Inflammatory effects of autologous, genetically modified autologous, allogeneic, and xenogeneic mesenchymal stem cells after intra-articular injection in horses

J. H. Pigott¹; A. Ishihara¹; M. L. Wellman²; D. S. Russell²; A. L. Bertone¹

¹Comparative Orthopedics Research Laboratory, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, Ohio, USA; ²Comparative Orthopedics Research Laboratory, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

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Summary
Objectives: To compare the clinical and inflammatory joint responses to intra-articular injection of bone marrow-derived mesenchymal stem cells (MSC) including autologous, genetically modified autologous, allogeneic, or xenogeneic cells in horses.

Methods: Six five-year-old Thoroughbred mares had one fetlock joint injected with Gey's balanced salt solution as the vehicle control. Each fetlock joint of each horse was subsequently injected with 15 million MSC from the described MSC groups, and were assessed for 28 days for clinical and inflammatory parameters representing synovitis, joint swelling, and pain.

Results: There were not any significant differences between autologous and genetically modified autologous MSC for synovial fluid total nucleated cell count, total protein, interleukin (IL)-6, IL-10, fetlock circumference, oedema score, pain-free range-of-motion, and soluble gene products that were detected for at least two days. Allogeneic and xenogeneic MSC produced a greater increase in peak of inflammation at 24 hours than either autologous MSC group.

Clinical significance: Genetically engineered MSC can act as vehicles to deliver gene products to the joint; further investigation into the therapeutic potential of this cell therapy is warranted. Intra-articular MSC injection resulted in a moderate acute inflammatory joint response that was greater for allogeneic and xenogeneic MSC than autologous MSC. Clinical management of this response may minimize this effect.

Introduction
Direct intra-articular injection of bone marrow-derived mesenchymal stem cells (BMDMSC) has demonstrated modest therapeutic efficacy in models of cartilage defects, osteoarthritis, cruciate ligament injury, and meniscus damage in rats, pigs and goats (1–3). In horses with superficial digital flexor tendinopathy, recent studies have demonstrated that intra-lesional BMDMSC may reduce the reinjury rate after returning to racing (4). Direct intra-articular BMDMSC injection with hyaluronan improved repair quality in a cartilage defect model in the stifle joint of horses, warranting further study of these cells for potential treatment of equine joint disease (5).

Mesenchymal stem cells (MSC) from equine bone marrow are considered to be multipotent as demonstrated by trilineage differentiation in vitro (6–8). These cells can be further characterized by the positive expression of MHCI, cluster of differentiation (CD)90, CD44, CD29 and the negative expression of major histocompatibility complex (MHC) class II (MHCII), CD14 and CD79α (9, 10). The properties of trilineage differentiation (chondrogenic, adipogenic and osteogenic), plastic adherence in culture, MHCII negative expression, and CD90 positive expression were selected for MSC characterization in this study. Mesenchymal stem cells can be expanded and genetically manipulated in standard culture conditions making them an attractive target for gene therapy (11, 12). Genetic alteration of cells allows for
the release of soluble factors that may provide an enhanced therapeutic response (13). Bone morphogenetic protein 2 (BMP-2) has demonstrated chondrogenic effects and safety both in vitro and in animal models, including horses, when placed in the articular environment (14–16). Bone morphogenetic protein 2 was therefore selected for the investigation of the inflammatory response to these genetically engineered autologous BMDMSC as a potential therapeutic delivery method for anabolic growth factors to the joint.

Few studies have characterized joint inflammation after intra-articular injection of MSC in any species, with limited reports in the horse (17–19). A recent short-term (72 hrs.) study compared the inflammatory effects of autologous or allogeneic placenta-derived MSC after intra-articular injection of 7.5 x 10⁶ cells in horses. Although the changes in several of the outcome parameters measured were not statistically different, the injection of allogeneic MSC was associated with a greater degree of inflammation for approximately the first 36 hours than the autologous MSC or saline control (17). Our study further investigated the joint clinical and inflammatory reaction to BMDMSC after intra-articular injection of a greater dose of cells (15 x 10⁶ cells), and groups of cells representing potential sources for intra-articular cell therapy; autologous (Auto) BMDMSC, a genetically modified autologous (Auto-BMP-2) BMDMSC, an allogeneic (Allo) BMDMSC or a xenogeneic (Xeno) BMDMSC. Our hypotheses were that there would be no significant difference in the clinical and synovial inflammatory responses among these cell groups and that the BMP-2 soluble gene product would be detectable in the synovial fluid. One goal of this work was to demonstrate that intra-articular cells could potentially be used to deliver anabolic growth factors to joints and that various cell sources, more convenient than autologous cells, would be tolerated by the joint in terms of an acceptable clinical response.

**Materials and methods**

**Horses and study design**

Six five-year-old Thoroughbred mares, free of musculoskeletal abnormalities in the metacarpal(tarsal)phalangeal (fetlock) joints as determined by palpation, range-of-motion, and flexion tolerance of the fetlock joint, were included in the study. Twenty-eight days prior to MSC injection, the horses had a randomly chosen fetlock joint injected with 1 mL of Gey’s balanced salt solution (GBSS) and clinical and synovial fluid parameters were measured for 28 days as per the MSC protocol. Subsequently (day 0), fetlock joints were assigned in a block design such that every horse had each of the four fetlock joints treated with one of four BMDMSC treatments: 1) Auto, 2) Auto-BMP-2, 3) Allo (from a different study horse) or 4) Xeno (human). Fetlocks were assigned such that the BMDMSC treatments were equally divided between forelimbs and hindlimbs. Clinical and synovial fluid parameters were evaluated serially on days 1, 2, 7, 14, 21 and 28 after GBSS injection and subsequently on days 1, 2, 7, 14, 21 and 28 after MSC injection. Horses were confined to stall rest during the study period and limbs were not bandaged throughout the study. Non-steroidal anti-inflammatory drugs were not administered so as to not interfere with the inflammatory response and to provide an unaltered clinical response to the injections. All procedures were approved by the Institutional Animal Care and Use Committee at The Ohio State University.

**Mesenchymal stem cells isolation and characterization**

Bone marrow-derived mesenchymal stem cells from these six study horses were isolated, expanded in primary culture and cryopreserved prior to the start of this study. Briefly, 60 mL of bone marrow was obtained from the sternum while the horses were under general anesthesia and this was concentrated using a gravitational marrow separator with subsequent culture in monolayer (20). Mesenchymal stem cells were identified based on plastic adherence under standard cell culture conditions: Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS) at 37°C in a 5% CO₂ atmosphere. Mesenchymal stem cells were divided into aliquots of 1x10⁶ cells and were placed in 1 mL of FBS containing 10% dimethyl sulfoxide and frozen at −80°C for future use. Upon thawing, the cells were passaged two to four times, harvested by trypsinization, counted using a haemacytometer, and viability was confirmed with trypan blue exclusion stain. Xenogeneic BMDMSC were similarly expanded and processed.

The multipotential nature of these BMDMSC (equine and human) was confirmed by differentiation into osteogenic, adipogenic, and chondrogenic lineages in vitro using the manufacturer’s recommended protocol for human MSC. For osteogenic differentiation, cells were maintained in monolayer culture for approximately 14 days and stained for production of mineral with von Kossa (21). For adipogenic differentiation, cells were maintained in monolayer culture for 21 days and stained for lipid production with oil red O. For chondrogenic differentiation, cells were expanded in monolayer culture and approximately 2.5 x 10⁶ cells were formed into a pellet. Cells were maintained in chondrogenic culture for 21 days and then fixed in formalin, sectioned (4 μm) and stained for glycosaminoglycan production and chondrocyte morphology with toluidine blue.

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a GBSS: Sigma Aldrich, St. Louis, MO, USA
b Human Bone Marrow Stromal Cells: Lonza Inc., Walkersville, MD, USA
c MarrowStim™ Concentration Kit: Biomet Biologics LLC., Warsaw, IN, USA
d DMEM: Gibco Life Technologies, Grand Island, NY, USA
e Penicillin-streptomycin: Gibco Life Technologies, Grand Island, NY, USA
f FBS: Sigma Aldrich, St. Louis, MO, USA
g DMSO: Sigma Aldrich, St. Louis, MO, USA
h Trypsin: Gibco Life Technologies, Grand Island, NY, USA
i Trypan blue: Sigma Aldrich, St. Louis, MO, USA
j Oil red O staining protocol for adipogenic cultures (Protocol SC 00011): ThermoFisher Scientific, Pittsburgh, PA, USA
k Comparative Pathology and Histology/Immunohistochemistry: Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA
Bone marrow-derived MSC (equine and human) were further characterized based on cell surface expression of MHCII and CD90. Briefly, BMDMSC from the same culture flask were trypsinized, washed twice with phosphate buffered saline, counted by haemacytometer, and one million cells were placed in two separate tubes. To serve as flow cytometry control cells to compare to MSC, peripheral blood mononuclear cells (PBMC) were obtained from the same horses and isolated by Ficoll density gradient from 30 mL of heparinized venous blood per horse by following established company protocols. Briefly, 15 mL of blood mixed with 15 mL of phosphate buffered saline (PBS) containing 2% FBS was carefully layered on top of 15 mL of Ficoll (2 tubes per horse to process a total of 30 mL blood) and centrifuged at room temperature for 30 minutes. The upper plasma layer was removed, and the PBMC layer at the plasma-Ficoll interface was removed and retained. The PBMC were washed twice with PBS, counted using a haemacytometer and placed in two separate tubes (1 million cells/tube) as a control cell type. Mesenchymal stem cells and PBMC were mixed with anti-equine MHCII monoclonal antibody (0.5 μL/million cells), anti-mouse secondary antibody (0.5 μL/million cells), and 7-aminoactinomycin D (7AAD: 3.5 μL/million cells) fluorescent chemical or with anti-human CD90 primary monoclonal antibody (0.5 μL/million cells), and 7AAD (3.5 μL/million cells). The cells were then incubated for two hours at 4°C in a dark atmosphere, washed twice with PBS, and analyzed with a flow-cytometry analyzer. For each sample, all cells were gated and analyzed using forward and side light scatter. The numbers of viable MHCII positive or CD90 positive cells were calculated by counting the total cells and MHCII(+) 7AAD(-) cells in the first tubes, and the total cells and CD90(+) 7AAD(-) cells in the second tubes. Negative controls consisted of 7AAD without cells and non-stained cells while positive controls consisted of killed cells (5 minutes with alcohol) with 7AAD and stained PBMC.

**Production of BMP-2 by transduced BMDMSC**

Cryopreserved BMDMSC for each horse were thawed, seeded at approximately 500,000 cells per culture flask (25 cm²), maintained until approximately 80–90% confluence, and then transduced with an adenovirus (Ad) serotype 5 vector with an E1 deletion encoding the human BMP-2 gene (Ad-BMP-2). At 100 multiplicity of infection (MOI), 1, 2, 7, 14, 21, and 28; BMP-2 protein production in this media was confirmed with ELISA. The ELISA analysis was performed following company recommended protocols, including generation of a standard curve and optical density measurements obtained using an ELISA microplate reader.

**Generation of hbmp-2 engineered autologous BMDMSC**

Two days prior to MSC injection of the joints, a subset of equine MSC (80–90% confluence in a 75 cm² flask, approximately 4 million cells) were designated as the Auto-BMP-2 group and were transduced with 100 MOI Ad-BMP-2 after estimating cell numbers per flask with an ocular lens grid square (1 cm x 1 cm). The cells were maintained in culture for two days until peak gene expression was achieved. Cells were then harvested via trypsinization, counted using a haemacytometer, and viability confirmed using trypan blue stain, and approximately 15 x 10⁶ cells were transferred to another flask. This injection dose was then washed twice in GBSS. The cell pellet was resuspended in GBSS to create an injection volume of 1 mL and an injection dose of 15 x 10⁶ cells transduced with the human BMP-2 gene (hbmp-2). The other MSC groups (Auto, Allo, Xeno) were trypsinized, counted, and washed in a similar manner in preparation for injection.

**Intra-articular MSC injection**

Intra-articular injections were performed in the standing horse after sedation (xylazine hydrochloride 0.2–1.1 mg/kg IV) through a lateral collateral sesamoidean ligament approach for every joint. Each joint was aseptically prepared prior to injection using 2% chlorhexidine scrub followed by an isopropyl alcohol rinse. At day 0, all six horses had a dose of approximately 15 x 10⁶ cells injected into the fetlock joint within approximately 60–90 minutes from trypsinization. All joints were injected by a single investigator (JHP) blinded to the MSC group being administered.

**Synovial fluid analyses**

Synovial fluid (1 mL) was aspirated from injected fetlock joints on days 0, 1, 2, 7, 14, 21 and 28 after MSC injection after a 28 day washout period between GBSS and MSC injections. A single investigator (JHP), blinded to the MSC treatments, performed all arthrocenteses throughout the sampling period. Approximately 0.3 mL of synovial fluid was aliquoted into an EDTA tube for cytology. The remaining fluid was placed in a microcentrifuge
tube, spun at 12,000 rpm for five minutes, and the supernatant was frozen at –80°C for ELISA analysis of interleukin-6 (IL-6) \(^u\), interleukin-10 (IL-10) \(^u\), and BMP-2 \(^u\). All ELISA protocols were followed as described previously for BMP-2. The BMP-2 control samples consisted of synovial fluid from joints injected with non-transduced autologous BMDMSC and baseline synovial fluid in the autologous groups before injection. Analysis of synovial fluid was performed within

![Graphs](image-url)

**Figure 1** (A–F) Mean ± standard error of the mean (SEM) synovial fluid analysis for mesenchymal stem cell (MSC) injected joints compared to controls. **A**) Total nucleated cell count (cells/µL) with inset highlighting days 7–28. **B**) Neutrophil cell count (cells/µL). **C**) Monocyte cell count (cells/µL) with inset highlighting days 7–28. **D**) Total protein concentration (g/dL). **E**) Interleukin-6 concentration (pg/mL). **F**) Bone morphogenetic protein-2 (BMP-2) concentration (pg/mL). For (A–E), data from the autologous (Auto) and the genetically modified autologous-bone morphogenetic protein-2 (Auto-BMP2) groups were not significantly different and were combined for graphics only. Different symbols (*, †) indicate significantly different (p <0.05) groups (A–E) or time points (F). All mesenchymal stem cell groups were significantly different than Gey’s balanced salt solution (GBSS) injected joints up to or including day 7. Xeno = xenogeneic; Allo = allogeneic; IL = interleukin.
30–60 minutes of collection for total nucleated cell count and total protein concentration via refractometry. Differential nucleated cell counts were performed by a board certified veterinary clinical pathologist (MLW) blinded to the treatment groups.

**Clinical monitoring**

Pulse, respiration, temperature, and lameness at the walk were monitored throughout the study. Clinical signs of joint inflammation were monitored on the same days as synovial fluid collection and included joint swelling (circumference and oedema score), and evidence of pain (pain-free range of joint motion) as previously described (16, 23). Pain-free range-of-motion was assessed through fetlock flexion until the horse raised its head or moved its limb in resistance while measuring degree of flexion with a handheld goniometer. Fetlock circumference was measured with a tape at the middle aspect of the proximal sesamoid bones. Both measurements were taken in triplicate and averaged. Distal limb oedema was assessed on a 0 to 4 scale (0 = no swelling; 1 = minimal swelling localized to the injection site; 2 = mild swelling localized to the fetlock joint; 3 = moderate swelling extending proximally towards the carpus or tarsus; 4 = marked swelling extending to or above the carpus or tarsus. All observations were made by an investigator (JHP) blinded to the MSC group administered.

**Statistical analysis**

Repeated-measure analysis of variance (ANOVA) was used to evaluate three factors; 1) MSC group, 2) time, and 3) limb. Limb was not a significant factor in the analysis. For the significant factors of MSC treatment and time, multiple post-test comparisons were performed using Proc Mixed statistical models for all continuous outcomes and Proc Genmod statistical models for all categorical outcomes. Variables were considered nested within horse, with the horse factor treated as a random variable, and the distribution of data assessed by use of a subset of normality. Data were assessed for normality using the Shapiro-Wilk analysis. Significance level was set at p < 0.05 for all analyses.

**Results**

**Mesenchymal stem cells isolation and characterization**

Mesenchymal stem cells were successfully isolated from bone marrow and expanded in culture. Characterization of BMDMSC (equine and human) via flow cytometry re-
revealed that the majority of cells were MHC class II negative (95 ± 0.86%) and CD90 positive (98 ± 0.69%). The multipotential nature of BMDMSC from the equine and human sources was confirmed by trilineage differentiation of all samples with positive staining for mineral, glycosaminoglycan and lipid formation in vitro (data not shown). Stem cell stain uptake was assessed subjectively and was robust for all groups similar to other published reports (7, 9).

**Synovial fluid analysis**

All six horses successfully completed the study and synovial fluid was obtained from all horses at each time point. There was no significant difference between Auto and Auto-BMP-2 groups both within and across time points for all outcome assessments. All BMDMSC injected groups produced greater inflammation than the GBSS control that peaked at day 1 as measured by total nucleated cell count, neutrophil and monocyte counts, total protein and IL-6 concentrations (Figure 1A-E). All synovial fluid parameters for the GBSS vehicle control group returned to normal reference ranges by day 7. Synovial fluid neutrophil count, total protein and IL-6 concentrations returned to normal reference ranges or baseline (IL-6) by day 14 for all MSC injected joints. Total nucleated cell count, lymphocyte, and monocyte counts returned to normal reference ranges by day 28 for all MSC injected joints.

Allogeneic and Xeno injections produced a significantly greater peak of inflammation at 24–48 hours as measured by total nucleated cell count, neutrophil, lymphocyte and monocyte counts, total protein and IL-6 concentrations and returned to normal reference ranges by the end of the study (Figure 1A-E). Synovial fluid lymphocyte and monocyte counts in the Allo group were significantly greater than the Auto and Auto-BMP-2 groups on day 14 (p <0.05), although not on days 7 or 21 (Figure 1C inset showing monocytes only). Interleukin-10 was not detectable except in one horse with increased IL-10 concentration for all four MSC groups that remained greater than GBSS across the study period. Bone morphogenetic protein 2 was significantly greater in synovial fluid from joints injected with auto-BMP-2 on days 1 and 2 than auto controls (Figure 1F). At the time of injection, ELISA analysis demonstrated high levels of BMP-2 in culture media with each cell producing approximately 0.019 pg/cell BMP-2 (data not shown).

**Clinical monitoring**

Physical exam parameters of temperature, pulse, and respiration were within normal limits throughout the study. A mild to moderate lameness response at a walk was observed within 12 hours that was not different among MSC treatments. This response was variable, with some horses demonstrating no lameness, with resolution in all by 24–48 hours post-injection. All MSC groups had a significantly greater clinical inflammatory response than the GBSS control (Figure 2A-C). Allogeneic and Xeno groups produced a significantly greater peak of clinical inflammation at 24–48 hours, measured as increased joint circumference, increased oedema score, and reduced range of pain-free motion (Figure 2A-C). Joint clinical parameters returned to baseline for all MSC groups by the termination of the study except for distal limb oedema, which persisted and consisted of mild oedema at or directly around the site of injection. The pattern of distal limb oedema was predominately proximal to the pastern and was greater than the GBSS control for all MSC groups (Figure 3).

**Discussion**

Our study showed that direct intra-articular injection of 15 million cultured BMDMSC produced three main findings: marked yet transient clinical inflammation that was on average 25% greater for allogeneic and xenogeneic MSC than autologous MSC, gene product of detectable concentrations in equine synovial fluid after injection of genetically modified cells, and no difference in the inflammatory response between autologous MSC and a genetically modified autologous MSC.

To the authors’ knowledge, this is the first study reporting a significantly greater acute inflammatory stress response (<48 hrs.) after intra-articular injection of MSC from an allogeneic or xenogeneic source compared to autologous MSC injection. The cause of this greater immediate stress response of the joint to allogeneic and xenogeneic MSC is unknown, but could be a response to cell size and function variability, non-self protein secretion, or any other innumerable changes that occur when propagated biologic material is placed into a sensitive joint environment. The difference in inflammatory response requires further study in order to better elucidate the significance of the finding. For our study, all BMDMSC were cultured and processed similarly including multiple wash
steps prior to injection and therefore, cell processing was not considered the reason for difference among MSC groups. Importantly, the measureable differences in our non-self MSC groups were deemed clinically acceptable and represented a range from 10% (nucleated cell count and oedema score) to 100% (IL-6) increase in the measured parameter when compared to the autologous group, but only in the 24–48 hour window after injection.

Our findings confirmed the previous published results of an acute synovial inflammatory response after intra-articular MSC injection in horses, characterized by a marked yet transient influx of neutrophils (approximately 80%) (17). Our findings of greater total nucleated cell number and greater joint circumference in horses injected intra-articularly with allogeneic cells than with autologous cells confirmed a difference noted in a previous report, although not statistically significant (17). All of our MSC groups produced a degree of inflammation that resulted in mild, transient discomfort that corresponded with the acute inflammatory response. Our study also demonstrated greater limb swelling predominantly proximal to the MSC injected joint compared to the GBSS vehicle control limbs. This limb swelling improved significantly in all MSC groups throughout the study period. Horses were not bandaged during the 30 days post-MSC injection, were not administered anti-inflammatory drugs, and were not exercised in order to assess the full clinical response and identify differences among our MSC injections uninfluenced by physical effects or medications. Typical clinical practice of post-injection bandaging and administration of non-steroidal anti-inflammatory drugs would be expected to ameliorate the clinical inflammatory response.

After MSC injection, increased levels of IL-6 and IL-10 were detected for all MSC groups. For the allogeneic and xenogeneic MSC injected joints, IL-6 concentration was significantly higher than in the autologous MSC injected joints. Increased IL-6 concentration has been demonstrated in co-culture experiments of human MSC with allogeneic PBMC and may function to augment T helper cells (24, 25). Increased IL-10 has also been demonstrated in human stem cell co-culture models, the role of which may help suppress the immune cell response (24).

Additional contributing factors to the inflammatory response may include the dose of injection or the overall sensitivity of the fetlock joint to these cells. Our injection dose consisted of 15 x 10⁶ MSC in fetlock joints, a dose comparable to previous reports of 3 x 10⁶ MSC in equine fetlock joints, 7.5 x 10⁶ MSC in equine radiocarpal joints, approximately 10.5 x 10⁶ MSC in equine middle carpal joints, and 20 x 10⁶ MSC in equine medial femorotibial joints (5, 17–19). However, a study directly evaluating the safety and comparative efficacy of various cell doses is lacking. In rats after a relatively high dose MSC injection (10 x 10⁶) in a model of intra-articular tissue injury, free bodies of scar tissue containing fibroblastic-like cells were observed in both the sham and tissue injured knee joints whereas joints injected with 1 x 10⁶ MSC did not demonstrate this finding, suggesting an MSC dose effect (1). However, despite evidence of a marked acute inflammatory stress response after intra-articular injection with MSC in our study (<48 hours), this reaction was transient with joints appearing significantly improved by seven days with no evidence of scar tissue or a decrease in range-of-motion at the end of the 28 day study period. This inflammatory response after intra-articular stem cell injection may be altered in a joint with pre-existing injury or osteoarthritis.

Our study is the first to report that MSC joint injections can function to deliver measurable soluble gene product in equine synovial fluid. The genetic modification of MSC using the hbmp-2 gene significantly increased BMP-2 protein in synovial fluid of auto-BMP-2 injected joints for at least the first two days and less than seven days post-injection. The lack of detection after this time period was probably due to cell migration, cell death, or a decrease in protein production. For our study we chose to transduce the BMDMSC using an adenoviral vector due to the high transduction efficiency of that vector in MSC and robust gene expression typical of that vector. The transient expression has been documented for at least 28 days and therefore was sufficient for our experimental study (26).

The genetic engineering of cells using an adenovirus vector and the human BMP-2 gene did not produce a greater inflammatory response than autologous cells. Direct injection of adenoviral vector, encoding for BMP-2 gene expression, can produce joint inflammation comparable to our MSC injections but with more robust BMP-2 expression (16). Direct injection of vector however is associated with antibody reaction to vector and the risk of unwanted vector transduction of bystander cells (16). The benefits and limitations of growth factor delivery by engineered cells, direct vector injection or recombinant protein injection remain to be compared.

Mesenchymal stem cell characterization is under further research in order to better elucidate markers specific to this cell population. Most consistently, plastic adherence and trilineage differentiation in vitro, combined with the positive and negative expression of certain cell surface glycoproteins have helped to characterize this population to date. The authors recognize that several other glycoprotein markers have been identified on human stem cells including but not limited to positive expression of CD90, CD73, CD105 and MHCII while negative to CD34, CD45, CD11b, CD14, CD19, CD75a and MHCII (27). Many of these markers have not been evaluated in equine bone marrow derived stem cells with cross reactivity unconfirmed. We chose a subset of these markers based on other published reports in horses (6, 9).

The effect of passage and culture conditions on MSC functional and morphological characteristics are of limited study. An in vitro assessment of human BMDMSC demonstrated that higher passage stem cells (passage 7 or 10) lose their immunosuppressive potential and ability to divide (28). Mesenchymal stem cells used in our study were low passage (passage 2–4) in order to decrease any risk of morphological or functional changes that can take place with higher passage cells and long-term culture. To the authors’ knowledge, a study evaluating MSC functional differences amongst low passage cells has not yet been performed.
In conclusion, intra-articular injection of BMDMSC, using this protocol, produced a transient joint inflammatory response that peaked at 24–48 hours and resolved clinically within seven to 28 days without treatment. The comfort of these horses was deemed clinically acceptable in that the findings of the physical examinations and the appetite were normal, despite a mild, transient (<48 hours) variable lameness in some limbs at the walk. Allogeneic and xenogeneic MSC injections produced a modest, but detectably greater inflammatory response than autologous MSC in our study. Further studies are needed to investigate the relevance or persistence of this difference in other models, with lower doses of MSC or in horses with joint disease. The genetic alteration of MSC with the hbpmp-2 gene resulted in a detectable, acute increase in BMP-2 protein that warrants further investigation into the potential therapeutic effect of this anabolic protein in joints.

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Conflict of interest
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