The meniscus is a semilunar fibrocartilaginous tissue found in each stifle joint that participates in complex biomechanics of the stifle joint. The meniscus helps in load bearing and transmission of loads, shock absorption, joint stabilization, lubrication, and maintaining joint congruity. After a meniscus is injured, there is poor healing potential because of the largely avascular nature of the fibrocartilaginous meniscal tissue. Loss of or damage to this anatomic load-bearing structure could lead to cartilage degeneration and osteoarthritis.

OBJECTIVE
To assess the ability to regenerate an equine meniscus by use of a collagen repair patch (scaffold) seeded with mesenchymal stem cells (MSCs) derived from bone marrow (BM) or adipose tissue (AT).

SAMPLE
6 female Hispano-Breton horses between 4 and 7 years of age; MSCs from BM and AT were obtained for the in vitro experiment, and the horses were subsequently used for the in vivo experiment.

PROCEDURES
Similarities and differences between MSCs derived from BM or AT were investigated in vitro by use of cell culture. In vivo assessment involved use of a meniscus defect and implantation on a scaffold. Horses were allocated into 2 groups. In one group, defects in the medial meniscus were treated with MSCs derived from BM, whereas in the other group, defects were treated with MSCs derived from AT. Defects were created in the contralateral stifle joint but were not treated (control samples).

RESULTS
Both types of MSCs had universal stem cell characteristics. For in vivo testing, at 12 months after treatment, treated defects were regenerated with fibrocartilaginous tissue, whereas untreated defects were partially repaired or not repaired.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that MSCs derived from AT could be a good alternative to MSCs derived from BM for use in regenerative treatments. Results also were promising for a stem cell–based implant for use in regeneration in meniscal lesions.

IMPACT FOR HUMAN MEDICINE
Because of similarities in joint disease between horses and humans, these results could have applications in humans. (Am J Vet Res 2016;77:779–788)
The aforementioned cells, which are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and myocytes, represent an attractive potential means of regenerating damaged connective tissues, including intra-articular structures of the stifte joint (eg, a meniscus). There has been a focus on BM-MSCs and AT-MSCs because they are thought to include multipotent progenitor cells and are easily and readily available. The multidifferentiation potential of MSCs has aroused clinical interest into whether these cells could be used for regenerative treatments.

Numerous materials have been evaluated as scaffolds for meniscal repair or regeneration. There has been growing interest in the use of biocompatible and biodegradable biomaterials to regenerate damaged tissue, such as injured articular or meniscal cartilage. Stem cell–3-D-graftable biomaterials to regenerate damaged tissue, such as injured articular or meniscal cartilage. Multidifferentiation potential of MSCs has aroused clinical interest into whether these cells could be used for regenerative treatments.

Several in vitro studies relating to the chondrogenic potential of BM-MSCs and AT-MSCs have been conducted, but limited information has been collected with regard to parallel in vivo experiments in horses. On the basis of results for those previous studies, we hypothesized that AT-MSCs could be considered as a good alternative to BM-MSCs for regenerative treatments.

Therefore, the objective of the study reported here was to evaluate the in vitro proliferative capacity of AT-MSCs and BM-MSCs when these cells were seeded on a collagen-derived scaffold. It was hypothesized that this scaffold could serve as a 3-D pattern for stem cells to direct new tissue formation, which would result in more mature tissues with an enhanced matrix content and biomechanics comparable to those of native meniscus tissue. A second objective was to investigate the in vivo regenerative capacity when AT-MSCs and BM-MSCs were implanted in a defect of an injured meniscus in horses. It was believed that BM-MSCs and AT-MSCs would have similar abilities to regenerate a damaged meniscus.

**Materials and Methods**

**Sample**

Six female Hispano-Breton horses between 4 and 7 years of age were used for both portions of the study. Harvest of BM and AT was performed on each horse, and samples were used for the in vitro portion of the study. Subsequently, each of the 6 horses was used in the in vivo portion of the study. Protocols of this study were in accordance with the Guidelines of the Council of the European Union (86/609/EU) and adhered to Spanish regulations for the use of laboratory animals (BOE 67/8509-12, 1998). They were approved by the Scientific Committee of the University of León.

**Harvest, isolation, and culture of MSCs**

Horses were sedated by IV administration of romifidine (0.04 mg/kg) and butorphanol tartrate (0.015 mg/kg). Approximately 15 g of AT was harvested from the region over the superficial gluteal fascia; the tissue was immediately used for AT-MSC isolation in accordance with a previously published protocol. Harvest of BM and BM-MSC isolation were performed as described elsewhere.

After BM and AT were harvested, each horse received a medication (15 to 20 mL, IM, q 24 h for 2 days) for prophylaxis against postoperative infection. The medication contained benzylpenicillin procaine (200 U/mL), dexamethasone sodium phosphate (200 mg/mL), and dexamethasone 21-sodium phosphate (0.50 mg/mL). Horses received ketoprofen (2.2 mg/kg, IV, q 24 h for 3 to 5 days) for postoperative pain relief.

**Characterization of MSCs by use of flow cytometry**

All MSCs obtained were expanded in primary culture in 25-cm² culture flasks. Flasks were incubated at 37°C in a humidified 5% CO₂ environment until they reached 80% confluence when detached with trypsin. Cells (1 x 10⁶ MSCs) were incubated with markers for MSCs (primary mouse anti-CD73, anti-CD90, and anti-CD105 antibodies); markers were used at a dilution of 1:100. Cells were then stained with streptavidin-alexa 488 and streptavidin-alexa 647 secondary antibodies; antibodies were used at a dilution of 1:100. Cells were suspended in cold buffer solution and analyzed by use of flow cytometry. Only secondary antibodies were used in control samples, and no marker cells were used to select the gate for analysis of cells. Procedures were also performed on cell lines described in another study.

Standard labeling protocols were used, and manufacturers' instructions were followed. A minimum of approximately 10,000 events were used for fluorescence capture and analyzed by use of commercially available software.

**Confocal characterization of MSCs**

Cells were subcultured on 8-well chamber slides (2 x 10³ cells/well). Cells were fixed by incubation with 2% paraformaldehyde for 15 minutes and then incubated with primary mouse anti-CD73, anti-CD90, and CD105 antibodies (dilution, 1:100) overnight at 4°C. Cells subsequently were incubated with secondary biotinylated anti-mouse antibodies (dilution, 1:100) and
then were stained with streptavidin–alexa 488 and streptavidin–alexa 647 antibodies (dilution, 1:100). Finally, chamber slides were mounted by use of mounting medium that contained 4’,6-diamidino-2-phenylindole and examined with a confocal microscope.

**Multidifferentiation potential of BM-MSCs and AT-MSCs in vitro**

Isolated BM-MSCs and AT-MSCs (1 x 10^6 cells) were cultured under conditions conducive to adipogenesis, osteogenesis, and chondrogenesis to assess multipotentiality. For adipogenic induction, 500µM isobutyl-methyl-xanthine, 150µM indomethacin, 1µM dexamethasone, and 10µM insulin were added to culture medium that contained 1% antimicrobial-antimycotic solution and 10% fetal bovine serum. Cells were cultured for 15 days, and cultured cells then were stained with oil red O. For osteogenic induction, BM-MSCs and AT-MSCs were cultured under osteogenic culture conditions for 15 days in a solution that contained 10mM β-glycerophosphate, 10µM L-ascorbic acid 2-phosphate, and 0.1µM dexamethasone in high-glucose Dulbecco modified Eagle medium with 1% antimicrobial-antimycotic solution. After incubation was completed, osteogenesis was confirmed by use of von Kossa and alizarin red stains to highlight calcification of extracellular matrix. For chondrogenic induction, cell cultures were incubated in chondrogenic medium containing 6.25 µg of insulin-transferrin sodium selenite/mL, 50nM L-ascorbic acid 2-phosphate, and 10 ng of transforming growth factor–β1/mL in high-glucose Dulbecco modified Eagle medium plus 1% antimicrobial-antimycotic solution. Cells were incubated for 21 days, and chondrogenesis was confirmed by use of alcian blue stain and immunohistochemical analysis for aggrecan. Cells were rinsed with PBS solution and fixed with 4% paraformaldehyde. Immunohistochemical analysis for aggrecan stain was performed by use of a rabbit anti-breivican or anti-aggrecan polyclonal primary antibody (dilution, 1:100) and a biotinylated goat anti-rabbit secondary antibody (dilution, 1:100). Staining was performed with streptavidin–alexa 488 antibody (dilution, 1:100). Cells were viewed with a confocal microscope after staining.

**Seeding of BM-MSCs and AT-MSCs into a scaffold**

A collagen repair patch was used as a scaffold to evaluate regeneration of meniscal tissues. This scaffold was chosen because of its biocompatibility, versatility for use, and numerous mechanical features and because it was chemically cross-linked and thus resistant to enzymatic degradation. Pieces of the biomaterial (0.5 cm²) were used. Harvested BM-MSCs and AT-MSCs were resuspended in complete medium, and 100 µL of cell suspension (0.5 X 10^6 cells) was seeded onto the scaffold in a 24-well plate. Samples were incubated for 2 hours in a humidified atmosphere of 95% air and 5% CO₂ at 37°C, and then slowly added to each well. Medium was changed twice each week. Samples for the in vitro experiment were cultured for 6, 9, 12, 15, 18, or 21 days. As an experimental control sample, scaffolds without MSCs were cultured for the same time periods.

**Scanning electron microscopy**

To assess integration between MSCs and the scaffold, stem cell–matrix constructs were prepared for scanning electron microscopy. Samples were fixed in 2% glutaraldehyde, buffered with 0.1M phosphate buffer (final pH, 7.4), and then incubated for 8 to 16 hours on the basis of sample size. Samples were washed in buffer and then fixed by incubation in 0.1M phosphate buffered 1% osmium tetroxide (pH, 7.4) for 2 to 8 hours on the basis of sample size. Samples were dehydrated in serial ethanol solutions and infiltrated with amyl acetate; samples then were dried in a critical-point drying apparatus by replacing amyl acetate with gold-palladium. Samples were examined by use of a scanning electron microscope at 20 kV.

**Proliferation assay**

Cell proliferation in the scaffolds was evaluated by use of DNA quantification and a cell viability assay. To enable us to assess total DNA content, the scaffold was cut into thin strips and digested by incubation with type I collagenase 0.075% at 37°C for 1 hour or until no visible scaffold material remained. The digested material was then centrifuged at 2,000 X g for 30 minutes to precipitate remaining proteins. Supernatant was purified with phenol-chloroform–isoamyl alcohol. The DNA was purified and measured spectrophotometrically by use of a spectrophotometer.

The cell viability assay was designed to provide a quantitative measure of the proliferation of various cells of humans and other animals. Absorbance for cells incubated with the assay dye solution was measured by use of a microplate reader at a wavelength of 570 nm. The number of viable cells was correlated with the amount of dye reduction and, in accordance with the manufacturer’s instructions, was expressed as a percentage of dye reduction.

**In vivo implantation experiment**

Four weeks after surgery for the harvest of MSCs, horses underwent surgery for creation of a meniscal defect in both stifle joints. Horses were sedated by IV administration of ketamine hydrochloride (2 mg/kg) and romifidine (0.05 mg/kg); horses then received a continuous IV infusion of guaifenesin (0.9% NaCl) solution (25 mg/kg). Endotracheal intubation was performed, and anesthesia was induced by the administration of 5% isoflurane in oxygen. Anesthesia was maintained by administration of 1.5% to 2.5% isoflurane in oxygen. Intraoperative monitoring consisted of ECG, pulse oximetry, noninvasive measurement of blood pressure, and capnography.
Anesthetized horses were positioned in dorsal recumbency with a femorotibial joint angle of 90° and the tibia in a horizontal position. Arthroscopic surgery was performed by use of an approach described elsewhere with a forward-facing 30°-oblique arthroscope. A pressure pump was used to ensure stable joint pressure. An arthroscope portal for the medial aspect of the femorotibial joint was made axial to the intermediate patellar ligament and 2 cm proximal to the cranial border of the tibia. An instrument portal was created over the medial femoral condyle at a location medial to the arthroscope portal.

A lesion (10 mm in length and 6 mm in depth) was created in the cranial part of the medial meniscus of each horse (Figure 1). Lesions were made with a motorized synovial resector.

Horses were allocated by use of a simple randomization procedure into 2 groups (3 horses/group). The 3 horses of one group received treatment with BM-MSCs, whereas the 3 horses of the other group received treatment with AT-MSCs. One stifle joint in each horse was arbitrarily selected for treatment with cultured MSCs loaded onto a collagen scaffold; the contralateral stifle joint in each horse was not treated with MSCs and served as a cell-free control joint.

The MSCs used for autologous meniscal implantation had been harvested from the horses 4 weeks prior to the surgery for creation of the meniscal defect. They had been cultured for 21 days and subsequently inside the scaffolds for 7 days to initiate a good proliferation rate, which was continued after implantation inside the meniscal defects.

A collagen scaffold seeded with AT-MSCs or BM-MSCs was inserted into the assigned meniscal defect (Figure 2). The scaffold was sutured in place by use of 2 spinal needles (20 gauge, 3.5 inches in length) and 2–0 polypropylene with an inside-out technique that consisted of 2 sutures passed through arthroscopic cannulas and tied directly over the joint capsule. Sutures were retrieved through a cannula in the arthroscopic portal to avoid entrapment in soft tissues. A vertical suture orientation was used to evenly appose the meniscus to the joint capsule.

After surgery was completed, horses were administered romifidine, placed in a padded box stall, and allowed to recover from anesthesia. Each horse received a medication (15 to 20 mL, IM, q 24 h for 7 days) for prophylaxis against postoperative infection and received ketoprofen (2.2 mg/kg, IV, q 24 h for 3 to 5 days) for postoperative pain relief.

Horses were housed separately in a stable for 6 weeks after surgery and then were housed as a group in university facilities. Horses were allowed exercise (hand walking for 10 minutes twice daily) beginning 2 weeks after surgery.

**Macroscopic assessment of meniscal repair**

Horses were euthanized 12 months after surgery. Horses were sedated by IV administration of xylazine.
or romifidine to minimize violent thrashing and provide better-controlled recumbency during euthanasia. Horses then were euthanized with an overdose of barbiturate (sodium pentobarbital administered IV). Both stifle joints of each horse were harvested, grossly evaluated, and photographed with a digital camera. All samples were evaluated by an experienced investigator (FGM) to ascertain macroscopic evidence of macroscopic regeneration; the investigator was not aware of the source of the samples.

**Histochemical analysis of wounded meniscus sections**

Samples were excised in blocks that included all implanted tissue and surrounding tissues. Samples were initially fixed in 4% paraformaldehyde and prepared for histologic examination. Samples were incubated in neutral-buffered 10% formalin for 48 hours and then embedded in paraffin. Tissues were cut in the radial plane into 8-µm-thick sections; sections were subsequently stained with safranin O fast green for histologic examination via light microscopy. Safranin O fast green detects acidic proteoglycans that are contained in cartilaginous tissue. Quantification of regenerated meniscus was performed by measuring the amount of safranin O fast green staining by use of image software.  

**Statistical analysis**

Statistical analyses were performed with statistical analysis software. Each experiment was performed with 3 replicates (each horse represented a replicate). Results were reported as mean ± SEM. Paired data were compared by use of a Student $t$ test. Values of $P < 0.05$ were considered significant.

**Results**

**Culture, proliferation, characterization, and multipotentiality of BM-MSCs and AT-MSCs**

An abundance of multipotent cells were isolated from AT and BM obtained from the horses. Harvested tissues yielded spindle-shaped cells that adhered to plastic culture dishes under standard conditions. Cells obtained from both AT and BM appeared morphologically similar; morphological differences between AT-MSCs and BM-MSCs were not evident. Although AT-MSCs had a more homogeneous population that reached confluence before BM-MSCs did, BM-MSCs were capable of forming single-cell colonies (Figure 3). These colonies coalesced to cover the entire flask. It was possible to observe on a graph of the growth curve that for days 2 through 4 of culture, AT-MSCs had a different pattern of proliferation than did BM-MSCs, whereas after culture for 6 days, the pattern of proliferation appeared similar until proliferation reached a plateau, which was slightly higher in AT-MSCs than in BM-MSCs.

Cultured AT-MSCs and BM-MSCs possessed established properties of multipotent MSC. Expression of CD73, CD90, and CD105 as specific surface markers of MSCs was analyzed. Immunofluorescent characterization of equine MSCs confirmed stem cell characteristics, and expression of all markers was evident (Figure 4). Positive results were > 93% for CD90 and CD105 but not for CD73.

Cultured AT-MSCs and BM-MSCs were capable of differentiating into adipocytes, osteocytes, and chondrocytes. Colonies cultured in adipogenic medium stained well with oil red O. Colonies cultured in osteogenic medium had an abundance of mineral deposits in the extracellular matrix. Colonies cultured in chondrogenic medium were smooth glistening pellets that stained with alcian blue. The chondrogenic pellets also had immunofluorescence against aggrecan, which is an abundant proteoglycan in cartilage matrix (Figure 5).

**Growth of MSCs in collagen scaffolds**

Proliferation of MSCs in collagen scaffolds was evaluated on the basis of double-stranded DNA content and results of the cell viability assay. Cell density in each scaffold was measured at various times (6, 9, 12, 15, 18, and 21 days) after initiation of culture. The highest cell density in scaffolds was reached after culture for 15 days; cell density then decreased until day 21. Scanning electron microscopy revealed cell adhesion to the surface of the scaffold. Cell adhesion to the scaffold steadily increased; after culture for 21 days, AT-MSCs and BM-MSCs spread over

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**Figure 3**—Photomicrographs of AT-MSCs (A) and BM-MSCs (B) after 5 days of culture and a graph of the growth curve for MSCs (C). In panels A and B, notice that AT-MSCs and BM-MSCs formed monolayers of spindle-shaped cells that adhered to plastic culture flasks but that AT-MSCs grew homogeneously and BM-MSCs formed colonies; bar = 1,000 µm. In panel C, notice that the pattern of proliferation differed between AT-MSCs (black line) and BM-MSCs (gray line) on days 2 through 6 and that the plateau was higher for AT-MSCs than for BM-MSCs. Values reported are mean ± SEM (n = 3 horses/group). Day 0 = Start of cell culture.
the surface and formed large confluent cell layers that covered all seeded areas of the scaffold (Figure 6). Proliferation observed in the scaffolds was extremely similar for AT-MSCs and BM-MSCs, except on day 12 of culture, when total DNA content was significantly higher in AT-MSCs cultures (Figure 7).

Figure 4—Photomicrographs of cell cultures of equine MSCs and expression of the CD73, CD90, and CD105 surface markers as determined by use of immunohistochemical analysis and confocal microscopy and histograms of results for positive staining. Cells were incubated with primary mouse anti-CD73, anti-CD90, and anti-CD105 antibodies, stained with streptavidin–alexa 488 and streptavidin–alexa 647 secondary antibodies, and evaluated by use of flow cytometry. Notice in the histograms that positive results were > 93% for CD90 and CD105 but not for CD73. Bar = 100 µm.

Figure 5—Photomicrographs of AT-MSCs (A–E) and BM-MSCs (F–J) after culture in adipogenic (A and F), osteogenic (B, C, G, and H), and chondrogenic (D, E, I, and J) induction medium. In panels A and F, notice the adipogenic differentiation after 15 days of culture as indicated by lipid droplets stained with oil red O. In panels B, C, G, and H, notice the presence of mineralization after 15 days of culture as indicated by staining with alizarin red and von Kossa stains. In panels D and I, presence of proteoglycans is evident after 21 days in culture as indicated by staining with alcian blue. In panels E and J, presence of aggrecan (green) in the chondrogenic nodules is evident. Cells were cultured with rabbit anti-brevican or anti-aggrecan polyclonal primary antibody and then a biotinylated goat anti-rabbit secondary antibody; staining was performed with streptavidin–alexa 488 antibody. Bar = 100 µm for A–D and F–I and 60 µm for E and J.
There were also significant differences in proliferation between AT-MSCs and BM-MSCs on days 9, 12, and 18, as determined by use of the cell viability assay.

Implantation of MSCs and effects on meniscal regeneration

Macroscopic findings were evaluated in the menisci 12 months after creation of the defects. The control (untreated) menisci had grossly visible, full-thickness defects that remained in all samples (Figure 8). These defects were not filled or were only partially filled with thin fibrous tissue. Defects remained in only 1 of the 6 menisci treated with MSCs. There were no macroscopic differences between results for AT-MSCs and BM-MSCs; gross inspection revealed that all defects were completely covered with at least a partial-thickness tissue.

Histologic examination confirmed that there was no regeneration or only partial regeneration with fibrous tissue in control defects. Serial sections of menisci were stained with safranin O fast green, which detected the location of any acidic proteoglycan contained in cartilaginous tissue. The quantity of regenerated meniscal tissue (as determined on the basis of staining with safranin O fast green) was greater in meniscus defects treated with MSCs than in control defects (Figure 9). This suggested the development of fibrocartilaginous regenerated tissue. Intense staining with safranin O fast green was evident in meniscal defects treated with both AT-MSCs and BM-MSCs. Results for the MSC-treated defects were significantly \( P < 0.001 \) different from results for the control defects (Figure 10). However, there was no significant difference in results between defects treated with AT-MSCs or BM-MSCs.

Discussion

The objective of the study reported here was to investigate whether MSCs could regenerate meniscal tissues and whether there was a difference between BM-MSCs and AT-MSCs. The BM-MSCs and AT-MSCs were cultured, and growth kinetics, stem cell markers, and differentiation potential were examined. Several biological materials have been evaluated for their ability to improve healing of meniscal injuries, but the outcomes were disappointing. In the present study, a commercially available collagen repair patch was used as a scaffold for MSCs.

The AT-MSCs are an alternative cell source that share many properties with BM-MSCs. They are more easily collected than BM-MSCs, and the proportion of cells obtained is several times as high as that for BM-MSCs. In vitro and in vivo comparisons of BM-MSCs and AT-MSCs were conducted in the present study. During the first few days of culture in an in vitro experiment, AT-MSCs had a slightly different pattern of proliferation than for BM-MSCs, whereas after culture for 6 days, the pattern became similar until proliferation reached a plateau. A similar phenotype and capacity for multilineage differentiation toward osteogenic, adipogenic, and chondrogenic lineages was detected. It was observed that both BM-MSCs and AT-MSCs had positive results that exceeded 93% for CD90 and CD105 surface markers, as measured by use of flow cytometry; in contrast, positive results for the CD73 surface marker did not exceed 11%. The low percentage for CD73 could have...
been attributable to the fact that the antibodies were not specific against equine markers. Confocal microscopy revealed an intense positive response for all the surface makers, including CD73.

On the basis of their multipotent differentiation capacity, MSCs may offer a good possibility for use in treatment of injured menisci. Results obtained in the present study suggested that MSCs have the potential to differentiate toward fibrocartilage and thus could validate the use of MSCs to regenerate an injured meniscus. To investigate this possible use in more detail, Hispano-Breton horses were used. These animals are of considerable economic importance. Furthermore, it may also be possible to transfer the results for horses to clinical applications in humans on the basis that there are similarities in anatomic and biomechanical characteristics of the femorotibial joints of humans and horses.

The study reported here indicated that MSC constructs derived by use of a collagen scaffold can effectively regenerate tissues in meniscal lesions or defects. Although the potential plasticity of MSCs was confirmed in in vitro assays of adipogenic, osteogenic, and chondrogenic differentiation in the present study, undifferentiated MSCs were implanted to maximize tissue adhesiveness and enable secure placement in the stifle joints. Further more, it may also be possible to transfer the results for horses to clinical applications in humans on the basis that there are similarities in anatomic and biomechanical characteristics of the femorotibial joints of humans and horses.

The regenerated tissue mediated by scaffold-MSC constructs had proteoglycan production and fibrocartilaginous tissue characteristics 12 months after implantation. The implanted MSCs appeared to have responded in vivo, which led to the effective development of fibrocartilaginous tissue in the damaged menisci. It is worth mentioning that the meniscal tissues regenerated by implantation of MSCs produced proteoglycans as indicated by positive results for safranin O fast green staining. On the other hand, there was a substantial paucity of proteoglycans in the untreated control joints.

Similar to results in previous studies conducted to investigate articular cartilage repair in animals, implantation of MSC constructs in the present study promoted regeneration of meniscal tissue in the stifle joint of horses. In 1 study, intra-articular administration of autologous BM-MSCs as a treatment for meniscal lesions provided little benefit over treatment with arthroscopic surgery alone. Similar results were obtained in another study by the injection of BM cells in canine tissues. Because MSCs can easily be cultured from BM and other sources, it was originally thought that after delivery of culture-expanded MSCs to an injured host, they would migrate to the injury site and directly differentiate into the cells of an appropriate phenotype and function, thus contributing to regeneration of the injured tissue. However, this has not proven successful because MSCs remain in the synovium, and fewer cells are able to reach the defect than when a scaffold seeded with cells is used. Results for the study reported here are in accordance with those of a study in which beneficial effects on meniscal regeneration were detected by use of a stem cell-collagen scaffold implant. Regeneration of punch defects in the avascular zone of the meniscus has been
achieved with a combination of biodegradable composite matrices and BM-MSCs. The authors are not aware of previous experiments that revealed effects of meniscal regeneration in vivo by the use of MSCs in horses. In the aforementioned study, investigators assessed effects for postoperative intra-articular administration of BM-MSCs in a femorotibial lesion. To our knowledge, there have been no reports on the use of MSC-scaffold constructs implanted directly into a meniscal defect.

The present study had some limitations, such as lack of a control treatment with only the scaffold inserted into the defect (ie, no MSCs) to evaluate the effect of the scaffold alone. Furthermore, the number of horses was not sufficient to perform a statistical analysis between the regenerative potential of AT-MSCs and BM-MSCs, and there were a low number of reagents available on the equine research market, which limited mediator analyses. Nevertheless, the results reported here can serve as a basis for additional studies into the characteristics of the tissue regenerated by MSCs and the use of MSCs in regenerative treatments.

In the study reported here, BM-MSCs and AT-MSCs shared similar immunophenotypes and capacities for in vitro multilineage differentiation. Thus, it was concluded that the regenerative capacities of BM-MSCs and AT-MSCs were similar. Therefore, AT-MSCs can be considered a good alternative to BM-MSCs for use in regenerative treatments. These results also provided an important basis for investigation of the contribution of equine MSCs to regeneration of tissue in meniscal defects and whether the information obtained for horses may be transferred to humans.

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The authors declare that there were no conflicts of interest.

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Footnotes

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