

## Abstract

•Bone-marrow derived mesenchymal stem cells are capable of differentiating into multiple tissue types and are currently a commonly utilized novel therapeutic treatment of musculoskeletal injuries.



•Equine sternum and ilium are the common sites for bone marrow aspirates; however, there have been no comparisons in the differentiation

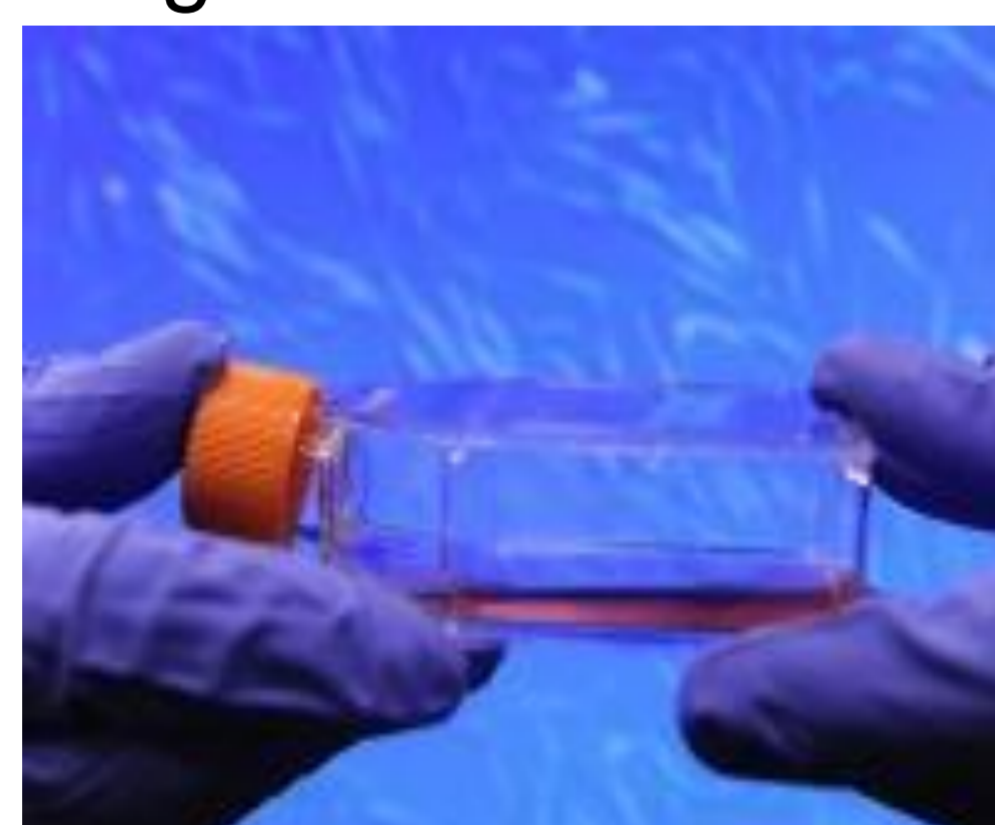
propensity of these cells from each site

•The aim of this study was to compare MSCs acquired from equine sternum and ilium and evaluate osteogenic and chondrogenic potential of different aspirate sites

## Introduction

•Equine musculoskeletal disease is common in sport horses and is often career ending without intervention

•Regenerative therapy utilizing bone marrow-derived mesenchymal stem cells (BMDMSCs) is a novel approach to healing damaged tissues.



•BMDMSCs can be cultured in vitro to produce many different tissue types, including bone and cartilage.

•Bone marrow aspirates are usually acquired from equine sternum or ilium.



•The purpose of this study was to compare differences in MSCs from equine sternum and ilium.

•We hypothesized that there are no significant differences in the differentiation capacity of MSCs taken from sternum and ilium; but there are differences between the first and second aspirate pull from the same location.

## Materials and Methods

### Bone-Marrow Aspirates for Cell Acquisition

- Seven horses, between 3-5 years of age, had sternum and ilium aspirates performed.
- Two 5 mL aspirates were taken from each site without needle redirection.
- Marrow was spun down and serum was collected. Cells were grown in monolayer with DMEM.
- Cells were trypsonized after reaching confluency and stored in liquid nitrogen at passage 3.
- MSCs removed from cryopreservation were allowed to grow to confluency prior to differentiation assays.

Figure 1: Osteogenic Stains

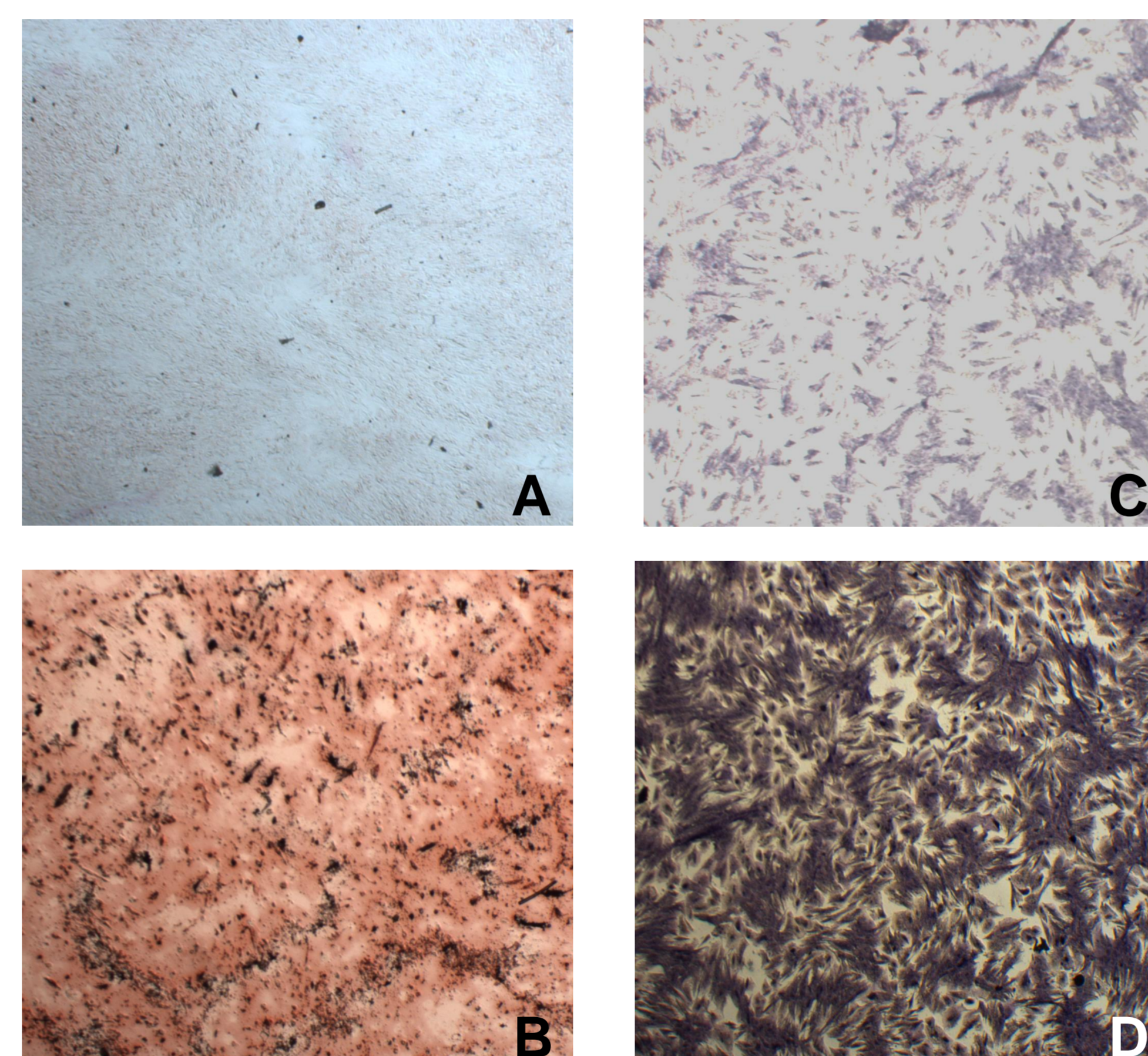


Figure 1: Alizarin red and Alkaline Phosphatase Staining indicating osteogenesis. Cells stained with Alizarin Red without osteogenic media (A) and with osteogenic media (B). Cells stained with Alkaline Phosphatase stain without osteogenic media (C) and with osteogenic media (D).

### Osteogenic Differentiation

- Osteogenic differentiation was conducted in 24 well plates with a cell density of 80,000 per cm<sup>2</sup>.
- MSCs were allowed to grow to confluency within wells (24 hours) treated with aMEM and FGF. Cells were then treated with aMEM supplemented with dexamethasone, ascorbic acid and glycerol phosphate.
- After 7 days of exposure to differentiation media, MSCs were lifted for Alkaline Phosphatase ELISA and stained with Alizarin Red and Alkaline Phosphatase stains.

## Materials and Methods

### Chondrogenic Differentiation

•MSCs were suspended in low melting agarose gel and surrounded with chondrogenic differentiation media (high glucose DMEM + 1% ITS, dexamethasone, ascorbate 2 phosphate, and TGF beta).

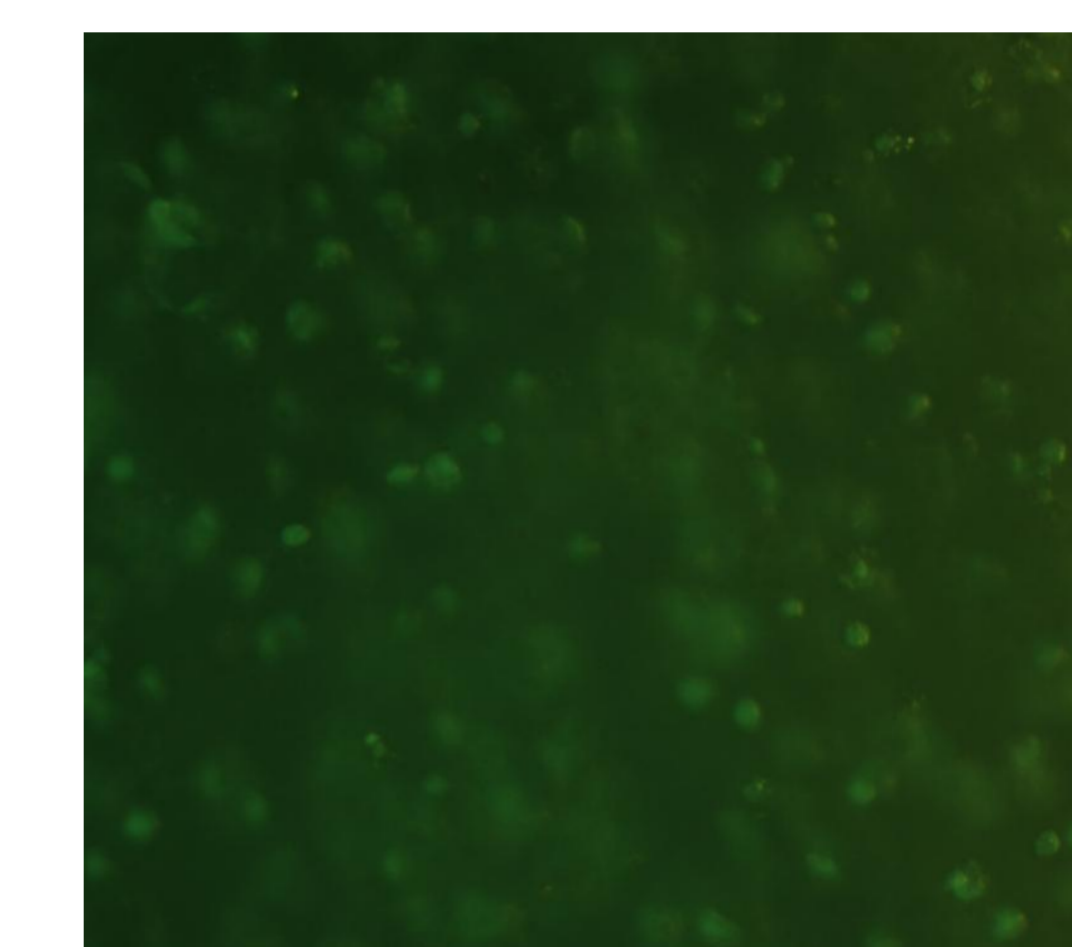


Figure 4. Live MSCs within Agarose

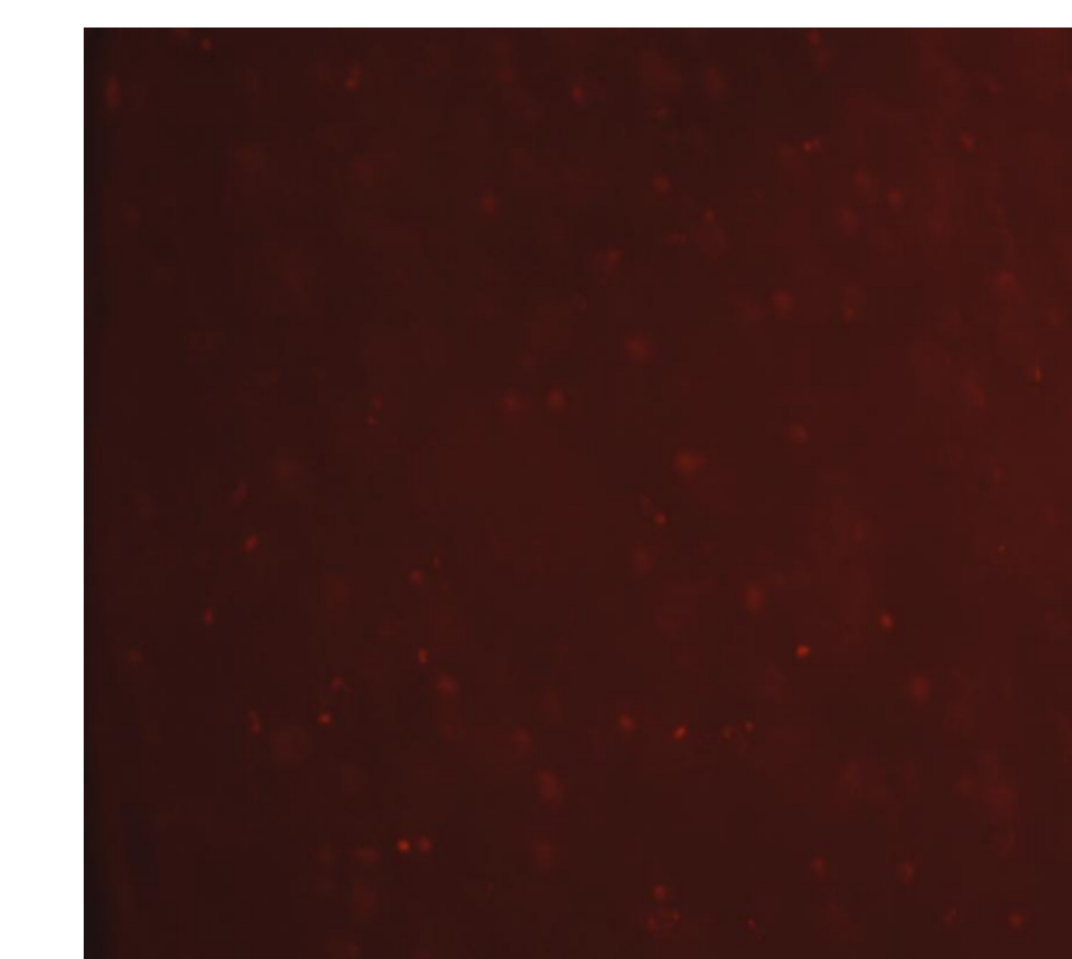


Figure 5. Dead MSCs within Agarose

- Agarose gel wells were sliced after 12 and 24 hours for live dead staining.
- Wells containing viable cells within gels, were allowed to grow in differentiation media for 21 days, with media changes every 3<sup>rd</sup> day.
- Gels were then snap frozen, sectioned and stained with Alcian blue to observe chondrogenic potential.
- DMMB assays were performed to quantify the sulfated GAG present in each gel well as well as surrounding media.
- Immunohistochemistry was conducted for Collagen Type II

## Discussion and Conclusion

Initial observations indicate there may be no difference in MSCs from equine sternum or ilium with regard to their propensity to differentiate into osteocytes or chondrocytes. Further, cells from the first aspirate appeared more prolific however propensity to become chondrogenic or osteogenic was not noted. Clinicians can draw from either sternum or ilium when attempts at regenerative therapy are pursued without concern that one site would be more appropriate than another.

## Acknowledgements



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