Injuries leading to tendon, ligament, or joint degeneration are common in all uses of horses, from recreation to competition to farmwork.¹ Treatments for orthopedic injuries include resting, therapeutic shoeing, physical therapy, or local/parenteral pharmacotherapy with anti-inflammatory and biologic agents.²−⁴ Regardless of treatment modality, there is a high risk of tendon and ligament injury recurrence as a result of the development of fibrotic tissue with impaired strength instead of functional tissue, and a limited reparative magnitude of articular cartilage in joints. Regenerative medicine aims to heal and restore normal aspects of the tissue affected by using scaffolds, growth factors, or cell-based therapies.⁵

Mesenchymal stromal cells (aka mesenchymal stem cells or MSCs) are progenitor cells that can be isolated from bone marrow as well as other tissue sources (eg, placenta, adipose) and have demonstrated some positive results as a regenerative medicine treatment of orthopedic injuries in research and clinical practice.⁶ After intra-articular injection, MSCs have been shown in laboratory animal models to localize to injured tissue and may contribute to the final regenerated ligament structure.⁷ Furthermore,
MSCs may have beneficial effects from nonprogenitor functions such as altering the tissue environment to promote improved cellular repair.8,9 In a blinded clinical trial using intra-articular MSCs to treat horses in the early stages of joint disease, MSC treatment results in fewer negative clinical signs in the short-term, and long-term MSC-treated horses were more likely to return to work.10 In addition, after MSC treatment of tendon and ligament injuries, both a lower re-injury rate compared to conventional therapy and a 77% return to work has been reported.8,11 There is not a standardized technique for MSC treatment, and treatment methods vary considerably across research studies and clinical practice. MSC treatment protocol variables can include cell dose, route of administration (local or systemic), number of administrations (single or multiple), cell origin (bone marrow or adipose tissue), or sample origin (autologous cells, where donor and recipient are the same animal; or allogeneic cells, where donor and recipient are different animals).9

This retrospective study focuses on evaluating the characteristics of orthopedic equine patients considered for MSC therapy, the response to treatment in those animals, and whether there was a difference in outcome depending on the treatment protocol used. Treatment protocol features tested for an impact on outcome included therapeutics prior to MSC therapy, MSC origin (autologous or allogeneic), MSC dose, number of applications, application site, MSC tissue origin, and concurrent therapy. Given the numerous protocol features, our initial hypothesis was that using an autologous MSC protocol to treat horses with tendon, ligament, or joint injuries will have a greater proportion of clinical improvement in lameness than using an allogeneic MSC protocol.

Materials and Methods

This retrospective study included internal cases from the William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California (UC), Davis, and external cases of horses treated with MSCs prepared by the Regenerative Medicine Laboratory at UC Davis, from May 2016 to August 2019. The protocols used in this study were approved by the UC Davis Institutional Animal Care and Use Committee. An investigational new animal drug was filed with the US Food and Drug Administration, and annual reports logging the notice of claimed investigational use were submitted. Written informed consent was obtained from the owners for the participation of their animals in this study. Equine MSCs were tested for purity and identity using CD44 (clone CVS18; AbD Serotec), CD29 (clone 4B4LDC9LDH8; Beckman Coulter), and CD90 (clone DH24A; VMRD) and were confirmed to be negative for F6B (WBC label; provided by Dr. Jeffrey Stott, UC Davis).12 MSCs were thawed and cultured for a minimum of 48 hours prior to administration. Before administration, they were enumerated and confirmed to be > 90% viable based on trypan blue, and negative for endotoxin (PYROGENT Plus LAL Gel Clot Assay; Lonza) and mycoplasma (Myco- Scope PCR Kit; Genetiks). A sample was submitted each time for aerobic bacterial culture and no treatment grew any organisms. Only horses receiving MSC therapy authorized by the owner, with or without concurrent treatment, for tendon, ligament, and/or joint lesions, were considered. Because of the number of variables in this retrospective data set, a non-MSC–treated control group to determine whether MSC treatment was effective was not identified. An attempt at comparing data to a retrospective matched control would induce additional variables and still would not produce definitive claims of MSC treatment efficacy. Data were collected from electronic and hard-copy records, and from forms completed by the veterinarian responsible for the cases that included animal signalment (age, sex, breed, and use), recent history, clinical signs, complementary diagnostic evaluation, final diagnosis, and treatment performed. Outcome data from the treatment were acquired from recheck reports completed by UC Davis veterinary services, available on the online database, or by the referring veterinarian responsible for the external case.

For MSC application, MSCs were either administered to the site of injury (for a joint, via intra-articular injection; for a tendon/ligament, into the soft tissue lesion), administered regionally (via the vein or artery supplying the lesion), or both to the site and regionally. The horses with no recheck information were excluded from final analyses. MSC therapy was evaluated regarding cell origin (allogeneic or autologous), route of administration (site of injury or regional artery/vein), number of applications (1 or 3), and cell number (low dose, < 2.0 X 10⁷ cells; intermediate dose, 2.0 X 10⁷ and 4.0 X 10⁷ cells; and high dose, > 4.0 X 10⁷).

The outcome was classified into effective therapy or ineffective therapy, with effective being those with lameness improvement, reported by the veterinarian as “lower degree lameness” or with a lower lameness grade when compared to evaluation before therapy, and ineffective being the cases with no improvement or worsening lameness. The patient needed a recheck with the same clinician prior to and after MSC therapy to evaluate the effectiveness of the therapy. A secondary outcome was structural improvement observed and reported by UC Davis veterinary staff by imaging techniques, if the same technique was used before and after MSC application. This secondary outcome was also classified as effective if structural improvement was reported, or ineffective otherwise. The images were not blinded, but the determination of improvement was made by a board-certified radiologist rather than the lameness clinician on the case.

Statistical analysis was performed using SAS Studio® (OnDemand for Academics; SAS Institute). Descriptive statistics were used initially to characterize the data set. Bivariate analysis comparing outcome groups was done with Fisher’s exact test for the risk factors age, structure injured, MSC therapy features, and presence of previous or concurrent
therapy, and were considered statistically different at \( P < .05 \). This study had a fixed number of horses enrolled, so a study power calculation was performed with G*Power Software (version 3.1, Heinrich-Heine-Universität Düsseldorf) using the “MSC origin” variable for the calculation. A difference of 20% in the proportion of horses showing lameness improvement—between autologous and allogeneic groups—was specified for clinical relevance. Sample size calculations to guide future studies were performed based on our preliminary findings.

**Results**

A total of 65 horses were included in the study, with MSC treatments that began in 2016 for 34 horses (52.3%), in 2017 for 10 (15.4%), in 2018 for 19 (29.3%), and in 2019 for 2 (3.1%). Animals receiving MSC therapy were distributed mostly over the younger age groups (1 to 5, 6 to 10, and 11 to 15 years old), with only 5/65 horses (7.7%) being older than 15 years. The majority were male (41/65, 63.1%); and Warmbloods (19/65, 29.2%), Quarter Horses (18/65, 27.7%), and Thoroughbreds (16/65, 24.6%) were the most prevalent breeds. Fourteen owners (21.5%) reported the intended use for the horses, and all were used for competition (eg, dressage, jumping, barrel racing) except for 1 horse that was used for trail riding. A comprehensive description of the horses’ signalment is presented in Table 1.

Clinical signs before receiving MSC treatment were described for 37 horses (56.9%) included in the study. All 37 presented with lameness (grade 2/5 or greater, according to American Association of Equine Practitioners scale), and 14 horses (21.5%) presented with additional clinical signs, including local swelling or enlargement, tendon sheath effusion, pain during palpation, local heat, joint effusion, or a stilted gait. Thirty-eight horses (58.5%) underwent complementary diagnostic tests, in addition to the initial physical examination, to identify the location and origin of the orthopedic injury. Of the horses receiving additional diagnostics, 1 was evaluated solely on flexion tests; 5 were diagnosed with flexion tests and an imaging technique; 7 were evaluated with diagnostic analgesia (nerve blocks) followed by an imaging technique; 4 underwent flexion testing, nerve blocks, and imaging; and 21 received only imaging as an additional diagnostic. The imaging modalities used are described in Table 2. Based on the clinical signs and complementary diagnostics, the injury was localized to a tendon in 27/65 horses (41.5%), a ligament in 17/65 (26.1%), both a tendon and ligament concurrently in 5/65 (7.7%), or a joint in 16/65 (24.6%). A summary of these variables can be seen in Table 1.

MSC therapy was the primary treatment modality for 11 of the horses included in the study (16.9%), whereas 24 horses (36.9%) were treated using another therapeutic method prior to MSC treatment, and the remaining 30 (46.2%) had no annotation regarding therapies prior to MSC treatment (Table 1). Additional therapies included resting, systemic anti-inflammatory, extracorporeal shockwave, laser, bandaging, icing, intra-articular or tendon sheath injections (hyaluronic acid, antimicrobial, and/or corticosteroids), and surgical articular debridement alone or in combination. Of the 24 animals receiving treatment prior to MSC application, 9 had no change in their clinical condition before application of MSCs, 5 began improving prior to MSC therapy but were not completely healed, 2 demonstrated worsening clinical signs prior to MSC therapy, and no data were available for the remaining 8 animals.

**Table 1—Signalment, clinical signs, diagnostics method, lesion site, and treatment history for 65 horses treated from 2016 to 2019.**

| Variable                  | Frequency (n = 65) | Prevalence (%) |
|---------------------------|--------------------|---------------|
| Age, years                |                    |               |
| 1–5                       | 14                 | 21.5          |
| 6–10                      | 13                 | 20.0          |
| 11–15                     | 25                 | 38.5          |
| > 15                      | 5                  | 7.7           |
| Unknown                   | 8                  | 12.3          |
| Sex                       |                    |               |
| Male                      | 41                 | 63.1          |
| Female                    | 24                 | 36.9          |
| Breed                     |                    |               |
| Quarter Horse             | 18                 | 27.7          |
| Warmblood                 | 19                 | 29.2          |
| Thoroughbred              | 16                 | 24.6          |
| Paint                     | 1                  | 1.5           |
| Draft                     | 1                  | 1.5           |
| Arabian                   | 3                  | 4.6           |
| Other                     | 6                  | 9.2           |
| Unknown                   | 1                  | 1.5           |
| Use                       |                    |               |
| Competition (not specified)| 4                  | 6.1           |
| Dressage                  | 4                  | 6.1           |
| Jumping                   | 2                  | 3.1           |
| Trail riding              | 1                  | 1.5           |
| Barrel racing             | 3                  | 4.6           |
| Unknown                   | 51                 | 78.5          |
| Clinical signs            |                    |               |
| Lameness                  | 37                 | 56.9          |
| Other signs               | 14                 | 21.5          |
| Unknown                   | 28                 | 43.1          |
| Diagnostic tools          |                    |               |
| Imaging                   | 37                 | 56.9          |
| Flexion test              | 10                 | 15.4          |
| Diagnostic analgesia      | 12                 | 18.5          |
| (nerve block)             |                    |               |
| Location of lesion        |                    |               |
| Tendon                    | 27                 | 41.5          |
| Ligament                  | 17                 | 26.1          |
| Tendon and ligament       | 5                  | 7.7           |
| Joint                     | 16                 | 24.6          |
| Treatment before (a)      |                    |               |
| Yes                       | 24                 | 36.9          |
| No                        | 11                 | 16.9          |
| Unknown                   | 30                 | 46.2          |

*aOther signs observed included swelling, sheath effusion, enlargement, pain during palpation, local heat, joint effusion, and a stilted gait. bTreatment before use of mesenchymal stromal (stem) cells consisted of resting, systemic anti-inflammatory, extracorporeal shockwave, laser, bandaging, icing, intra-articular or tendon sheath injection, and/or surgical articular debridement.
When MSC treatment was recommended, the treatment protocol used was determined by the attending veterinarian. The treatments varied by site of application, number of applications, origin of MSCs (autologous vs allogeneic), and number of cells used. Table 2 describes the features of the MSC therapy protocols used in the 65 horses included in the study. Concurrent with MSC treatment, 8 animals (12.3%), all diagnosed with a joint injury, received another type of therapy, such as extracorporeal shockwave or intra-articular injections of hyaluronic acid, antimicrobials, corticoids, and/or platelet-rich plasma. There were horses that underwent more than 1 imaging diagnostic technique, resulting in a sum of more than 65 or 100%.

Treatment efficacy at recheck was determined based on a lameness exam for 22 of the 26 horses with recheck information after 1 to 6 months of MSC therapy, or on structural pattern improvement assessed by imaging techniques for 13 of the 26, as shown in Table 2. Based on recheck lameness examination, 13/22 (59.1%) had an effective MSC treatment (improvement of clinical signs); based on imaging, 10/13 (76.9%) presented an effective result (structural pattern improvement assessed by imaging). Of the 13 animals with imaging recheck examinations, 10 horses were rechecked on ultrasound, 1 with MRI, 1 with CT, and 1 with radiographs. Only 9 animals (13.8%) had both recheck lameness exam and recheck imaging data available, of which 7 were in agreement (77.8%) showing an effective outcome, whereas 2 were in disagreement (22.2%), with 1 horse presenting lower degree lameness but no improvement in CT scans and 1 horse showing improvement in the ultrasound exam but a higher degree of lameness.

### Table 2—Mesenchymal stromal (stem) cell (MSC) treatment features (origin, dose, and number and site of applications), concurrent therapy, recheck information, and imaging techniques used for 65 horses treated from 2016 to 2019.

| Variable                     | Frequency (n = 65) | Prevalence (%) |
|------------------------------|-------------------|----------------|
| MSC origin                   |                   |                |
| Autologous                   | 26                | 40.0           |
| Allogenic                    | 39                | 60.0           |
| MSC dose                     |                   |                |
| Low                          | 17                | 26.1           |
| Intermediate                 | 31                | 47.7           |
| High                         | 17                | 26.1           |
| No. of MSC applications      |                   |                |
| 1                            | 37                | 56.9           |
| 2                            | 3                 | 4.6            |
| 3                            | 25                | 38.5           |
| MSC application site         |                   |                |
| Location of injury           | 45                | 69.2           |
| Regional                     | 15                | 23.1           |
| Combination                  | 5                 | 7.7            |
| MSC tissue source            |                   |                |
| Bone marrow                  | 60                | 92.3           |
| Adipose                      | 5                 | 7.7            |
| Concurrent therapy           |                   |                |
| Yes                          | 8                 | 12.3           |
| No                           | 27                | 41.5           |
| Unknown                      | 30                | 46.2           |
| Recheck information          |                   |                |
| Lameness examination         | 22                | 33.8           |
| Imaging assessment           | 13                | 20.0           |
| Not available                | 39                | 60.0           |
| Imaging techniques           |                   |                |
| Radiography                  | 19                | 29.2           |
| Ultrasonography              | 29                | 44.7           |
| Scintigraphy                 | 5                 | 7.7            |
| Tenoscopy                    | 1                 | 1.5            |
| Arthroscopy                  | 7                 | 10.8           |
| CT                           | 5                 | 7.7            |
| MRI                          | 8                 | 12.3           |

### Table 3—Bivariate analysis using Fisher’s test for the variables likely related to effectiveness of mesenchymal stromal (stem) cell (MSC) therapy for the 22 horses with clinical improvement outcome information.

| Variable                     | Effective (n = 13), Frequency (n = 13), Prevalence (%) | Ineffective (n = 9), Frequency (n = 9), Prevalence (%) | Two-sided P value |
|------------------------------|--------------------------------------------------------|--------------------------------------------------------|-------------------|
| Age, years                   |                                                         |                                                        | .799              |
| 1–5                          | 4 (30.8) 2 (22.2)                                      |                                                        |                   |
| 6–10                         | 3 (23.1) 3 (33.3)                                      |                                                        |                   |
| 11–15                        | 4 (30.8) 3 (33.3)                                      |                                                        |                   |
| > 15                         | 2 (15.3) 0 (0.0)                                       |                                                        |                   |
| Unknown                      | 0 (0.0) 1 (11.1)                                       |                                                        |                   |
| Location of lesion           |                                                         |                                                        | .920              |
| Tendon                      | 3 (23.1) 1 (11.1)                                       |                                                        |                   |
| Ligament                     | 3 (23.1) 2 (22.2)                                       |                                                        |                   |
| Tendon and ligament          | 1 (7.7) 1 (11.1)                                        |                                                        |                   |
| Joint                        | 6 (46.1) 5 (55.5)                                       |                                                        |                   |
| Treatment before             |                                                         |                                                        | .648              |
| Yes                          | 8 (61.5) 4 (44.4)                                       |                                                        |                   |
| No                           | 4 (30.8) 4 (44.4)                                       |                                                        |                   |
| Unknown                      | 1 (7.7) 1 (11.1)                                        |                                                        |                   |
| MSC origin                   |                                                         |                                                        | = 1.000           |
| Autologous                   | 9 (69.2) 6 (66.6)                                       |                                                        |                   |
| Allogenic                    | 4 (30.8) 3 (33.3)                                       |                                                        |                   |
| MSC dose                     |                                                         |                                                        | = 1.000           |
| Low                          | 5 (38.5) 4 (44.4)                                       |                                                        |                   |
| Intermediate                 | 6 (46.1) 4 (44.4)                                       |                                                        |                   |
| High                         | 2 (15.3) 1 (11.1)                                       |                                                        |                   |
| No. of MSC applications      |                                                         |                                                        | .655              |
| 1                            | 3 (23.1) 3 (33.3)                                       |                                                        |                   |
| 2                            | 0 (0.0) 0 (0.0)                                         |                                                        |                   |
| 3                            | 10 (76.9) 6 (66.6)                                      |                                                        |                   |
| MSC application site         |                                                         |                                                        | .845              |
| Location of injury           | 7 (53.9) 6 (66.6)                                       |                                                        |                   |
| Regional                     | 3 (23.1) 2 (22.2)                                       |                                                        |                   |
| Combination                  | 3 (23.1) 1 (11.1)                                       |                                                        |                   |
| MSC tissue source            |                                                         |                                                        | .616              |
| Bone marrow                  | 10 (76.9) 8 (88.9)                                      |                                                        |                   |
| Adipose                      | 3 (23.1) 1 (11.1)                                       |                                                        |                   |
| Concurrent therapy           |                                                         |                                                        | = 1.000           |
| Yes                          | 5 (38.5) 3 (33.3)                                       |                                                        |                   |
| No                           | 8 (61.5) 5 (55.5)                                       |                                                        |                   |
| Unknown                      | 0 (0.0) 1 (11.1)                                        |                                                        |                   |

*Concurrent therapy with MSCs consisted of shockwave or intra-articular injections of hyaluronic acid, antimicrobials, corticoids, and/or platelet-rich plasma. There were horses that underwent more than 1 imaging diagnostic technique, resulting in a sum of more than 65 or 100%.*
Table 4—Bivariate analysis using Fisher's test for the variables likely related to the effectiveness of mesenchymal stromal (stem) cell (MSC) therapy for the 13 horses with structural improvement based on imaging outcome information, which was based on recheck ultrasound, CT, MRI, and radiographs.

| Variable                        | Effective (n = 10), n (%) | Ineffective (n = 3), n (%) | Two-sided $P$ value |
|---------------------------------|---------------------------|---------------------------|---------------------|
| Age, years                      |                           |                           |                     |
| 1–5                             | 4 (40.0)                  | 1 (33.3)                 | .8437               |
| 6–10                            | 2 (20.0)                  | 1 (33.3)                 |                     |
| 11–15                           | 3 (30.0)                  | 1 (33.3)                 |                     |
| > 15                            | 1 (10.0)                  | 0 (0.0)                  |                     |
| Location of lesion              |                           |                           | 1.000               |
| Tendon                          | 5 (50.0)                  | 1 (33.3)                 |                     |
| Ligament                        | 3 (30.0)                  | 1 (33.3)                 |                     |
| Joint                           | 2 (20.0)                  | 1 (33.3)                 |                     |
| Treatment before                |                           |                           | .018                |
| Yes                             | 9 (90.0)                  | 0 (0.0)                  |                     |
| No                              | 0 (0.0)                   | 2 (66.7)                 |                     |
| Unknown                         | 1 (10.0)                  | 1 (33.3)                 |                     |
| MSC origin                      |                           |                           | .192                |
| Autologous                      | 4 (40.0)                  | 3 (100.0)                |                     |
| Allogenic                       | 6 (60.0)                  | 0 (0.0)                  |                     |
| MSC dose                        |                           |                           | .706                |
| Low                             | 6 (60.0)                  | 2 (66.7)                 |                     |
| Intermediate                    | 3 (30.0)                  | 0 (0.0)                  |                     |
| High                            | 1 (10.0)                  | 1 (33.3)                 |                     |
| No. of MSC applications         |                           |                           | .070                |
| 1                               | 7 (70.0)                  | 0 (0.0)                  |                     |
| 2                               | 0 (0.0)                   | 0 (0.0)                  |                     |
| 3                               | 3 (30.0)                  | 3 (100.0)                |                     |
| MSC application site            |                           |                           | 1.000               |
| Location of injury              | 4 (40.0)                  | 1 (33.3)                 |                     |
| Regional                        | 4 (40.0)                  | 2 (66.7)                 |                     |
| Combination                     | 2 (20.0)                  | 0 (0.0)                  |                     |
| MSC tissue source               |                           |                           | 1.000               |
| Bone marrow                     | 8 (80.0)                  | 3 (100.0)                |                     |
| Adipose                         | 2 (20.0)                  | 0 (0.0)                  |                     |
| Concurrent therapy$^a$          |                           |                           | 1.000               |
| Yes                             | 2 (20.0)                  | 1 (33.3)                 |                     |
| No                              | 6 (60.0)                  | 2 (66.7)                 |                     |
| Unknown                         | 1 (10.0)                  | 0 (0.0)                  |                     |

$^a$Concurrent therapy with MSCs consisted of shockwave or intra-articular injections of hyaluronic acid, antimicrobials, corticoids, and/or platelet-rich plasma.

Of the 9 animals that received treatment prior to MSC application and improved based on imaging, none improved in the time between this treatment and the MSC therapy; improvement was observed only after MSC injection. Considering both primary and secondary outcome evaluations (lameness exam and image assessment), the horses could be classified into four possible groups: MSCs were effective in both, MSCs were ineffective in both, MSCs were effective based on the lameness exam but ineffective based on imaging, and MSCs were ineffective based on lameness but effective based on imaging. No statistical significance was observed among groups regarding lesion site or MSC therapy variables (cell origin, sample origin, dose, route, and number of applications).

**Discussion**

The improvement following MSC treatment in 59.1% to 76.9% of horses (depending on the outcome measure) with diverse ages, breeds, sex, and lesion locations suggests many potential signalments and clinical presentations may be candidates for MSC therapy. A variety of protocols for MSC treatment have been used, and in our study there were no significant differences in outcomes noted. Statistical significance was detected between horses receiving additional treatment before the application of MSC, indicating that horses that were previously treated but did not recover from the injury had a better outcome, than horses in which MSC was the primary therapy. This could be a result of synergies across the treatment modalities or there could be confounding factors that deserve attention in future studies. For example, even when MSC treatment is instituted as the primary therapy, delays in administration of the therapy resulting from cell isolation and culture time could affect the final outcome. The investigation of variables in MSC treatment protocols is important, given the increasing interest in using MSCs for their regenerative potential, but there is not a definitive methodology to follow, nor specifications on which lesions or animal characteristics are indicative of use.

Lameness evaluation is a clinically relevant marker of orthopedic injury improvement and was used as our primary outcome as severe lesions in tendons, ligaments, and joints present with lameness as the main clinical sign. Lameness grade improvement was observed for tendons (75%), ligaments (60%), concurrent tendon and ligament lesions (50%), and joint lesions (54.5%), showing the potential of MSC treatment for injury to these structures. The subjectivity of lameness evaluation is a potential source of information bias resulting from the expectations a clinician has to observe a positive response to the chosen therapy, as well as differences in interpretation and grading of lameness among veterinarians. Imaging techniques provide a more objective evaluation for diagnosis and lesion assessment, but present limitations such as cost, accessibility, and availability that...
become a drawback when used in retrospective studies as the sole outcome evaluation method. For this reason, improvement through imaging assessment was a secondary outcome in our investigation, and lesion improvement was found in all tissue types evaluated; tendons (83.3%), ligaments (75.0%), and joints (66.7%). In addition, imaging assessment of structural improvement can also be affected by information bias; however, using a radiologist not directly involved in patient care, as in our study, is likely to yield a more objective evaluation. Recording lameness exams and anonymizing images is ideal in designing a prospective study to evaluate MSC efficacy. The most definitive and objective outcome of MSC treatment efficacy is histology documenting improvement at the cellular level; however, this outcome is difficult to obtain in clinical patient populations because it requires a more invasive sample. Other studies have also documented improvement after MSC treatment of these tissues,\(^6\,8,10,21-24\) consistent with our findings, but without a control group we cannot attribute healing definitively to MSC treatment.

It is important to remember that management of orthopedic disorders commonly involves an extensive number of therapeutic methods, from systemic anti-inflammatories to physical therapy or surgical procedures, chosen and combined according to the lesion’s characteristics and the benefits of each therapy.\(^1,4,20,25\) For this reason, attempts to evaluate one isolated treatment (ie, MSC treatment) in non-controlled studies can result in subjects receiving multiple concurrent or previous therapies affecting the final outcome, as shown in our results by horses receiving previous treatments presenting more effective outcomes than horses that did not receive any before MSC application. This highlights the importance of considering these variables in any research related to orthopedic treatments using MSCs, and performing multivariable analysis when sample sizes are amenable.

An important classification for MSC therapies is whether the cells originated from the same animal receiving the cells (autologous) or from a different animal (allogeneic). In vitro studies have documented similar immunomodulatory properties for both autologous and allogenic MSCs, indicating a therapeutic potential for inflammatory-associated disorders.\(^26\) On the other hand, in vivo studies have shown disparities in their results.\(^27-31\) The results from our study found improvement after MSC therapy when autologous (9/15, 60.0% clinical signs improvement; 4/7, 57.1% imaging improvement) or allogenic (4/7, 57.1% clinical signs improvement; 6/6, 100% imaging improvement) cells were used, with no difference between groups. Currently the literature is inconclusive regarding the recommended number of MSC applications,\(^29,32,33\) the number of cells (dose),\(^34,35\) and the tissue source from which MSCs are derived to achieve a positive outcome with minimal side effects.\(^36\) Although the vast majority of the MSCs in this study were derived from bone marrow (60/65, 92.3%), autologous adipose-derived MSCs were used in 5 patients (7.7%). Four of these horses had clinical rechecks (18.1%) and 2 had imaging rechecks (15.4%). The decision to derive MSCs from adipose tissue is often made based on the age of the patient—in our study the 5 horses with adipose tissue-derived MSCs were 14 to 20 years old with a median age of 15 years—as a result of concerns that bone marrow collection will be unsuccessful in an older horse. In addition, some owners have concerns about the invasive nature of bone marrow collection, and thus prefer adipose collection. This is one of many MSC treatment variables where convenience and owner/clinician preference is driving selection rather than evidence-based data. However, we were unable to find a statistical difference in the final outcome when comparing these MSC therapy variables (dose, number and site of applications, and tissue source), thus we were unable to provide any further recommendations from this study regarding how best to design these MSC therapy protocols.

Difficulties in acquiring follow-up information for these conditions, which have a prolonged time period for progression, affected the sample size of our study and became a limitation, resulting in a \(P\) value of .677 and a study power of 55.8% for our main hypothesis: determining whether using autologous MSCs to treat horses with tendon, ligament, or joint injuries will have a greater improvement in lameness than using allogeneic MSCs. Although the strict inclusion criteria accepting only recheck information from specific veterinarians made the sample size even smaller, it also strengthened our results by reducing potential information bias and providing more credible results regarding the effectiveness of the MSC therapy compared to an owner survey, for example. Our results are useful as a reference to guide study design and sample size calculation. For example, if we want to see a difference of 20% between groups of horses with orthopedic disorders treated with autologous and allogeneic MSCs to consider the lameness improvement significant, and our results show a 60% effectiveness of the autologous MSC treatment, future studies would require a sample size of 170 horses, if the group size ratio was 1:1; or 198 horses, if the ratio was similar to our study’s ratio of 2.14 (to have results with a type I error of 0.05 and a power of 80%). In addition, a larger sample size would enable robust statistical testing such as multivariate logistic regression. Last, including a control group not receiving MSCs in a prospective study would be the only way to document the effectiveness of this regenerative medicine modality and not only compare MSC application protocols. It is our opinion that attempting to extract a control group in a retrospective MSC study would induce additional variables and still would not produce definitive claims of MSC treatment efficacy.

In conclusion, although more research is needed to accept or reject our hypothesis that autologous MSCs would yield a greater improvement in lameness than allogeneic MSCs, our study allowed a preliminary evaluation of multiple variables likely to influence the outcome of MSC therapy. It also
highlighted the importance of considering other therapeutic methods used before MSC application as direct influencers to the progression of orthopedic injuries in horses after MSCs are used. Furthermore, the descriptive information included can possibly assist future research in the regenerative medicine field, serving as a guide for hypothesis formation and study design. Our study highlights the clinical reality of how MSCs are currently being used in equine practice, and serves to direct future prospective clinical studies to answer definitively whether MSC treatment is efficacious in treating orthopedic disease.

Acknowledgments

Financial support was provided by the Harriet E. Pfleger Foundation and the Center for Equine Health at UC Davis, with funds provided by the State of California satellite wagering funds and contributions by private donors. The authors declare there were no conflicts of interest.

References

1. Souza NR, Luna SPL, Pizzigatti D, Martins MTA, Possebon FS, Aguilar ACS. Relationship between type and local of orthopedic injuries with physical activity in horses. Ciência Rural. 2017;47(2):e201512118. doi:10.1590/0103-8478cr20151218.

2. Frisbie DD, Stewart MC. Cell-based therapies for equine joint disease. Vet Clin North Am Equine Pract. 2011;27(2):335–349. doi:10.1016/j.cveq.2011.06.005.

3. Ferris DJ, Frisbie DD, McIlwraith CW, Kawcak CE. Current joint therapy usage in equine practice: a survey of veterinarians. 2009. Equine Vet J. 2011;43(5):530–535. doi:10.1111/j.2042.3306.2010.00324.x.

4. Smith R, Schramme M. Tendon injury in the horse: current theories and therapies. Equine Pract. 2003;25(9):529–539. doi:10.1136/inpract.25.9.529.

5. Fortier LA, Smith RKW. Regenerative medicine for tendinous and ligamentous injuries of sport horses. Vet Clin North Am Equine Pract. 2008;24(1):191–201. doi:10.1016/j.cveq.2007.11.002.

6. Frisbie DD, Smith RKW. Clinical update on the use of mesenchymal stem cells in equine orthopedics. Equine Vet J. 2010;42(1):86–89. doi:10.2746/042516409X477263.

7. Agung M, Ochi M, Yanada S, et al. Mobilization of bone marrow-derived mesenchymal cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. Knee Surg Sport Traumatol Arthrosc. 2006;14(12):1307–1314. doi:10.1007/s00167-006-0124-8.

8. Van-Loon VJF, Scheffer CJW, Genn HJ, Hoogendoorn AC, Greve JW. Clinical follow-up of horses treated with allogeneic equine mesenchymal stem cells derived from umbilical cord blood from normal equine tendons and ligament disorders. Vet Q. 2014;34(2):92–97. doi:10.1080/01652176.2014.949390.

9. Schnabel LV, Fortier LA, McIlwraith CW, Nobert KM. Therapeutics use of stem cells in horses: which type, how and when? Vet J. 2013;197(3):570–577. doi:10.1016/j.tvjl.2013.04.018.

10. 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.

11. Smith RK. Mesenchymal stem cell therapy for equine tendinopathy. Disabil Rehabil. 2008;30:1752–1758. doi:10.1080/09638280701788241.

12. Barberini DJ, Aleman M, Aristizabal F, et al. Safety and tracking of intrathecal allogeneic mesenchymal stem cell transplantation in healthy and diseased horses. Stem Cell Res Ther. 2018;9(1):96. doi:10.1186/s13287-018-0494-6.

13. Dyson SJ, Arthur RM, Richardson D. Suspensory ligament desmitis. Vet Clin North Am Equine Pract. 1995;11(2):177–215. doi:10.1016/s0749-0739(17)30319-x.

14. Jeffcott LB. Osteochondrosis: an international problem for the horse industry. J Equine Vet Sci. 1996;16(1):32–37. doi:10.1016/s0737-0806(96)80065-3.

15. Chesen B, Babareiner RM, Chaffin K, Carter K. Tendinitis of the proximal aspect of the superficial digital flexor tendon in horses: 12 cases (2000–2006). J Am Vet Med Assoc. 2009;234(11):1432–1436. doi:10.2460/javma.234.11.1432.

16. Keegan KG, Dent EV, Wilson DA, et al. Repeatability of subjective evaluation of lameness in horse. Equine Vet J. 2010;42(2):92–97. doi:10.2746/042516409X479568.

17. Leelamankong P, Estrada R, Mahlmann K, Rungpsi R, Lischer C. Agreement among equine veterinarians and between equine veterinarians and inertial sensor systems during clinical examination of hindlimb lameness in horses. Equine Vet J. 2020;52(2):326–331. doi:10.1111/evj.13144.

18. Dowling BA, Dart AJ, Hodgson DR, Smith RKW. Superficial digital flexor tendinitis in the horse. Equine Vet J. 2000;32(5):369–378. doi:10.2746/04251640077591138.

19. Vanel M, Olive J, Gold S, Mitchell RD, Walker L. Clinical significance and prognosis of deep digital flexor tendinopathy assessed over time using MRI. Vet Radiol Ultrasound. 2012;53(5):621–627. doi:10.1111/j.1740-8261.2012.01961.x.

20. Dyson S. Diagnosis and management of common suspensory ligament lesions in the forelimbs and hindlimbs of sport horses. Clin Tech Equine Pract. 2007;6(3):179–188. doi:10.1053/j.ctep.2007.08.004.

21. Carvalho AM, Badiad PR, Alvarez LEC, et al. Equine tendinopathy therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. Stem Cell Res Ther. 2013;4(4):85. doi:10.1186/scrt236.

22. Smith RKW, Werling NJ, Dakin SG, Alam R, Goodship AE, Dudhia J. Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. PLoS One. 2013;8(9):e75697. doi:10.1371/journal.pone.0075697.

23. Vandenberg A, Broeckx SY, Beerts C, et al. Tenogenically induced allogeneic mesenchymal stem cells for the treatment of proximal suspensory ligament desmitis in horses. Front Vet Sci. 2015;2:49. doi:10.3389/fvets.2015.00049.

24. Broeckx S, Suls M, Beerts C, et al. Allogeneic mesenchymal stem cells as a treatment for equine degenerative joint disease: a pilot study. Curr Stem Cell Res Ther. 2014;9(6):497–503. doi:10.2174/1574888x09666140826110601.

25. Contino EK. Management and rehabilitation of joint disease in sport horses. Vet Clin Equine. 2018;34(2):345–358. doi:10.1016/j.cveq.2018.04.007.

26. Colbath AC, Dow SW, Phillips JN, McIlwraith CW, Goodrich LR. Autologous and allogeneic equine mesenchymal stem cells exhibit equivalent immunomodulatory properties in vitro. Stem Cells Dev. 2017;26(7):503–511. doi:10.1089/scd.2016.0266.

27. Joswig AJ, Mitchell A, Cummings KJ, et al. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in equine model. Stem Cell Res Ther. 2017;8(1):42. doi:10.1186/s13287-017-0503-8.

28. Owens SD, Kol A, Walker NJ, Borjesson DL. Allogeneic mesenchymal stem cells treatment induces specific alloantibodies in horses. Stem Cells Int. 2016;2016:5830103.

29. Pezzanite LM, Fortier LA, Antczak DF, et al. Equine allogeneic bone marrow-derived mesenchymal stromal cells elicit antibody response in vivo. Stem Cell Res Ther. 2015;6(1):54. doi:10.1186/s13287-015-0053-x.

30. Carrade DD, Owens SD, Gallupo LD, et al. Clinicopathologic findings following intra-articular injections of autologous

AJVR
and allogeneic placentally derived equine mesenchymal stem cells in horses. *Cytotherapy.* 2010;13(4):419–430. doi:10.3109/14653249.2010.536213

31. Brandão JS, Alvarenga ML, Pfeifer JPH, et al. Allogeneic mesenchymal stem cell transplantation in healthy equine superficial digital flexor tendon: a study of the local inflammatory response. *Res Vet Med.* 2018;118:423–430. doi:10.1016/j.rvsc.2018.03.012

32. Kol A, Wood JA, Holt DDC, et al. Multiple intravenous injections of allogeneic equine mesenchymal stem cells do not induce a systemic inflammatory response but do alter lymphocyte subsets in healthy horses. *Stem Cell Res Ther.* 2015;6(1):73. doi:10.1186/s13287-015-0050-0

33. Ardanaz N, Vázquez FJ, Remacha AR, et al. Inflammatory response to the administration of mesenchymal stem cells in an equine experimental model: effect of autologous, and single and repeat doses of pooled allogeneic cells in healthy joints. *BMC Vet Res.* 2016;12:65. doi:10.1186/s12917-016-0927-5

34. Hare JM, Traverse JH, Henry TD, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (Prohycymal) after acute myocardial infarction. *J Am Coll Cardiol.* 2009;54(24):2277–2286. doi:10.1016/j.jacc.2009.06.055

35. Wolbank S, Peterbauer A, Fahrner M, et al. Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng.* 2007;13(6):1173–1183. doi:10.1089/ten.2006.0313

36. Mohamed-Ahmed S, Fristad I, Lie SA, et al. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Res Ther.* 2018;9(1):168. doi:10.1186/s13287-018-0914-1