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CLINICAL RESEARCH

Allogeneic chondrogenic-induced mesenchymal stem cells for the treatment of tarsometatarsal lameness in horses

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Abstract

Objective: To assess the efficacy of commercial intra-articular blood-derived allogeneic-induced mesenchymal stem cells (CIMSCs) to treat tarsometatarsal lameness in horses.

Study design: This was a retrospective cohort study.

Animals: Records from 167 adult light breed horses with bilateral tarsometatarsal lameness.

Methods: Horses with tarsometatarsal lameness were retrospectively selected from medical records. Diagnosis followed subjective graded lameness assessment before and after intra-articular analgesia, with graded radiographic tarsal examination. Horses were excluded if they were diagnosed or treated for any other concurrent lameness conditions during the study. Time to last follow-up and time of recurrence of lameness was recorded at veterinary re-assessment.

Results: A total of 67 horses were recruited to the CIMSC-treated group and 100 to the corticosteroid (CS)-treated group. Median age was 9 years, with no difference in signalment, use or radiographic grade between groups. First re-examination was 38 days (95% CI: 38–49), with no difference between groups, CIMSC 42 (35–45), control 34 (25–42). Median follow-up was 438 days for CIMSC, 546 for controls. Symptoms of lameness recurred in 86/100 controls compared to 17/67 (25%) CIMSC. Median time to lameness recurring in CIMSC was 336 days (95% CI: 239–400), control 90 days (95% CI: 80–108), $p < .0001$. Cox proportional hazard ratio for treatment was 8.35, 95% CI: 4.67 to 14.92, $p < .0001$.

Conclusions: Lameness was abolished in all treated horses. It recurred significantly less often, and later, in CIMSC-treated horses.

Clinical significance: Intra-articular CIMSC treatment results in prolonged soundness in horses with tarsometatarsal lameness.

1 | INTRODUCTION

Distal tarsal joint osteoarthritis is reported to be one of the most common causes of lameness in horses.[1,2] Lameness and radiographic signs both vary from mild to severe but do not seem to correlate with one another.[3] Traditional treatment options provide symptomatic pain relief by means of anti-inflammatory drugs, either administered systemically or locally.[3–5] Corticosteroids are commonly used and have wide-ranging anti-inflammatory effects in the joint environment at every level, including gene upregulation.[6,7] They are considered to exert their effects predominantly through cytoplastic receptors, blocking the prostaglandin cascade by means of inhibiting cyclooxygenase 1 and 2, as well as phospholipase A2.[8] Thus there are beneficial analgesic effects through blockage of inducible inflammatory mediators, but also deleterious effects through the concurrent blockage of the constitutive homeostatic mechanisms mediated through cyclooxygenase-1. This may create an additional risk of overloading a treated joint, potentially increasing damage to chondrocytes.[9] The association of prior local injection of corticosteroid with catastrophic musculoskeletal injuries is recognized as significant in racehorses.[10] Interest in alternative regenerative biological therapies has grown partly out of these safety concerns, with autologous conditioned serum, platelet-rich plasma and mesenchymal stem cells (MSCs) derived from fat, bone marrow and systemic blood all described.[11–14] Multipotent MSCs have significant immunomodulatory effects, suppressing inflammation when exposed to proinflammatory mediators,[15] whilst their potential attraction and subsequent incorporation into damaged joint tissues may also be beneficial.[16] The solution may be chondrogenic-induction, a technique whereby undifferentiated mesenchymal stem cells are cultured in a modified induction medium to preferentially develop into chondrocytes, consequently being attracted to damaged equine cartilage.[17,18] A commercially-licensed product for osteoarthritis in horses was recently released for use in Europe (Arti-Cell Forte).[12,19] A double blind placebo-controlled study demonstrated positive results in comparison to saline control in 75 adult Warmblood horses with clinical fetlock disease: from week 3 to 18, lameness scores, flexion test responses and joint effusion scores were all flexion test responses and joint effusion scores were all significantly improved in horses treated with the product.[20] Further studies published as part of a successful European market authorization (EMA) demonstrated safety and proof of concept in a larger population of horses using a metacarpophalangeal groove model.[12,19] This product is not currently approved by the FDA for use in the USA. An earlier unrelated study already demonstrated the potential benefits of MSCs for the treatment of TMT joint lameness. Sixteen horses were treated with intra-articular adipose-derived MSCs: 10 received MSCs, three betamethasone and three saline. At 30 days lameness had only resolved in the betamethasone group; at 60 days lameness had resolved in both MSC and betamethasone groups; at 90 and 180 days, only the MSC horses remained sound.

The aim of this retrospective study was to determine the effect of CIMSCs to treat naturally-occurring lower tarsal joint lameness. A control group treated using intra-articular corticosteroid was also recruited. The null hypothesis was that there would be no difference in symptomatic outcome or duration of soundness between horses treated with CIMSCs and those treated with corticosteroids.

2 | MATERIALS AND METHODS

2.1 | Study design

All horses presented to a single referral center for the treatment of clinical lameness or poor performance attributed to tarsometatarsal (TMT) joint disease were identified and selected retrospectively over a 4 year period, through clinical records. Individual animals were treated by using intra-articular CIMSC and a separate control group with intra-articular corticosteroids, with allocation to the two groups determined nonrandomly by owner preference. All animals recruited had to have been re-examined a minimum of once; those without follow-up, or with additional or concurrent orthopedic lameness conditions, were excluded. Horses treated with corticosteroids which did not improve at any time were excluded. Long-term follow-up was obtained by means of further veterinarian follow up on subsequent visits.

2.2 | Diagnostic method

All horses underwent full passive and dynamic physical examinations prior to diagnostic analgesia. Dynamic assessment was undertaken before and after every diagnostic analgesic block. This included straight line trotting in hand on a hard surface, full hindlimb flexion tests, lunging on a circle at trot on both hard and soft surfaces on both left and right reins, and cantering on the soft on both reins. Lameness was graded according to the AAEP scale 0–5. Many of the horses classified as grade 2 lame were difficult to see on the straight line but were consistently visible on lunging. Several factors were used to identify the subtle lameness in these horses and assign a more severely affected side. These included the presence and degree of transient ipsilateral lameness after tarsal flexion testing, the degree to which hindlimb cranial

phase was reduced on a hard surface with subsequent improvement on the soft, the reluctance of the horse to pick up and maintain a normal canter, leading with the inside hind leg. An inertial-based objective lameness device was not used. All horses underwent diagnostic analgesia in left and right hindlimbs simultaneously. During the examination, the distal limb and subtarsal areas were first blocked to exclude pain from this area, using low VI point and deep branch lateral plantar perineural analgesia, respectively. The TMT joint was then injected aseptically using a standard lateral approach.[2] Centrodistal (CD) joints were not injected. The diagnosis of TMT lameness was made following subjective resolution of lameness after injection with 60 mg mepivacaine per joint, at a maximum of 10 min post-block. Xylazine sedation (0.04–0.07 mg/kg IV) was used as warranted by the temperament of the horse.

Lateromedial and dorsoplantar radiographs[21] were obtained in all cases and retrospectively graded using a modified system derived from.[22] For these two projections, a grading system assessing four separate radiographic variables of "periarticular modeling," "subchondral bone lysis," "subchondral bone sclerosis," and "joint narrowing" on a scale of 0 to 2 (0 = none, 1 = mild, 2 = marked) was created. Radiographs were independently scored by two experienced equine orthopedic clinicians who were not radiologists but were fully blinded to all clinical details including treatment method. The more severely affected limb was used to grade the individual horse. An overall score of radiographic severity was then generated for each horse by adding the two clinician scores together, which could therefore theoretically range from 0 to 16. No horses underwent three-dimensional imaging or gamma scintigraphy.

2.3 | Treatment protocol

Treatment method was based on owner preference and was not randomized. In all cases, both hindlimbs were treated simultaneously in the TMT joint but not the CD joint. Aseptic injection technique was the same as described for the block, other than horses were heavily sedated using intravenous detomidine 0.01–0.02 mg/kg and butorphanol 0.02–0.03 mg/kg.

A commercially manufactured blood-derived CIMSC product was utilized (Arti-Cell Forte®, Boehringer Ingelheim Animal Health UK Ltd., Bracknell, UK). This product is presented in the form of two separate vials: one containing 1.4 to 2.5 × 10^6 CIMSCs suspended in 1 mL of Dulbecco's modified Eagle medium low glucose (DMEM-LG) with 10% dimethylsulfoxide (DMSO) to improve cell survival following cryopreservation; the other, 1 mL of equine allogenic plasma containing some platelets, reported to improve MSC viability. The range dosage of CIMSCs quoted is that provided the manufacturer of the product and was not controlled. No filtration of the allogenic plasma was undertaken.

The method of manufacture is detailed as part of the supporting documentation for the EMA.[18,20,23] Undifferentiated MSCs are harvested and cultured from the buffy coat of equine whole blood using a centrifugation at 1000 *g* for 20 min. After dilution in phosphate buffered saline (PBS), the resulting suspension is layered on a Percoll gradient (density 1.080 g/mL) and centrifuged at 600 *g* for 15 min. The interphase is collected, washed three times in PBS with centrifugation at 200 *g* for 10 min. The cells are then planted at 16 × 10^4 cells/cm^2 in a T_{75} flask in a culture medium containing DMEM-LG, supplemented with 30% fetal calf serum, 10–11 M low dexamethasone and antibiotic—antimycotic drugs, refreshed twice weekly. Putative MSCs are maintained at 37°C and 5% CO_2. At 60% confluency, cells are trypsinized with 0.25% trypsin–EDTA (P_0) and further cultured for 9 further passages (P_9) in expansion medium. Subsequent chondrogenic induction is carried out by means of culture with DMEM LG, 20% FCS, 1% AB/AM and growth factors including TGF-β_1 and IGF-1. These allogeneic CIMSCs are resuspended in DMEM LG and transported on dry ice.[24] Characterization and confirmation of chondrogenic induction was carried out by light microscopy utilizing hematoxylin and crystal violet staining. Biochemical induction was also ascertained using selected gene expression of cartilage oligometric matrix protein (COMP), and the presence of cell surface markers (CD45, MHC II, CD29, CD44, and CD90). Allogeneic plasma was collected from a donor by means of collection into a citrate phosphate dextrose adenine-1 container before freezing.

The two components, CIMSC and equine allogeneic plasma were defrosted in line with manufacturer's guidelines in ≤98 °F (37°C) water, combined by aspiration into a single syringe and then injected straight away. One dose was supplied per single tarsometatarsal joint, meaning two doses per horse. A minimum of 48 h was left between blocking and treatment to avoid any interaction with drug preservatives, the actual delay between diagnostic analgesia and CIMSC treatment was recorded. Horses were supplied a single intravenous dose of 1.1 mg/kg flunixin meglumine at the same time as injection with intra-articular CIMSC.

Control horses were injected in the same way into the tarsometatarsal joint, usually on the same day as the block. One of two drugs was selected for use during the study: triamcinolone acetonide (Kenalog Intra articular®, Bristol-Myers Squibb Pharmaceuticals Unlimited Company, Dublin, Republic of Ireland) (TA) at 10 mg per joint total

dose (20 mg per horse), or methylprednisolone acetate (Depo-Medrone 40 mg/mL suspension for injection®, Pfizer Limited, Kent, UK) (MPA) 20 to 40 mg per joint total dose (40 to 80 mg per horse). No other drugs, such as antibiotics, were injected in the joint and no intravenous flunixin was supplied to these horses. Choice of corticosteroid was based on type and use of horses and was not randomized. Competition horses were generally treated with TA; those out of competition or used for noncompetitive disciplines were treated with MPA.

2.4 | Rehabilitation protocol

Owners were supplied written discharge instructions following treatment. This included instructions to monitor for adverse side effects and seek veterinary attention in the event any were observed. These included local swelling, heat, pain and deteriorating lameness.

Stem cell-treated horses followed a plan based on manufacturer recommendations (Arti-Cell Forte®, Boehringer Ingelheim Animal Health UK Ltd). They received 3 days strict box rest with minimal walking only to assess comfort. Ten to 15 min of walking exercise was then encouraged from day 4 to the end of day 14 post treatment, either in hand, under saddle or on a walker. Some received oral sedatives to facilitate this. Small paddock turnout was then instructed, with increasing exercise levels to start including trotting, with faster work-up to week 8 allowing return to normal athletic duties.

Horses treated with corticosteroids were recommended to have 1 week off on field rest, then to spend a further week on relatively easy/gentle straight-line work. After 14 days they were encouraged to resume normal work. Supplementary manual therapy and corrective shoeing were recommended for all horses in the study. Where undertaken, manual therapy was carried out by external paraprofessionals and consisted of therapeutic massage of painful hypertonic muscles and joint mobilization techniques both in the affected limbs and spinal areas of the back.[25] This was not standardized or controlled.

2.5 | Reassessment and follow-up

All horses were recommended to return for their first reassessment at 6 weeks and at subsequent intervals where possible; the timing of all reassessments was recorded. Full passive and dynamic physical examinations were repeated in exactly the same way as described in 2.2. All reassessments were recorded. Diagnostic intra-articular analgesia was repeated in CIMSC horses experiencing symptomatic recurrence at reassessment to ascertain location. Control horses were blocked or remedicated. Individual horses left the study at the time point of first recurrence of lameness, or last follow up. Horses that went lame for other reasons at follow-up were excluded. The number of days to recurrence of lameness symptoms, or to the last day of follow up, created a "number of days sound" value for every horse.

2.6 | Statistical analysis

Data were collated on a spreadsheet (Excel®, Microsoft Corporation, 98052–6399 Washington, USA) and statistical analysis undertaken using specialist software (MedCalc Software Ltd., 8400 Ostend, Belgium, R Core Team [2022]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Continuous data for age and follow-up days was tested for normality using a d'Agostino Pearson test. Interobserver agreement was tested for radiographic grade scores by calculating a weighted kappa value. Categorical data were compared by means of χ^2 and continuous data using Mann–Whitney U tests to compare groups. Survival analysis then measured and compared the duration of soundness for the different treatments by means of Kaplan–Meier plots and Cox proportional hazards modeling. Statistical significance was set at $p \leq .05$.

3 | RESULTS

3.1 | Case data

A total of 355 horses were identified from which 167 met the inclusion criteria: 67 in group CIMSC and 100 controls. Of the 188 horses excluded, 137 were excluded on the basis of having additional sources of pain at initial diagnosis: 100 proximal suspensory region pain, 38 cervical OA, 25 stifle pain and six lower limb. A total of 20 horses were excluded on the basis of having gone lame at follow-up for a different reason: three in CIMSC, one proximal suspensory region pain and two stifle injuries; 17 controls, six proximal suspensory, two stifle and nine no specific diagnosis made. An additional 31 horses were excluded on the basis of having had no follow-up after corticosteroid treatment.

Median age at treatment was 9 years, range 4 to 20, with no difference between the two groups, $p = .52$. Breeds represented were: 46 Thoroughbred and Thoroughbred crosses, 29 Warmbloods, 29 Sports horses, 27 Cobs and their crosses, 27 ponies, seven draft breeds and two Arabs. Sex distribution was 101 geldings and 66 mares. Uses varied, with 20 used for primarily for dressage, 32 for jumping,

26 for athletic work (racing, eventing, endurance and mounted games); the remaining 89 were classed as general-purpose horses. There was no difference between test and control groups in the breed type $\chi^2(6) = 2.73$, $p = .84$, proportion of mares and geldings $\chi^2(1) = 0.11$, $p = .74$ and use $\chi^2(3) = 4.08$, $p = .25$.

Lameness grade at presentation was median 2, range 2 to 4. The CIMSC horses were lamer overall, $p = .02$. A total of 63 horses were considered more severely affected left hind, 79 right hind and eight equally affected, with no difference between groups, $\chi^2(2) = 0.89$, $p = .64$.

3.2 | Radiology

All 67 CIMSC horses and 97 of 100 controls had radiographs available for grading. Weighted kappa for the two observers was 0.65, 95% CI: 0.57–0.73, indicating fair inter-observer agreement. Median grade was 5 ± SD 3.3, range 0–16. There was no correlation between lameness and radiographic grade, Spearman rank correlation $p = .56$.

In both treatment groups the median was grade was 5, ±SD4.2 in group CIMSC and ± SD2.6 in the controls. There was no difference in radiographic severity between the two groups, $p = .66$, Figure 1. Likewise, there was no detectable difference in radiographic grade between TA and MPA treated horses, $p = .80$.

3.3 | Clinical outcome

Horses in the CIMSC group were treated median 14 days after diagnostic analgesia, 95% CI: 9–16. No horses in either group were reported by owners to have experienced any detectable adverse reaction to treatment. First re-examination was 38 days (95% CI: 38–49), with no difference between groups: CIMSC 42 (35–45), control 34 (25–42), $p = 0.11$. All CIMSC horses and 79 controls

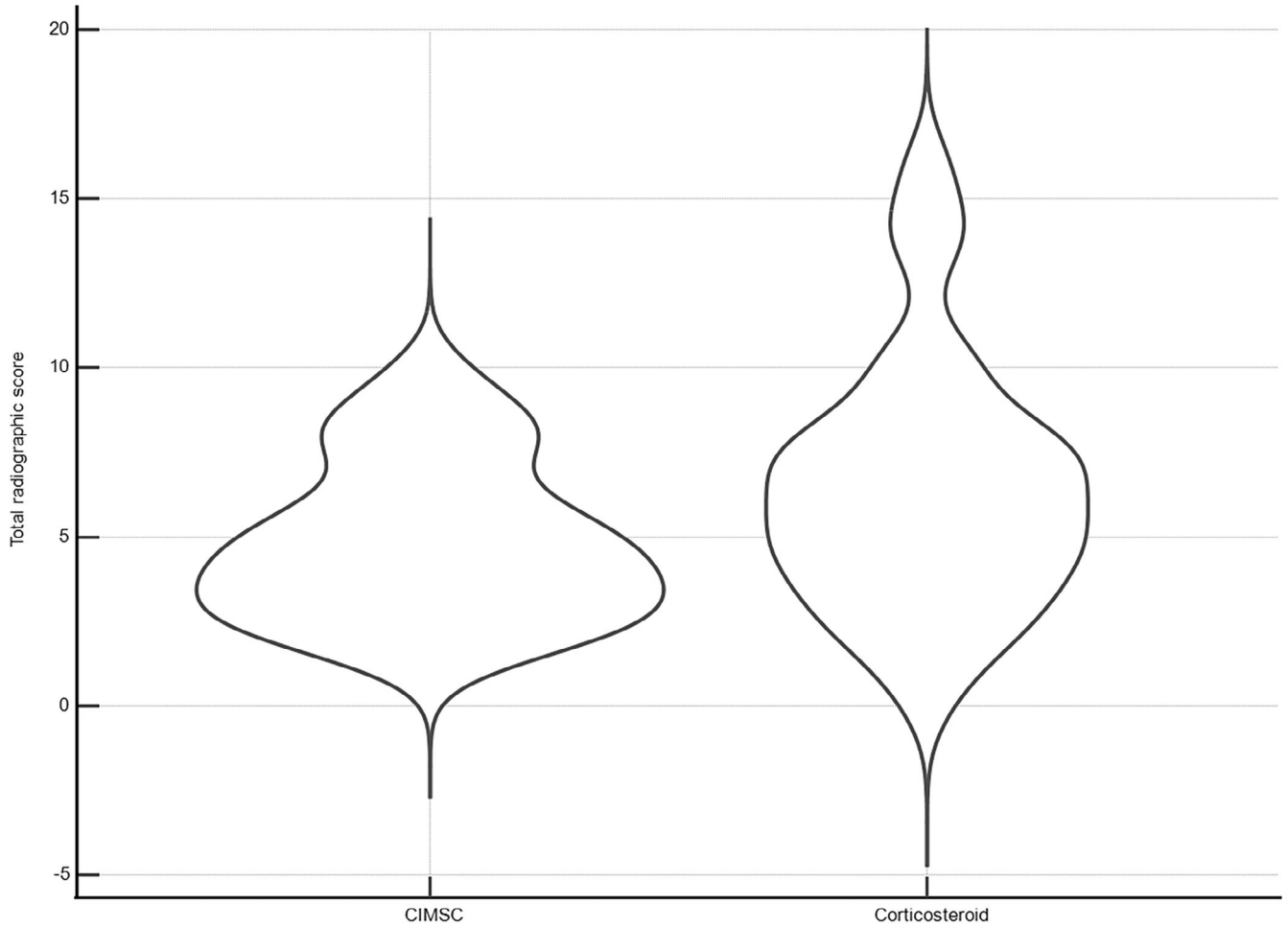

FIGURE 1 Violin plot showing the range of radiographic severity for the 67 horses treated with chondrocyte-induced mesenchymal stem cells (CIMSC) and 100 control horses treated with intra-articular corticosteroids. Median grade was 5 in both and there was no significant difference, $p = .6581$.

went sound after treatment for median 148 days (95% CI: 111–181). A further 21 control horses were reported to have gone sound after treatment but only re-presented when lameness recurred. Median follow-up was 438 days for CIMSC horses (95% CI: 344–542), 546 for controls (95% CI: 407–690), Mann–Whitney U = 2641.5, $p = .03$. Horses in group CIMSC were sound for 336 days (95% CI: 239–400), control horses for 90 days (95% CI: 80–108), Mann–Whitney U = 5183, $p < .0001$. Horses that stayed sound during the study varied significantly, with 17 (25%) CIMSC going lame again, compared to 86 (86%) control horses. Success rate for horses remaining sound was therefore 75% for CIMSC versus 14% for corticosteroids, $p < .0001$. Of the 17 CIMSC horses recurring, three had repeat treatment with CIMSC, 12 were treated with intra-articular corticosteroids, one managed on oral firocoxib and two were retired. Of the 86 control horses recurring, 55 were remedicated with corticosteroids, 11 managed with polyacrylamide hydrogel and 20 managed on oral NSAIDs.

Figure 2 shows a Kaplan–Meier plot of time to recurrence of lameness or censoring as a function of treatment condition. The two control conditions did not differ but are plotted separately in Figure 2 for interest. Cox proportional hazard modeling indicated that controlling for age, breed, sex, lameness grade and radiographic severity, hazard ratio for treatment was 8.35, 95% CI: 4.67–14.92, $p < .0001$.

Within the control group, 66 horses were treated with MPA and 34 with TA. There was no detectable difference in outcome between these two drugs: 55 (83%) MPA-treated horses recurred, compared to 31 (91%) for TA, χ^2 $p = .28$. Similarly, if the two control treatments were separated in the Cox test, they produced extremely similar hazard ratios relative to CIMSC: 8.84 (4.81–16.22) for MPA and 7.59 (3.92–14.70) for TA.

4 | DISCUSSION

This study demonstrated that compared to corticosteroids in a clinical setting, intra-articular treatment with CIMSC resulted in long-lasting soundness in a high proportion of horses with naturally-occurring TMT lameness. Corticosteroids inhibit cartilage homeostasis at the same time as inhibiting the painful inflammatory pathways mediated through cyclo-oxygenase, impairing any intrinsic repair mechanisms.[8] Mesenchymal stem cells (MSCs) also modulate inflammation[26,27] but are also attracted to damaged tissues including menisci, cruciate ligaments and cartilage[16] where in contrast to corticosteroids, they might provide an alternative potential repair mechanism. Previous studies have demonstrated symptomatic benefits of intra-articular MSCs in horses, therefore the benefits we observed in this cohort of horses with naturally occurring disease support those preliminary studies.[17,28] Malformation of the distal tarsal cuboidal bones through osteochondrosis,[29] or excessive concussion to them through use or surface,[30] are both considered likely means of joint injury leading to pain and OA. Genetic predisposition is also a likely factor, with high

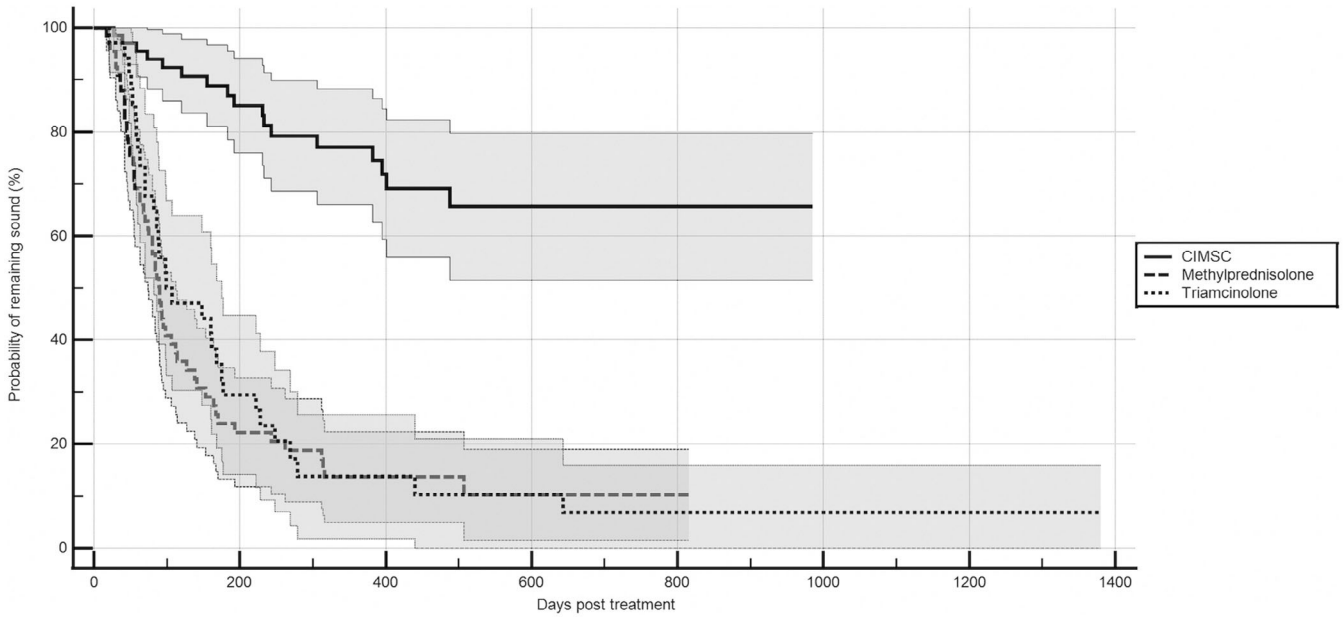

FIGURE 2 Kaplan Meier plot comparing the duration of soundness for chondrocyte-induced mesenchymal stem cells (CIMSC)-treated horses compared to controls treated with corticosteroids, shaded areas indicating 95% confidence intervals. Cox proportional hazard ratio was 8.35 (95% CI: 4.67–14.92, $p < .0001$) controlling for a range of background variables. No difference in results between triamcinolone and methylprednisolone in control horses was present, but these groups are shown separately for information.

rates of disease in breeds like Icelandic ponies.[31,32] In horses with profound active osteoarthritis, where changes extend deeply into the subchondral bone, the repair benefits of articular CIMSCs may be limited.

A number of important limitations of a retrospective clinical study of this type need to be kept in mind when interpreting the results presented. Owners and clinicians were not blinded to treatment and the very high cost of stem cell treatment in comparison to corticosteroids increased the risk of bias. This could affect owner's reported positive results at the time of re-assessment during history taking, subsequently increasing the risk of clinicians being tempted to overlook mild lameness in these same horses when re-assessing them. This form of bias cannot be excluded in this type of retrospective study. Because the majority of the horses undergoing treatment were funded by a third-party insurance company, differences in treatment cost were much less important than actual physical performance for these owners. Additionally, in the author's experience, the limited time (12 months) statute on these insurance policies increased motivation to seek regular reassessments and for owners to remain critical and vigilant of lameness. Arguably, this factor acted to decrease the risk of unduly optimistic interpretation of soundness following either treatment.

Using a commercially-produced proprietary "off-the-shelf" live MSC product carries significant concerns for many based on questions over provenance, safety and effectiveness. As noted in the EMA documentation, studies proving fundamental mechanism of action, distributions and patterns of distribution of equine MSCs are scarce. The presence of DMSO as an excipient in the Dulbecco's modified Eagle medium has the potential to act as an anti-inflammatory in its own right.[33] The conclusion of the CVMP assessment report for Arti-Cell Forte was that the final quantity of 5% DMSO, as well as DMEM-LG, was so low that no clinically significant pharmacological effect was anticipated.[23] The presence of platelets in the allogeneic plasma component might also have been anticipated to have some benefit. Given the very low levels, within the normal plasma range for horses, this was again considered insignificant for the treatment of OA. Nevertheless, some may still disagree with the interpretation of the current evidence as assessed by the CVMP committee, potentially undermining the positive results of CIMSC that we report.

Rehabilitation time varied significantly between groups, with CIMSC horses taking longer, creating a significant potential source of bias in this study. Evidence exists to indicate that a much longer response to intra-articular corticosteroid was obtained in people treated for knee synovitis with 24 h rest compared to those treated on an outpatient basis.[34] Some authors have extrapolated this to horses, though no published studies support it.[6] For the fairest possible comparison, horses would have followed exactly the same post-treatment regime, with control horses having more rest. Instead, in the absence of a generally accepted 'gold standard', the rehabilitation program followed standard textbook recommendations.[5] The importance of a period of physical rehabilitation involving exercise, strength training and manual therapy following treatment and rest for orthopedic injury and disease is well established in man but the advantages are less well defined in horses.[35]

The distal tarsal joints consist of the TMT and CD joints, two structures with direct physical communication in around one third of horses,[36] but between which both local anesthetic and corticosteroids have been shown to diffuse in therapeutic quantities in all horses.[37,38] There is debate as to the rates of communication and whether they differ in vivo, some studies having been carried out post-mortem.[1] A further potential limitation of this study is that only the TMT joint was injected in all horses. The CD joint was not treated with CIMSC in this study for practical reasons, the final volume being 2 mL. Early audited positive experiences with only treating the TMT had also led the authors to have confidence utilizing this approach in the larger cohort reported here. Whatever the reality and rate of communication, no studies exist to show therapeutic migration or diffusion of MSCs between these two joints and our results therefore have to be interpreted in this light.

Horses in this study had lameness localized to the TMT joint and many were bilaterally lame, complicating lameness detection. Although OA is reported to be the most common cause, evidence suggests that diagnostic analgesia of the small tarsal joints also numbs pain in the proximal suspensory,[1] and possibly vice versa blocking the deep branch of the lateral plantar nerve.[39,40] Intertarsal ligament tearing is a cause of lameness in the lower tarsal joints that was discovered following the introduction of magnetic resonance imaging (MRI),[2] whilst some fractures of the central and third tarsal bones could be missed on two orthogonal radiographs.[41] Though horses with some other causes of distal tarsal pathology were excluded, none had scintigraphy, computed tomography or magnetic resonance imaging. There was no evidence that radiographic severity or lameness degree had any effect on apparent success, and some horses treated with corticosteroids also experienced resolution of symptoms. Radiographic scoring has a long history of not correlating with clinical signs of lameness in distal tarsal osteoarthritis,[3] however complex and detailed they are.[31,42–44]

Few equine OA studies have utilized survival analysis to visualize the rate of success following a treatment over a long period like this. The benefit of using this method

of analysis is that each day sound contributed to the overall result, meaning horses with differing periods of follow-up were both able to contribute in a way that logistic regression modeling could not control. Older studies charting the clinical symptomatic results of intra-articular corticosteroids in horses with bone spavin are scant, but indicate a positive result by day 57[4] and by day 60.[28] Control horses in this study were followed up after this time, when their symptoms recurred. Survival analysis of culling rates of Icelandic ponies due to bone spavin has been reported,[32] but that study did not look at treatment methods, focussing instead on age as the main negative factor increasing risk of culling. Age did not have a detectable effect of outcome in this study, possibly through limited numbers.

In summary, a single intra-articular CIMSC treatment for tarsometatarsal lameness resulted in longer-lasting symptomatic relief of lameness compared to that observed after corticosteroids. Important limitations of a retrospective clinical study of this type need to be kept in mind when interpreting the results. Further studies are indicated to improve the selection method for horses with tarsometatarsal lameness.

AUTHOR CONTRIBUTIONS

Coomer RPC, MA, VetMB, CertES(Soft Tissue), Diplomate ECVS, MRCVS: Conceived and designed the study, participated in treatment, carried out data collection, participated in radiological grading, initial statistical analysis and writing the manuscript. Terschuur JA, MRCVS: Participated in treatment, radiological grading and contributed to manuscript writing. Pressanto M, PhD, MRCVS: Participated in treatment, radiological grading and contributed to manuscript writing. Walker I, BSc (Hons), DPhil: Carried out statistical analysis and contributed to manuscript writing.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared. In this retrospective study, archived material from the clinical records of patients were used. To use these records, their owners were informed, and written permission was given. No declared research grant from any funding agency in the public, commercial or not-for-profit sectors.

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REFERENCES

1. Bell BTL, Baker GJ, Foreman JH, Abbott LC. In vivo investigation of communication between the distal Intertarsal and Tarsometatarsal joints in horses and ponies. *Vet Surg*. 1993;22(4):289-292. doi:10.1111/j.1532-950X.1993.tb00400.x
2. Kawcak CE. Joint disease in the horse. In: McIllwraith CW, Frisbie DD, Kawcak CE, René van Weeren P, eds. *Tarsus*. Elsevier; 2016:340-353.
3. Gough M, Munroe G. Decision making in the diagnosis and management of bone spavin in horses. *In Pract*. 1998;20:252-299.
4. Labens R, Mellor D, Voûte L. Retrospective study of the effect of intra-articular treatment of osteoarthritis of the distal tarsal joints in 51 horses. *Vet Record*. 2007;161:611-616.
5. Dyson S, Ross M. The Tarsus. In: Ross M, Dyson S, eds. *Diagnosis and Management of Lameness in the Horse*. Elsevier; 2011:508-526.
6. Caron JP. Intra-articular injections for joint disease in horses. *Vet Clin North Am Equine Pract*. 2005;21(3):559-573. doi:10.1016/j.cveq.2005.07.003
7. Platt D. Review of current methods available for the treatment of bone spavin. *Equine Vet Educ*. 1997;9(5):258-264. doi:10.1111/j.2042-3292.1997.tb01320.x
8. Masferrer JL, Seibert K. Regulation of prostaglandin synthesis by glucocorticoids. *Receptor*. 1994;4(1):25-30.
9. Chunekamrai S, Krook L, Lust G, Maylin G. Changes in articular cartilage after intra-articular injections of methylprednisolone acetate in horses. *Am J Vet Res*. 1989;50(10):1733-1741.
10. Whitton RC, Jackson MA, Campbell AJD, et al. Musculoskeletal injury rates in thoroughbred racehorses following local corticosteroid injection. *Vet J*. 2014;200(1):71-76. doi:10.1016/j.tvjl.2013.09.003
11. Kisiday JD, Kopesky PW, Evans CH, Grodzinsky AJ, Mcllwraith CW, Frisbie DD. Evaluation of adult equine bone marrow- and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. *J Orthop Res*. 2008;26(3):322-331. doi:10.1002/jor.20508
12. Broeckx SY, Martens AM, Bertone AL, et al. The use of equine chondrogenic-induced mesenchymal stem cells as a treatment for osteoarthritis: a randomised, double-blinded, placebo-controlled proof-of-concept study. *Equine Vet J*. 2019;51(6):787-794. doi:10.1111/evj.13089
13. Carmona JU, Ríos DL, López C, Álvarez ME, Pérez JE, Bohórquez ME. In vitro effects of platelet-rich gel supernatants on histology and chondrocyte apoptosis scores, hyaluronan release and gene expression of equine cartilage explants challenged with lipopolysaccharide. *BMC Vet Res*. 2016;12(1):135. doi:10.1186/s12917-016-0759-8
14. Frisbie D, Kawcak C, Werpy N, Park R, McIlwraith CW. Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis. *Am J Vet Res*. 2007;68(3):290-296.
15. Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, Borjesson DL. Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Med*. 2012;4(1):1-12. doi:10.3727/215517912x647217
16. Agung M, Ochi M, Yanada S, et al. Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to

tissue regeneration. *Knee Surg Sports Traumatol Arthrosc*. 2006; 14(12):1307-1314. doi:10.1007/s00167-006-0124-8
17. Broeckx S, Zimmerman M, Crocetti S, et al. Regenerative therapies for equine degenerative joint disease: a preliminary study. *PLoS ONE*. 2014;9(1):e85817. doi:10.1371/journal.pone.0085917
18. Spaas JH, De Schauwer C, Cornillie P, Meyer E, Van Soom A, Van de Walle GR. Culture and characterisation of equine peripheral blood mesenchymal stromal cells. *Vet J*. 2013;195(1): 107-113. doi:10.1016/j.tvjl.2012.05.006
19. Broeckx SY, Spaas JH, Chiers K, et al. Equine allogeneic chondrogenic induced mesenchymal stem cells: a GCP target animal safety and biodistribution study. *Res Vet Sci*. 2018;117: 246-254. doi:10.1016/j.rvsc.2017.12.018
20. Broeckx SY, Seys B, Suls M, et al. Equine allogeneic Chondrogenic induced mesenchymal stem cells are an effective treatment for degenerative joint disease in horses. *Stem Cells Dev*. 2019;28(6):410-422. doi:10.1089/scd.2018.0061
21. Weaver M, Barakzai S. Radiography of the tarsus. In: Weaver M, Barakzai S, eds. *Handbook of Equine Radiography*. First. Edinburgh: Elsevier; 2010:79-93.
22. van Hoogmoed LM, Snyder JR, Thomas HL, Harmon FA. Retrospective evaluation of equine prepurchase examinations performed 1991-2000. *Equine Vet J*. 2003;35(4):375-381. doi:10.2746/042516403776014325
23. CVMP. Assessment report for Arti-Cell Forte. 2018 https://www.ema.europa.eu/en/documents/assessment-report/arti-cell-forte-epar-public-assessment-report_en.pdf. Accessed July 14, 2023.
24. Spaas JH, Broeckx SY, Chiers K, et al. Chondrogenic priming at reduced cell density enhances cartilage adhesion of equine allogeneic MSCs-a loading sensitive phenomenon in an organ culture study with 180 explants. *Cell Physiol Biochem*. 2015; 37(2):651-665. doi:10.1159/000430384
25. Haussler KK. Joint mobilization and manipulation for the equine athlete. *Vet Clin North Am Equine Pract*. 2016;32(1):87-101. doi:10.1016/j.cveq.2015.12.003
26. Mak J, Jablonski CL, Leonard CA, et al. Intra-articular injection of synovial mesenchymal stem cells improves cartilage repair in a mouse injury model. *Sci Rep*. 2016;6:23076. doi:10.1038/srep23076
27. Zayed M, Adair S, Dhar M. Effects of normal synovial fluid and interferon gamma on chondrogenic capability and immunomodulatory potential respectively on equine mesenchymal stem cells. *Int J Mol Sci*. 2021;22(12):6391. doi:10.3390/ijms22126391
28. Nicpoń J, Marycz K, Grzesiak J. Therapeutic effect of adipose-derived mesenchymal stem cell injection in horses suffering from bone spavin. *Pol J Vet Sci*. 2013;16(4):753-754. doi:10.2478/pjvs-2013-0107
29. Sigurdsson SF, Olstad K, Ley CJ, Björnsdóttir S, Griffiths DJ, Fjordbakk CT. Radiological, vascular osteochondrosis occurs in the distal tarsus, and may cause osteoarthritis. *Equine Vet J*. 2022;54(1):82-96. doi:10.1111/evj.13432
30. Tranquille CA, Blunden AS, Dyson SJ, Parkin TDH, Goodship AE, Murray RC. Effect of exercise on thicknesses of mature hyaline cartilage, calcified cartilage, and subchondral bone of equine tarsi. *Am J Vet Res*. 2009;70(12):1477-1483.
31. Björnsdóttir S, Axelsson M, Eksell P, Sigurdsson H, Carlsten J. Radiographic and clinical survey of degenerative joint disease in the distal tarsal joints in Icelandic horses. *Equine Vet J*. 2000; 32(3):268-272. doi:10.2746/042516400776563590
32. Björnsdóttir S, Árnason T, Lord P. Culling rate of icelandic horses due to bone spavin. *Acta Vet Scand*. 2003;44:161-169.
33. Sotelo EDP, Vendruscolo CP, Fülber J, et al. Effects of joint lavage with dimethylsulfoxide on LPS-induced synovitis in horses-clinical and laboratorial aspects. *Vet Sci*. 2020;7(2):57. doi:10.3390/VETSCI7020057
34. Chakrabarty K, Pharoah PDP, Scott DGI. A randomized controlled study of post-injection rest following intra-articular steroid therapy for knee synovitis. *Br J Rheumatol*. 1994;33:464-468.
35. Bergh A, Asplund K, Lund I, Boström A, Hyytiäinen H. A systematic review of complementary and alternative veterinary medicine in sport and companion animals: soft tissue mobilization. *Animals*. 2022;12(11):11440. doi:10.3390/ani12111440
36. Kraus-Hansen AE, Jann HW, Kerr DV, Fackelman GE. Arthrographic analysis of communication between the Tarsometatarsal and distal Intertarsal joints of the horse. *Vet Surg*. 1992; 21(2):139-144. doi:10.1111/j.1532-950X.1992.tb00032.x
37. Gough MR, Munroe GA, Mayhew IG. Diffusion of mepivacaine between adjacent synovial structures in the horse. Part 2: Tarsus and stifle. *Equine Vet J*. 2002;34(1):85-90. doi:10.2746/042516402776181088
38. Serena A, Schumacher J, Schramme MC, Degraves F, Bell E, Ravis W. Concentration of methylprednisolone in the centrodistal joint after administration of methylprednisolone acetate in the tarsometatarsal joint. *Equine Vet J*. 2005;37(2):172-174. doi:10.2746/0425164054223778
39. Contino EK, King MR, Valdés-Martínez A, Mcilwraith CW. In vivo diffusion characteristics following perineural injection of the deep branch of the lateral plantar nerve with mepivacaine or iohexol in horses. *Equine Vet J*. 2015;47(2):230-234. doi:10.1111/evj.12261
40. Hinnigan G, Milner P, Talbot A, Singer E. Is anaesthesia of the deep branch of the lateral plantar nerve specific for the diagnosis of proximal metatarsal pain in the horse? *Vet Comp Orthop Traumatol*. 2014;27(5):351-357. doi:10.3415/VCOT-13-12-0146
41. Tulamo R, Bramlage LR, Gabel AA. Fractures of the central and third tarsal bones in horses. *J Am Vet Med Assoc*. 1983; 182(11):1234-1238.
42. Dik KJ, Enzerink E, van Weeren PR. Radiographic development of osteochondral abnormalities, in the hock and stifle of Dutch warmblood foals, from age 1 to 11 months. *Equine Vet J Suppl*. 1999;31:9-15. doi:10.1111/j.2042-3306.1999.tb05308.x
43. Byam-Cook KL, Singer ER. Is there a relationship between clinical presentation, diagnostic and radiographic findings and outcome in horses with osteoarthritis of the small tarsal joints? *Equine Vet J*. 2009;41(2):118-123. doi:10.2746/042516408X345107
44. Labens R, Innocent GT, Voûte LC. Reliability of a quantitative rating scale for assessment of horses with distal tarsal osteoarthritis. *Vet Radiol Ultrasound*. 2007;48(3):204-211. doi:10.1111/j.1740-8261.2007.00230.x

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