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# Equine Bone Marrow Derived Mesenchymal Stem Cells: Isolation and Multilineage Differentiation

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## Abstract

**Objective-** To evaluate growth characteristics and differentiation capacity of equine mesenchymal stem cell (eMSCs) derived from bone marrow (BM).
**Study design-** In vitro experimental study.
**Animals-** Four young adult horses (2-5 years old)
**Procedure-** Cell morphology and growth characteristics of eMSCs harvested from BM were evaluated in standard culture conditions. eMSCs in passage 3 were subjected to osteogenic and adipogenic differentiation induction to investigate their differentiation potential.PCR analysis of differentiated cells was done to determine differentiation.
**Results-**The cells expanded to sufficient quantity for therapeutic purposes within days and they survived to later passages while sustained their fibroblast-like morphology. Positive osteogenesis was detected via Alizarin Red staining and adipogenesis was confirmed by Oil Red O staining. The cells were more potential to differentiate into osteoblasts rather than adipocytes. PCR analysis approve relative expression of osteogenic and adipogenic genes.
**Conclusions and Clinical relevance** - The results of this study further support eBM-MSCs as a cell population with the capacity to proliferate and differentiate down the osteogenic and adipogenic lineages. Equine BM is a potentials source of MSCs for cell-based regenerative therapies in horse.
**Keywords-** Equine, Bone marrow, Mesenchymal Stem Cell.

## Introduction

During the past decade, MSCs have been increasingly used as therapeutic aid in the field of veterinary medicine. They are employed clinically in wound repair, tissue engineering and in the field of regenerative medicine.[1,2] Human MSCs are plastic adherent cells which are positive for surface cell markers CD73, CD90, CD105 and have the potential to differentiate into at least 3 mesenchymal lineages, such as osteoblasts, adipocytes and chondrocytes.[3,4]
Nonhuman cells might not have the same surface markers as human MSCs, so, for now adherence to plastic and trilineage potential is likely sufficient to identify MSCs in horse and other species.[3]

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MSCs derived from BM and AT are the two most common equine stem cell types for cell-based regenerative therapies in horses.[5,6] High proliferative potential and early expansion to confluence has made these types of cells as proper options for tissue engineering purposes.[7]
Besides the widespread use of MSCs in regenerative medicine, the efficacy of these cells to healing of both human and non-human injuries is undetermined.[3] So in vitro differentiation studies are needed to determine MSCs as potential agents for regeneration of a specific tissue.[8]
In spite of significant importance of MSCs in horse, relatively most investigations on MSCs have been performed in species other than horses.[9] In equine medicine, stem cells have been evaluated as efficacious treatment options for some major injuries and conditions like tendinitis, desmitis and osteoarthritis.[7,10,11] Cartilage and tendon injuries are currently the most used model for stem cell therapies in horses.[3,12-15]

The objective of this study was to document the in vitro expansion and differentiation characteristics of equine BM-MSCs. This study was designed to evaluate osteogenic and adipogenic differentiation potential of BM-eMSCs but not chondrogenic differentiation, this is because "it is clear that typical mesodermal differentiation can be induced to form adipocye and osteocyte".[16]

## Materials and Methods

*Animals*

BM aspirates were collected from sternabrae of 4 horses (2-5 years old). The bone marrow collection was approved by the Institutional Animal Care and Use Committee of the University of Tehran.

*Bone marrow harvest*

Collection and isolation of eMSCs were performed by use of a modification of published protocols.[7,17]
For collection, horses were moved to standing stocks and sedated. A 10cm band overlying the sternum was clipped and scrubbed. The sternum is examined ultrasonographically to identify the three most caudal sternebrae by the appearance of their intersternebral spaces (Fig1). The most caudal one is avoided as it is thinner and there is high risk of penetrating into the deeper surface. The position of these intersternebral spaces is marked on the adjacent hair with a marker pen. The area over the sternum was aseptically prepared and local anesthesia (2% Lidocaine with Adrenaline, 5ml) was infiltrated into subcutaneous tissue and on ventral surface of sternum at the predicted entry point of aspiration needle. Under aseptic conditions, a small stab incision was made through the skin. Then the biopsy needle (Jamshidi biopsy needle, Medax medical devices, PoggioRusco, Italy, 11G×100MM) was introduced through the skin incision. Two 10ml of bone marrow was collected, using syringes preloaded with heparin (500iu/ml bone marrow aspirated, Fig 2).The BM aspirates were shipped to the University of Tehran, Faculty of Veterinary Medicine, Stem Cells Research Lab.

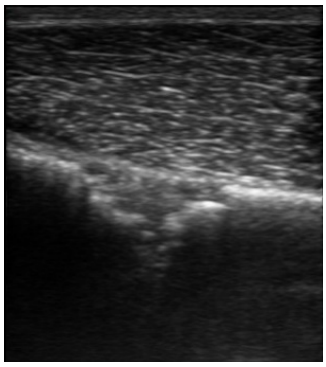

**Figure 1.** The ultrasonographic appearance of intersternal space between sternebrae 4 and 5.

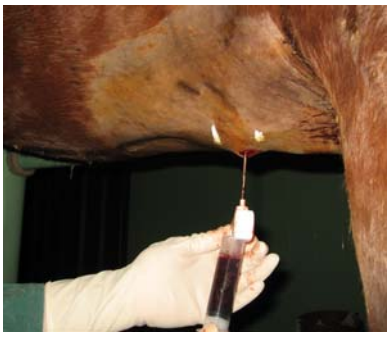

**Figure 2.** Successful aspiration of bone marrow requires attaching a heparin-loaded syringe to the inserted Jamshidi needle.

*Mesenchymal stem cell isolation and culture*

To isolate mononuclear cell fraction (MNCF), each BM aspirate was diluted with equal volumes of Dulbecco modified Eagle medium (DMEM, Gibco,UK). Atotal of 30ml diluted aspirate was loaded on 9ml of ficollhypaque (Lymphopreb, Oslo, Norway), centrifuged at 400g for 25minutes at room temperature and the interphase collected after aspirating and discarding the supernatant. The interphase (MNCF) was washed with 9ml PBS (phosphate buffered saline) and centrifuged at 2200rpm for 8 minutes. The supernatant was discarded and the cells were washed with PBS a second time at 2100rpm for 6 minutes. The cells were plated at a cell density of 100,000/cm² in T25flasks in 5ml DMEM containing fetal bovine serum (FBS, Gibco, UK) and 1% penicillin-streptomycin100x (Sigma-Aldrich, USA). Then, the cultures were incubated at 37°C in a humidifies atmosphere (95%) containing 5% CO2. The culture medium was completely replaced after 2 days in order to remove non-adherent cells. Medium was subsequently changed twice weekly. Once cells reached 80-90% confluence, adherent cells were washed twice in PBS, detached using (0.05% Trypsin/EDTA 1x, Sigma-Aldrich, USA) and replated according to standard cultural technique at 1:3 or 1:4 dilution (1st passage) and at a density of 3000cells/cm². In order to characterize the harvested cells as multipotential cells, multilineage differentiation was inducted (as described in introduction section, chondrogenic differentiation was not performed).

*Differentiation*

In this study, the passage 3 of MSCs were cultivated in 6 well plates to reach confluence for induction of osteogenic and adipogenic differentiation.

*Osteogenesis assay*

After 70-80% confluence was achieved in passage 3 of cell cultures, they were exposed to commercially available osteogenesis induction media (Gibco, UK). Cells were incubated at 37°C and 5% CO2 for 3 weeks

with medium changed twice weekly. Cultured cells were subsequently fixed with 70% methanol for 10 min and stained with Alizarin red solution to identify mineralization (calcium deposits in the matrix), which is an indicator of osteogenic differentiation.

*Adipogenesis assay*

When passage 3 of cultures reached 100% confluence, adipogenesis induction media was induced in culture using commercially available adipogenesis induction media (Gibco, UK). The cultures were incubated for 10 days at 37°C and 5%CO2. The medium was changed 3 times a week. At the end of the differentiation period, culture were fixed with 4%formalin at room temperature, washed by 70% ethanol and stained by Oil Red O solution to detect lipid vacuoles, suggesting adipogenic potential of our MSCs culture.

*RNA collection and PCR analysis*

On day 14, cells were briefly washed with PBS solution, and total RNA was collected and purified from cells that had been induced to differentiate by use of a high pure RNA isolation kit (Roche Diagnostics, Switzerland). The quantity of RNA samples was assessed spectrophotometrically using Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA). Then 1 µg of extracted RNA was reverse transcribed into cDNA using the BioRT cDNA First Strand Synthesis Kit (Hangzhou Bioer Technology, Japan) according to the manufacture's specifications. Quantitative real-time RT-PCR was performed on a light cycler instrument (Eco™ Real-Time PCR, Illumina, USA), using SYBR Premix Ex Taq technology (TakaraBio, Japan). SYBR Green master mix (10 µl), 2 µl of cDNA samples, 0.5 µl of forward and reverse primers (10 pmol) and 7 µl of nuclease-free water (Qiagen, Hilden, Germany) were mixed in a capillary tube (Roche Diagnostics, Switzerland) ito conduct PCR in a 20µl of reaction volume. Thermal cycling consist of an initial activation step for 30 s at 95_C, 40 cycles including a denaturation step for 5 s at 95_C and a combined annealing/extension step for 20 s at 60_C. Primers were designed through use of equine gene sequences (Appendix; Gen Bank accession Nos. XM_001502519.3, XM_003365243.1, XM_001492430.1 and XM_001489577 for core binding factor alpha1 (Cbfa1)/runt related transcription factor 2 (Runx2), transcription factor ostreix (Osx), peroxisome proliferator-activated receptor-gamma (PPAR-g) and lipoprotein lipase (LPL), respectively.
In this study equine bone marrow derived MSCs were characterized using recognized molecular markers for "stemness" namely: Cbfa1, Osx (osteogenesis markers), PPAR-g and LPL (adipogenesis markers). The sequences of primers are listed in Table1. The differentiated cells were characterized morphologically and relative expression of osteogenic and adipogenic genes (core binding for stemness was determined by Quantitative Real Time PCR (QRT-PCR).

## Results

*Cell morphology and culture characteristics*

After 3 days in culture, BM-eMSCs observed as 1-10 cell colonies of plastic adherent cells with a fibroblastic appearance (Fig2A). This elongated fusiform cells proliferated homogeneously in monolayer of swirling spindle-shaped cells.
BM derived cells became focally confluent in a requiring trypsinization in 18±3 days (fig 2B). We cultivated BM-MSCs up to passage 7.In the early passages, the BM-MSCs appeared as long, spindle shaped cells. Although the overall appearance of cell culture was not altered substantially during the passages, this morphology changed to a more flattened and elongated cell body (Fig 2C) Due to lack of contact inhibition in confluents cultures cells grew in multiple layers. Cultures at third passage yielded enough cells for our next experiment on equine lower extremity wound healing.

*Differentiation*

Cell differentiation of MSCs into adipocytes and osteoblast was observed in the inducted cultures under appropriate conditions.

*Osteogenic differentiation*

The first evidences suggesting osteogenic potential of the BM-eMSCs appeared as layered cell clusters surrounded by mineralized matrix in the culture, 4 days after induction of differentiation. By this time of osteogenic induction, the phenotype of cells altered to cubical star-shaped cells with sharp extension and they became larger in size. The mineralized culture stained red with Alizarin Red 8 days after induction, indicating the formation of calcium phosphate crystals by osteoblasts (Fig4A). Quantitative PCR analyses of Cbfa1/Runx2 and Osx mRNA expression revealed remarkable expression of these osteogenic marker genes comparing to controls (Fig5A).
These results are very important, since Runx2 and Osx transcriptional activities are essential for osteogenesis and consequent bone formation.[18]

*Adipogenic differentiation*

The first signs of adipogenic differentiation became apparent as lipid vacuoles in the differentiating cells 7days after induction of differentiation. By day 12 this droplets became more numerous and larger and stained red with Oil-Red-O staining (Fig4B).
QRT-PCR analysis indicated that the mRNA of adipocyte-specific genes including LPL, PPAR-gamma

were largely produced in adipogenic culture comparing to control ones (Fig5B). PPAR is key regulator of adipocyte differentiation and lipid storage and expression of LPL gene is essential for lipid mobilization and storage in adipose tissue so expression of these marker genes suggests a promising adipogenesis.[19, 20]

**Table 1.** Primers used for real-time PCR quantification

| Equus caballus | Accession number | Forward & Reverse primers | | Amplicon |
|---|---|---|---|---|
| **PPAR-g** | XM_001492430.1 | F: 5'-TAGCTCCCATCGCTTAGGTT-3'<br>R: 5'-CCAAAATGGCATCTCCGTGT-3' | 20<br>20 | 126 |
| **LPL** | XM_001489577 | F: 5'-CGAGCGCTCCATTCATCTCT-3'<br>R: 5'-CAGGCAGAGCCCTTTCTCAA-3' | 20<br>20 | 106 |
| **Cbfa1(Runx2)** | XM_001502519.3 | F: 5'-GCGCATTCCTCATCCCAGTA-3'<br>R: 5'-GGCTCAGGTAGGAGGGGTAA-3' | 20<br>20 | 176 |
| **Osx** | XM_003365243.1<br>XM_001494930.3 | F: 5'-CTCCCCACCTCCTGCAAC-3'<br>R: 5'-TCCACCACTGCCCTCCAAC-3' | 18<br>19 | 101 |
| **GAPDH** | NM_001163856.1 | F: 5'-CCCACTCTTCCACCTTCGAT-3'<br>R: 5'-CTCCTTCTCTTGCTGGGTGA-3' | 20<br>20 | 179 |

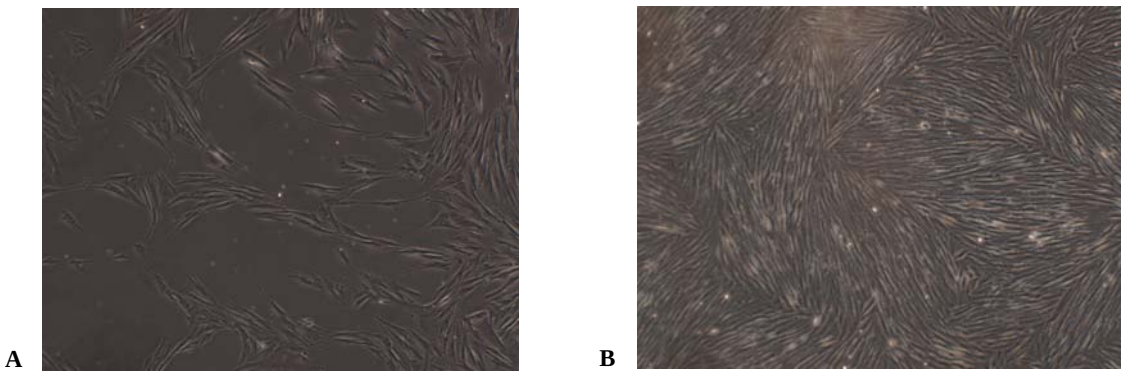


**Figure 3.** Morphology of Equine BM-MSCs at the beginning of the culture and at confluence (A) Plastic adherent cells appeared with elongated fibroblastic morphology (magnification 40x) ;(B) The cells became confluent after 18±3 days in culture (magnification20x).

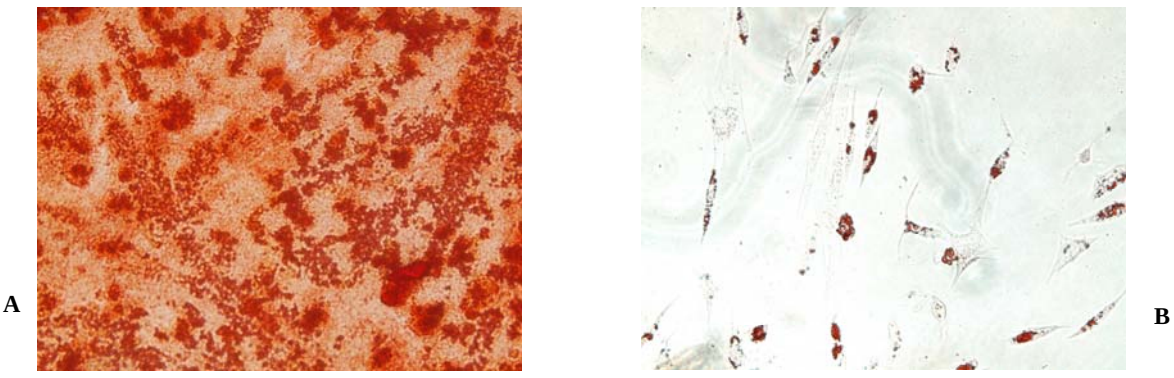


**Figure 4.** Cell differentiation of Equine BM-MSCs into adipocytes and osteoblasts. (A) Osteogenesis, 8 days after induction to osteogenesis, cell clusters and mineralised matrix that stained positive by alizarin red staining were detected; (B) Adipogenesis, 14 days after induction of differentiation, numerous lipid droplets (stained in red with Oil Red O staining) became apparent as cytoplasmic accumulations.

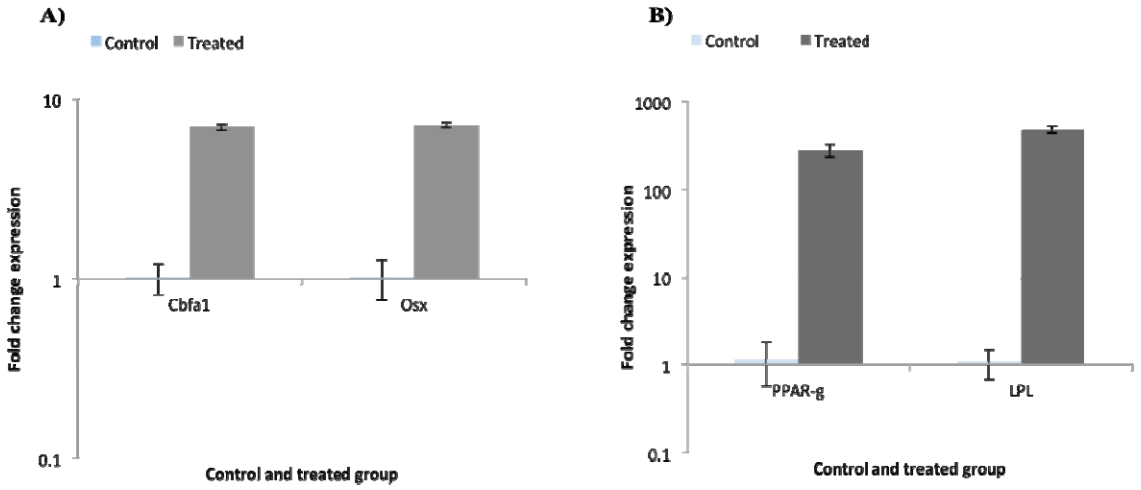


**Figure 5-** Fold change expression of osteogenic and adipogenic genes.(A) Fold change expression of osteogenic genes (Osx and Cbfa1/Runx2); (B) Fold change expression of adipogenic genes (PPAR and LPL).

## Discussion

MSCs can be harvested from several tissues such asBM,[7, 21-24] AT,[25] peripheral blood[23] and umbilical cord blood[15] in horse.

BM-MSCs and AT-MSCs are the 2 most common equine stem cell types used for cell therapies in horse.[5, 26] Technically, harvesting MSCs from BM and AT is easy but the technique employed for acquisition of MSCs from adipose tissue is more invasive due to standing surgical position of the animal and is riskier due to donor site morbidity.[24, 26]

Additionally, the harvest of MSCs is more cosmetically acceptable and the initial isolation process is less complicated compared with that of AT-MSCs.[25] Yield of MSCs from BM is lower when compared to AT, but their chondrogenic and osteogenic differentiation capacity is superior to AT-MSCs and they are investigated more in MSCs studies.[5, 6, 25, 27] Moreover, isolation of BM-MSCs is faster than AT-MSCs and in terms of purity, isolation from bone marrow yields more pure population cells in contrast to that of adipose tissue which is contaminated by fibroblasts and adipocytes.[24]

The focus of this study was to characterize BM-eMSCs based on adherence to plastic flasks, growth characteristics and osteogenic and adipogenic differentiation potential.

In this study, polystyrene adherent cells from bone marrow cultivated in standard media containing 15%FBS which is the least concentration with the highest mitogenic effect.[28] BM-eMSCs appeared as large elongated fibroblast like cells and divided like bone marrow stromal cells and tissue-specific cells from other species.[29, 30]

Bone marrow-derived stem cells have the capacity to proliferate even in high passages[31]. In the present study, the undifferentiated cells which were passaged 7 times maintained their overall morphological and growth characteristics. This finding was similar to that of previous studies.[7, 24] There was some inconsistency with the results of recent study on equine AT-MSCs which revealed morphological change of cells at later passages and detachment of them from plastic flasks at passage 5 to passage 6.[4]

According to previous investigations, the equine BM-derived MSCs were more capable of osteogenic differentiation than were adipose-derived cells and they produced more mineral in the culture treated with induction media comparing to untreated cells.[23, 25, 32]

In this study, morphological changes suggesting osteogenic differentiation was detected after 4days in the inducted culture. This finding was comparable to results of a study conducted by[7] who revealed that oseogenic differentiation of BM-MSCs occurred after 3-4 days.

However, two other studies have reported that time period required to achieve osteogenic differentiation of BM-eMSCsis 2or 3 weeks respectively.[18, 28]

In terms of adipogenic differentiation, elongated confluent fibroblastic cells started to change to oval-shaped cells containing large lipid vacuoles after 7days of exposure to induction medium which was in accordance with the findings of Baghban Eslaminejad.[28] BM-eMSCsshowed adipogenic potential 10 days after exposure to induction medium as described by Koch.[3]

The use of rabbit serum has been confirmed to enhance adipogenesis in BM-MSCS in man[33-35] and rodents.[33]There is some reports describing that BM-eMSCs show lower adipogenic differentiation capacity

than AT-MSCs. They also found adding 5% rabbit serum as a requirement for successful adipogenesis of equine BM-MSCs.[7, 25] Another study reported that addition of 15% rabbit serum to adipogenic induction medium has successfully induced adipogenesis of equine BM-MSCs.[26] According to Koch[3] " it appears that a concentration of 5% rabbit serum may be optimal in order to balance enhanced induction against increased cell detachment with higher serum concentrations".

However, similar to results of two other studies[21,22] adipogenic differentiation of BM-MSCs has been achieved without addition of rabbit serum in our study.

MSC frequency is directly related to age of the donor[7], so all of the equine donors we used were young animals for which a higher MSC frequency would be expected.

Currently, several research groups worldwide are investigating BM-MSCs as promising candidates for treatment of traumatic ligament and tendon injuries in the horse.[14, 36]

Despite the importance of managing lower extremity wounds in horses, the efficacy of MSC therapy for treatments of chronic non healing wounds of lower limb has not been studies in equine species.

Based on promising results of these experiments in other species[37-40], we would like to propose MSCs as potential treatments for wound healing in our successive experiment.

These data will be the basis of future efforts to standardize the isolation, expansion, differentiation and transplantation of equine Mesenchymal stem cells in clinical practice.

## Acknowledgments

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### List of Abbreviations

BM: Bone marrow
MSCs: Mesenchymal stem cells
AT: Adipose tissue
BM-MSCs: Bone marrow derived MSCs
AT-MSCs: Adipose tissue derived MSCs
eMSCs: Equine mesenchymal stem cells
BM-eMSCs: Bone marrow derived equine MSCs

## References


1. Stevens M, Stem cells in regenerative medicine. *The Pharmaceutical Journal* 2005; 275: 695-69.
2. Mimeault M, and Batra SK, Concise Review: Recent Advances on the Significance of Stem Cells in Tissue Regeneration and Cancer Therapies. *Stem cells*, 2006; 24(11):2319-2345.
3. Koch TG, Evaluation of equine cord blood multipotent mesenchymal stromal cells (published PhD dissertation), 2008; University of Guelph, Canada.
4. Braun J, Hack A, Weis-Klemm M, et al. Evaluation of the osteogenic and chondrogenic differentiation capacities of equine adipose tissue-derived mesenchymal stem cells. *Am J Vet Res* 2010; 71(10): 1228-1236.
5. Vidal MA, Robinson SO, Lopez MJ, et al. Comparison of Chondrogenic Potential in Equine Mesenchymal Stromal Cells Derived from Adipose Tissue and Bone Marrow. *Vet Surg* 2008; 37(8):713-724.
6. Colleoni S, Bottani E, Tessaro I, et al. Isolation, growth and differentiation of equine mesenchymal stem cells: effect of donor, source, amount of tissue and supplementation with basic fibroblast growth factor. *Vet Res Commun* 2009; 33(8): 811.
7. Vidal MA, Kilroy GE, Johnson JR, et al. Cell Growth Characteristics and Differentiation Frequency of Adherent Equine Bone Marrow–Derived Mesenchymal Stromal Cells: Adipogenic and Osteogenic Capacity. *Vet Surg* 2006; 35(7):601-610.
8. Rubin LL, Stem Cells and Drug Discovery: The Beginning of a New Era? *Cell* 2008;132(4): 549-552.
9. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999. 284:143-147.
10. Schnabel LV, Lynch ME, van der Meulen MCH, et al. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthopaed Res* 2009; 27(10): 1392-1398.
11. Smith RKW, Korda M, Blunn GW, et al. Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Vet J* 2003; 35(1): 99-102.
12. Crovace A, Lacitignola L, Rossi G, et al. Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. *Vet med internatiol*, 2010; 2010:250978-10.
13. Pacini S, Spinabella S, Trombi L, et al. Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue eng* 2007;13(12):2949-2955.
14. Wilke MM, Nydam DV, Nixon AJ, et al. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthopaed Res* 2007;25(7):913-925.
15. Koch TG and Betts DH, Stem cell therapy for joint problems using the horse as a clinically relevant animal model. *Expert Opinion on Biological Therapy* 2007;7(11): 1621-1626.
16. Sung HJ, Hong SC, Yoo JH, et al. Stemness Evaluation of Mesenchymal Stem Cells from Placentas According to Developmental Stage: Comparison to Those from Adult Bone Marrow. *J Korean Med Sci* 2010; 25(10):1418-1426.
17. Toupadakis CA, Wong A, Genetos DC, et al. Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord

blood, and umbilical cord tissue. *Am J Vet Res* 2010; 71(10):1237-1245.
18. Andrietti P, Antonella, Comprative Osteogenesis of Equine Mesenchymal Stem Cells Isolated from Bone Marrow, Adipose tissue and Synovium, in Veterinary Clinical Medicine, *MS thesis* 2012, University of Illinois: Urbana-Champaign.
19. Yu S, Matsusue K, Kashireddy P, et al. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *J Biol Chem* 2003; 278(1):498-505.
20. Gregoire FM, Smas CM, and Sul HS, Understanding adipocyte differentiation. *Physiol Rev* 1998; 78(3):783-809.
21. Fortier, LA, Nixon, AJ, Williams J. et al. Isolation and chondrocytic differentiation of equine bone marrow-derived mesenchymal stem cells. *Am J vet Res* 1998;59:1182-1187.
22. Arnhold, SJ, Goletz I, Klein H, et al**.** Isolation and characterization of bone marrow–derived equine mesenchymal stem cells. *Am J Vet Res* 2007; 68(10):1095-1105.
23. Koerner J, Nesic D, Romero JD, et al. Equine Peripheral Blood-Derived Progenitors in Comparison to Bone Marrow-Derived Mesenchymal Stem Cells. *Stem cells* 2006;24(6):1613-1619.
24. Violini S, Ramelli P, Pisani LF, et al. Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by in vitro exposure to BMP-12. *BMC cell biol* 2009; 10(1):29-29.
25. Vidal MA, Kilroy GE, Lopez MJ, et al. Characterization of Equine Adipose Tissue-Derived Stromal Cells: Adipogenic and Osteogenic Capacity and Comparison with Bone Marrow-Derived Mesenchymal Stromal Cells. *Vet Surg* 2007;36(7):613-622.
26. Bourzac C, Smith LC, Vincent P, et al. Isolation of equine bone marrow-derived mesenchymal stem cells: a comparison between three protocols. *Equine Vet J* 2010;42(6):519-527.
27. Kisiday JD, Kopesky PW, Evans CH, et al. Evaluation of adult equine bone marrow- and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. *J Orthopaed Res* 2008; 26(3):322-331.
28. Baghaban Eslaminejad MR, Taghiyar L, Dehghan MM, et al. Equine marrow-derived mesenchymal stem cells: isolation, differentiation and culture optimization. *Iran J Vet Res* 2009; 10(1):1-11.
29. Kadiyala S, Young RG, Thiede MA, et al. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *Cell Transplant* 1997(6):125-134.
30. Izadpanah R, Joswig T, Tsien F, et al. Characterization of multipotent mesenchymal stem cells from the bone marrow of rhesus macaques. *Stem Cells Dev* 2005(14):440-451.
31. Lee K, Majumdar MK, Buyaner D, et al. Human Mesenchymal Stem Cells Maintain Transgene Expression during Expansion and Differentiation *Mol Ther* 2001; 3(6):857-866.
32. Ahern BJ, Schaer TP, Terkhorn SP, et al. Evaluation of equine peripheral blood apheresis product, bone marrow, and adipose tissue as sources of mesenchymal stem cells and their differentation potential. *Am J Vet Res* 2011; 72(1):127-133.
33. Diascro DD, Vogel RL, Johnson TE, et al. High Fatty Acid Content in Rabbit Serum Is Responsible for the Differentiation of Osteoblasts Into Adipocyte-like Cells. *J Bone Miner Res* 1998; 13(1): 96-106.
34. Houghton A, Oyajobi BO, Foster GA, et al. Immortalization of Human Marrow Stromal Cells by Retroviral Transduction With a Temperature Sensitive Oncogene: Identification of Bipotential Precursor Cells Capable of Directed Differentiation to Either an Osteoblast or Adipocyte Phenotype. *Bone* 1998; 22(1):7-16.
35. Janderova L, McNeil M, Murrell AN, et al. Human Mesenchymal Stem Cells as an in Vitro Model for Human Adipogenesis. *Obesity* 2003; 11(1):65-74.
36. Smith RKW and Webbon PM, Harnessing the stem cell for the treatment of tendon injuries: heralding a new dawn? *Brit J Sport Med* 2005; 39(9): 582-584.
37. Nakagawa H, Akita S, Fukui M, et al. Human mesenchymal stem cells successfully improve skin-substitute wound healing. *Brit J Dermatol* 2005; 153(1):29-36.
38. Sasaki M, Abe R, Fujita Y, et al. Mesenchymal Stem Cells Are Recruited into Wounded Skin and Contribute to Wound Repair by Transdifferentiation into Multiple Skin Cell Type. *The J Immunol* 2008;180(4):2581-2587.
39. Li H, Fu X, Ouyang Y, et al. Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. *Cell Tissue Res* 2006; 326(3):725-736.
40. Wu Y, Chen L, Scot PG, et al. Mesenchymal Stem Cells Enhance Wound Healing Through Differentiation and Angiogenesis. *Stem cells* 2007; 25(10):2648-2659.

نشریه جراحی دامپزشکی ایران
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## چکیده

# سلولهای بنیادی مزانشیمی مشتق از مغز استخوان اسب: جداسازی و تمایز به چند رده سلولی

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**هدف-** بررسی خصوصیات رشد و قابلیت تمایز سلولهای بنیادی استخراج شده از مغز استخوان در اسب
**طرح مطالعه-** مطالعه‌ی تجربی خارج از محیط بدن
**حیوانات-** چهار رأس اسب بالغ جوان (سن ۵-۲سال)
**روش‌کار-** سلولهای بنیادی مزانشیمال استخراج شده از مغز استخوان از جهت ریخت شناسی و خصوصیات رشد در محیط کشت استاندارد مورد بررسی قرار گرفتند. جهت بررسی قابلیت تمایز آدیپوژنیک و استئوژنیک، فرایند القاء تمایز در مورد سلولهای پاساژ سوم انجام شد. آزمایش PCR سلولهای تمایز یافته برای تعیین تمایز انجام شد.
**نتایج-** طی چند روز، تکثیر سلولها منجر به تولید مقادیرکافی سلول برای اهداف درمانی شد. سلولها حتی در پاساژهای بالا زنده ماندند و شکل فیبروبلاستی خود را حفظ کردند. تمایز مثبت سلولهای بنیادی مرانشیمی به سلولهای استخوانی توسط رنگ آمیزی آلیزارین رد و برای تایید تمایز به سلولهای چربی رنگ آمیزی اویل رد انجام شد. آزمایش PCR نیز بیان نسبی ژن‌های آدیپوژنیک و استئوژنیک را تایید نمود. قابلیت سلولهای بنیادی مزانشیمال استخراج شده از مغز استخوان اسب در تمایز به بافت استخوانی در مقایسه با تمایز به بافت چربی بیشتر بود.
**نتیجه‌گیری-** نتایج این مطالعه نیز همانند مطالعات قبلی نشان داد که سلولهای بنیادی مزانشیمی استخراج شده از مغز استخوان در اسب به‌عنوان یک جمعیت سلولی با قابلیت تکثیر و تمایز به رده‌های استئوژنیک و آدیپوژنیک می‌باشند و مغز استخوان اسب می تواند به عنوان یک منبع بالقوه سلولهای بنیادی مزانشیمی برای درمان‌های بازسازی کننده با پایه‌ی سلولی در اسب مورد استفاده قرار گیرد.
**کلید واژگان-** اسب، مغز استخوان، سلولهای بنیادی مزانشیمی، تمایز