomly chosen SDFT was treated with 1 x 10^7 autologous BMMSCs suspended in BM supernatant, 4 weeks following injury. The contralateral SDFT received an equal volume of BM supernatant only. Horses were euthanised 12 weeks after treatment.
Study design:	Randomised, controlled, experimental study
Outcome studied:	Objective data – collagen fibril diameters were measured in transverse sections of SDFTs by transmission electron microscopy. Samples were obtained from healthy and injured regions of each SDFT.
Main findings: (relevant to PICO question):	• Intralesional administration of autologous BMMSCs did not affect the collagen fibril diameters of the treated and control SDFTs.
Limitations:	• Single outcome measure (collagen fibril diameter) was evaluated which may not accurately reflect the healing response at 12 weeks post-treatment • Short time frame, relative to clinical disease • No functional outcome assessments.

Godwin (2012)	
Population:	Clinical cases of overstrain-induced SDF tendinitis in thoroughbred racehorses.
Sample size:	n = 141
Intervention details:	Intralesional injection of 1 x 10^7 autologous BMMSCs in BM supernatant under ultrasonographic guidance.
Study design:	Case series – no controls, not blinded
Outcome studied:	Histological evaluation of BMMSC-treated SDFT samples from 8 horses, 6-12 months after treatment; evaluators were not blinded. Long-term follow-up of race records 2 years after treatment. Re-injury rates were calculated from information retrieved from telephone conversations with owners/trainers.
Main findings: (relevant to PICO question):	• Histological evaluation showed lesion repair and collagen crimp pattern restoration. • High percentage of cases returned to racing (98.2%) with a lower re-injury rate (27.4%) than previously reported in other studies of conservatively managed cases (46%).
Limitations:	• No 'in study' controls. Historical controls were used to determine

	the benefit of BMMSC therapy in reducing re-injury rates. • Unavoidable variations in severity of the lesions, intervals between injuries and therapy, and post-injection management/training practices

Marfe (2012)	
Population:	Clinical cases of overstrain-induced SDF tendinitis diagnosed with a distinct core lesion by ultrasonography.
Sample size:	n = 6
Intervention details:	Intralesional administration of autologous blood-derived stem cells (BDSCs) in 3 cases. Conventional conservative treatment was followed in 3 control cases.
Study design:	Clinical case-control study; not blinded
Outcome studied:	Ultrasonographic evaluation of the injured tendons prior to and 4 months following BDSC injections.
Main findings: (relevant to PICO question):	• Ultrasonographic evaluation of BDSC-treated cases showed complete in-fill of the injured site with linear collagen fiber alignment. • Control (conservatively treated) tendons showed disorganised fibrous tissue infill at the injured site.
Limitations:	• Very small group sizes • No objective/quantitative data of treatment outcomes or statistical analyses • No long-term follow-up

Lange-Consiglio (2013)	
Population:	Clinical cases of SDF tendinitis, DDF tendinitis and suspensory desmitis, diagnosed via ultrasonography.
Sample size:	n = 95
Intervention details:	Group A – 51 clinical cases treated with 5 x 10^6 allogenic amniotic membrane-derived mesenchymal cells. Group B – 44 clinical cases treated with 5 x 10^6 autologous BMMSCs.
Study design:	Prospective randomised controlled study, not blinded
Outcome studied:	Lameness and ultrasonographic evaluation at monthly intervals for 12-15 months. Follow-up information on return to previous activity and re-injury, at 2 years after treatment.
Main findings: (relevant to PICO question):	• No major ultrasonographic differences between groups • Group A – returned to work 4-5 months after treatment. Re-injury rate (4%) was lower than BMMSC group • Group B – returned to work after a recovery period that ranged

	between 4-12 months. Mean re-injury rate was higher than Group A (23.08%).
Limitations:	• No untreated control group • Wide ranges of ages, lesion sites and lesion severities, five performance disciplines that influence outcomes and re-injury rates

Smith (2013)	
Population:	Clinical cases of career-ending SDFT injury.
Sample size:	n = 12
Intervention details:	Six randomly selected horses were treated with intralesional injection of 1 x 10^7 autologous BMMSCs in 2 mL of BM supernatant, under ultrasonographic guidance. Remaining 6 'control' horses received an equal volume of saline. Horses were euthanised 6 months following treatment.
Study design:	Randomised, experimental study; not blinded.
Outcome studied:	Semi-objective data – Ultrasonographic evaluation, histological scores Objective data – Biochemical and biomechanical characteristics, were measured in specimens from treated and control SDFTs, 6 months following treatment
Main findings: (relevant to PICO question):	• Reduced cross-sectional area of BMMSC-treated SDFTs compared to saline controls at 6 months. • No difference in total collagen content of SDFTs • DNA and sulfated glycosaminoglycan contents of BMMSC-treated SDFTs were significantly lower than saline controls and similar to the contralateral normal SDFT. • Stiffness of BMMSC-treated SDFT was similar to normal SDFT; elastic modulus of BMMSC-treated SDFT higher than saline control but not significantly different. • Overall, improved histological characteristics of BMMSC-treated SDFT compared to saline control.
Limitations:	• Small group sizes • These cases had severe/end-stage lesions, which might not be reflective of responses in less severely injured cases. • No functional assessment, apart from biomechanical tests

Durgam (2016)	
Population:	Clinically healthy American Quarter horses
Sample size:	n = 8
Intervention details:	Collagenase-induced tendinitis was created in both forelimb SDFTs of all horses. At the same time, a small segment of lateral digital extensor tendon was surgically collected to isolate tendon-derived progenitor cells (TDPCs). Four weeks after collagenase injections, 1 randomly chosen SDFT in each horse was injected with 1 x 10^7 autologous TDPCs in 2 mls of saline and the contralateral SDFT received an equal volume of saline. All horses were euthanised 12 weeks after TDPC injections.
Study design:	Randomised, controlled, experimental study; no blinding
Outcome studied:	Subjective data –collagen and proteoglycan distributions in longitudinal histological sections Objective data – Biochemical, biomechanical and molecular characteristics, measured in both forelimb SDFTs and a normal hindlimb SDFT, 12 weeks following treatment. Fourier Transform-Second-Harmonic Generation (FT-S-HG) assessment of collagen alignment in longitudinal histological sections.
Main findings: (relevant to PICO question):	• Biochemical and molecular characteristics of the TDPC- and saline-treated SDFTs were not significantly different. • Yield and maximal stresses of TDPC-treated tendons were statistically similar to normal SDFT and higher than saline-treated tendons; however elastic modulus and stiffness of TDPC-treated SDFT were significantly lower than normal SDFT. • Subjectively, the collagen and proteoglycan distributions in histological sections of TDPC-treated SDFTs were similar to normal SDFT. These findings corroborated with quantitative FT-S-HG measurements of collagen alignment.
Limitations:	• Hindlimb SDFTs were used as controls • Small group sizes, although sufficient for statistical significance • Short time frame, relative to clinical disease • Biomechanical properties were determined in one-quarter longitudinal samples of the whole SDFT which may have influenced the results. • SDFT lesion site specimens collected for biochemical and molecular analyses might not be reflective of the entire injury.

Appraisal, application and reflection

Tendon injuries in horses range from acute tendon strains to chronic tendinopathy, and are both common and challenging problems in equine performance horse practice. The technique and feasibility of cell-based therapy (isolated from bone marrow) for treating equine SDFT injuries was first reported by Smith et al. (2003; 14). Since then, intralesional stem cell therapy, using cells from multiple tissue sources, has become

common and commercial services are now available to support cell-based therapies in horses. The purpose of this Knowledge Summary was to evaluate the available evidence from studies evaluating the efficacy of stem cell therapies for equine flexor tendon healing.

Appraisal

Eleven studies were identified that addressed this issue (note that the outcomes from references 2 and 3 were replicated in reference 10. Reference 10 was used to evaluate the collective outcomes from these manuscripts). These papers segregated into two distinct groups; six studies using experimental models of tendinopathy (references 1, 5, 10, 12, 13 and 17) and six case series of clinical tendinitis patients (references 4, 6, 9-11, and 15; reference 10 included data for both groups).

Accepting the focused nature of the question under review, there was a remarkable degree of variability in the source of 'stem cell' used in these studies (bone marrow [7], blood [1], amnion [1], adipose tissue [2], embryonic [1], and tendon [1]), the protocols used to generate the therapeutic cell populations, donor-recipient matching (three allogeneic sources and 9 autologous sources), the diluents used for the cell injections (saline [5], bone marrow supernatant [4], plasma [1], PRP [1], and fibrin/thrombin [1]) and, in the clinical studies, the specific disciplines the patients were engaged in. Collectively, these sources of variation confounded any coherent meta-analysis.

Of the six experimental studies, five used intratendinous collagenase injections to generate SDFT lesions (5, 10, 12, 13, 17) while Caniglia et al 2012 (1) created a core lesion with a synovial resector. In only two of these studies, were at least some of the outcome assessments conducted in blinded fashion (13, 17). While acknowledging that there are no other accepted alternative models, the extent to which either induced lesion reflects the cumulative tensile strain injury of clinical tendinitis cases is debatable. Self-evidently, neither model is generated by tensile overloading, and our own (5) and other's (3, 16) experience with the collagenase model has shown that individual horse's responses to collagenase vary considerably.

Accepting these concerns, in all six experimental studies, cell delivery improved the histological and 'matrix organisation' characteristics of the repair tissue, while the ultrasonographic findings were improved in three of the four studies that used ultrasonography for outcome assessment. In all but one experimental study (reference 5), histological outcomes were determined by a score derived from 'cell and tissue characteristics' grading schemes. There is clearly an element of subjectivity in these analyses, particularly given that only two were carried out in blinded fashion. Of more general concern, although it makes intuitive sense that a 'more histologically normal' repair tissue corresponds to a better functional outcome, the relationship between these outcomes is very poorly defined, particularly since higher level matrix organisation has not been addressed in any study to date.

With the possible exception of Lacitignolo et al 2008 (10; 24 weeks), the experimental studies were conducted over time frames far less (7-16 weeks) than the 'several months' interval generally required for clinical tendinitis cases to resolve. It is possible, and even perhaps to be expected, that many of the significant differences between cell-treated and control samples detected at early time points in the experimental studies disappear over longer time frames, as healing in the 'control' groups catches up.
Only two of the experimental studies (5,13) included biomechanical testing to provide some functional assessment of outcome. However, it is debatable how well single 'tensile load to failure' testing translates to the rapid, submaximal cyclic loading that flexor tendons are subjected to clinically. None of the experimental studies addressed the most clinically relevant issue; namely, do horses with tendinitis perform better after receiving cell-based therapies? The ultrasonographic, histological, biochemical, transcriptional and biomechanical analyses are, at best, indirect indices of 'healing quality' and functional return.

Collectively, outcomes from the experimental studies should be considered as B2 evidence.

Of the six clinical case series, three had very small case numbers and/or no control group and/or marginal outcome assessments (4, 10, 11) and can be considered to be little more than anecdotal (grade E) in value. The study by Godwin et al 2012 (6) is substantive by virtue of the large number of cases (141) enrolled in the analyses. There was no control/placebo group in this study; the authors used previously published outcomes from analogous patient groups for comparison. Further, the great majority of Godwin et al's patients (105) were National Hunt horses, and so their outcomes should be confined to this discipline. Accepting this, 98% of treated horses returned to racing (albeit after an 8+ month layoff) and the re-injury rate (27%) was approximately half that reported in two studies from similar National Hunt populations. Although the Evidentiary Value of the Godwin study should, at best, be classified as an uncontrolled prospective study (C), the clinical significance of the outcomes is high (grade 1 to 2).

Lange-Consiglio et al 2013 (9) compared the responses of 95 performance horses with tendon or ligament injuries randomly assigned to be treated with allogeneic amnion-derived stem cells (AMSC; 51 cases) or autologous bone marrow-derived stem cells (BMMSC; 44 cases) in a fibrin carrier. This study included show jumpers, dressage horses, eventers, trotters and thoroughbreds, with lesions in the superficial and deep digital flexor tendons and suspensory ligaments and a range of clinical signs and lesion severity, although these variables were distributed fairly evenly across both treatment groups. Horses treated with AMSCs returned to training earlier than horses receiving BMMSCs and the re-injury rates were considerably less in the AMSC group (4%) than the BMMSC group (23%). The overall BMMSC group re-injury rate was reassuringly similar to the rate reported by Godwin et al, above, although Lange-Consiglio's Thoroughbreds responded substantially better (12.5% recurrence) than Godwin's thoroughbreds (50%). The study by Lange-Consiglio et al 2013 should be considered B2.

In the final clinical study, by Smith et al 2013 (15), was a prospective, randomised, controlled clinical trial involving 12 horses with end-stage tendinitis. Further, the histological evaluations of the tendon samples were blinded. Although the horses in the study were not exercised above trotting speeds, there were substantial improvements in ultrasonographic, histological and biomechanical characteristics of healing tissue six months after Intralesional BMMSC treatment. From an experimental design perspective, this study provides the most compelling results supporting stem cell use for flexor tendinitis in horses and should be classified as B2.

Application

From an evidentiary perspective, the collective data supporting cell-based therapy for equine flexor tendinitis is not strong. In this review, we have raised concerns regarding the clinical applicability of the experimental tendinitis models, the relevance of indirect 'healing quality' outcomes to functional success, inadequate or nonexistent control groups and experimental design issues with published clinical trials. The clinical reality is that conventional approaches to managing flexor tendinitis/tendinopathy in performance horses require prolonged and intensive therapy and rehabilitation efforts yet carry a guarded prognosis. Any novel therapeutic option, whether cell-based, biologic or pharmaceutical, will likely be embraced by the equine veterinary community if that option holds some promise of an improved outcome. This perspective makes any attempt to conduct a randomised, controlled, prospective clinical trial challenging, particularly given the funding constraints that veterinary researchers routinely work under.

While acknowledging the questionable evidentiary value of some studies, there is a consistent collective body of evidence supporting the findings that cell-based therapies improve tendon matrix repair and reorganisation, both in experimental and clinical subjects, and significantly reduce recurrence rates following return to work. In light of these conclusions, and the lack of any more promising alternatives, cell-based therapies should be considered for treating equine flexor tendinitis cases and, by extension, for ligament injuries also. As the applications of these therapies evolve, there are many variables that need to be investigated and optimised. Some of the major issues are as follow:

1. Standardisation/optimisation of cell source(s), cell processing, and diluents
2. Optimisation of timing and dose(s) of cell delivery following injury
3. Agreement on effective post-therapeutic case management and rehabilitation protocols
4. Standardisation of meaningful outcome measures for specific equine disciplines

Conclusions and Reflection

Cell-based therapy, in both the human and veterinary fields, is a highly dynamic, but poorly defined field of discovery. It is clear that clinical applications of cell-based therapies have far outstripped our understanding of the biological mechanisms by which stem cells influence healing. As noted above, there is little or no rationale for deciding on many of the variables that influence cell-based therapy efficacy. Somewhat contentiously, the actual need for a 'stem cell' population, as opposed to any population of cells isolated from a tissue or fluid, has not been demonstrated through properly controlled experiments. Further, data from recent cell-tracking studies (5,7,16) clearly show that exogenous stem cells are cleared from the injection site within a few weeks and do not contribute to the pool of tenocytes and/or progenitor cell engaged in tendon repair/regeneration beyond this time. It is probable that secreted stem cell cytokines and/or trophic factors contribute substantially to stem cells' impact on tissue repair (8). Identifying these factors could simplify and standardise 'biologic therapy' considerably.

Regardless of the specific biologic therapy in question, randomised, appropriately controlled studies using a consistent and translatable experimental model or clinical caseload, standardised treatment and rehabilitation protocols and credible outcome assessments, will be required to make meaningful headway on clarifying efficacy. Given the costs and case numbers required for these clinical trials, multi-center/practice collaboration and significant financial support from private industry and foundations will be necessary. Depending on regulatory developments in human medicine, the veterinary community might also stand to benefit from biomedical research efforts focused on validating cell-based therapies for people.

Methodology Section

Search Strategy	
Databases searched and dates covered:	Search terms were applied in PubMed Central accessed on NCBI website (1910-2016), CAB abstracts database accessed on OVID platform (1973-2015) and Scopus
Search terms:	horse AND tendonitis OR tendinitis OR stem cell OR flexor tendon
Dates searches performed:	August 2016

Exclusion / Inclusion Criteria	
Exclusion:	Non-English language, review articles, case reports, conference proceedings/abstracts
Inclusion:	Experimental studies and clinical case series evaluating the effect of intralesional stem cell therapy on equine superficial digital flexor tendon healing.

Search Outcome					
Database	Number of results	Excluded – did not answer PICO question	Excluded – review articles	Excluded – not in English	Total relevant papers
NCBI PubMed	24	11	2	0	11
Scopus	18	8	0	1	9
CAB Direct	26	10	0	6	10
Total relevant papers when duplicates removed					11

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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