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## HORSE SPECIES: ADVANCES IN EQUINE STEM CELL BIOLOGY

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### 0382 Developmental progenitor cells of articular chondrocytes. J. N. MacLeod\*, *University of Kentucky, Lexington.*

Articular cartilage lesions frequently compromise diarthrodial joint function and limit the performance potential of equine athletes. Cartilage is a tissue with poor intrinsic repair capabilities, a primary reason why degenerative joint disease is often progressive. New cell-based therapeutic strategies for equine joint surface injuries are generating a high level of interest. Unfortunately, fibrocartilage formation and poor anchoring of the repair tissue into the surrounding healthy tissue continue to be major challenges. Based on structural and molecular comparisons of different cartilaginous tissues and studies using primary chondrocyte cultures, it is clear that not all chondrocytes are equivalent on a cell biology level. We are trying to advance cell-based therapies for joint surface lesions by considering the unique phenotype that defines normal articular chondrocytes relative to other chondrocyte cell types, as well as the developmental processes that generate these cells. During limb formation, a morphologically distinct zone of cells in the prechondrogenic mesenchyme initiates synovial joint formation. This mesenchymal tissue is known as the “interzone” and appears as a flattened layer of cells connected by gap junctions. Interzone cells exist during early fetal development in all mammals including horses, but are present only transiently before joint space cavitation. In a developmental biology context, the interzone is the normal progenitor of all synovial joint tissues including articular cartilage. Using an amphibian model system, we have demonstrated that interzone tissue can facilitate a remarkable repair of large articular cartilage defects and even generate an entirely new diarthrodial joint *de novo*. More recently, we have been able to characterize interzone tissue in early equine fetuses and isolate primary horse interzone cells that can be expanded in culture. Experiments are being conducted to compare gene expression profiles of interzone cells to different types of chondrocytes on a transcriptome level, while also studying their response to differentiation stimuli. We believe that interzone tissue represents a cell population already developmentally positioned to form articular cartilage— true progenitor cells of articular chondrocytes. As such, they may represent a far superior cell type to focus on for optimizing cell-based therapies to repair articular cartilage defects in the horse.

**Key Words:** horse, cartilage, interzone

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### 0383 Understanding the link between inflammation and muscle satellite cells in the horse.

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As an athletic animal, the horse performs a variety of activities throughout its life. With improvements in care, the equine population is living longer and remaining active and competing at increasingly older ages. Both advancing age and exercise result in increased concentrations of circulating and local cytokines, including interleukin (IL)-1 $\beta$ , IL-6, IL-8, interferon (IFN)- $\gamma$ , and tumor necrosis factor (TNF)- $\alpha$ . Athletic endeavors in the aged horse may further increase the pro-inflammatory environment in the muscle, decreasing the ability to react appropriately to exercise. Poor response to exercise limits the athletic ability of geriatric horses, thus reducing their useful life span and potentially increasing the risk of injury. Satellite cells are muscle stem cells that reside adjacent to muscle fibers in skeletal muscle and are at least partially responsible for both maintenance of muscle mass and muscle hypertrophy. Normally, these cells exist in a quiescent state, becoming active, proliferating and differentiating in response to specific stimuli. Growth factors and cytokines present during hypertrophy and following exercise affect satellite cell activity. While the specific effects of cytokines on satellite cells are not well established, cytokines can both positively and negatively influence satellite cell and myoblast proliferation and differentiation. Equine satellite cells are comparable to satellite cells isolated from other species, exhibiting a fibroblast-like morphology in culture after activation and expressing desmin, an intermediate filament protein specific to muscle cells. Further, they differentiate into multinucleated myotubes which express myosin heavy chain, a fundamental property of myogenic cells. Understanding the effect of cytokines on equine satellite cell function will allow us to determine the mechanisms responsible for the poor response to exercise. Preliminary data indicates that the pro-inflammatory cytokines IL-1 $\beta$  and IL-6 inhibit myogenesis. C2C12 myoblasts cultured with 1.0 ng/mL IL-1 $\beta$  exhibit impaired fusion compared with controls ( $P < 0.01$ ). Further, C2C12 myoblasts cultured with 10 ng/mL IL-6 exhibit decreased proliferation and decreased fusion compared with controls ( $P < 0.01$ ). Ongoing work is examining the effects of these cytokines on satellite cells from young and adult horses. The pro-inflammatory environment in aged horses may inhibit exercise induced satellite cell activity, thereby diminishing exercise induced hypertrophy. As more horses are surviving and competing into their 20's, more research is required to understand the response of these animals to exercise during normal aging.

**Key Words:** cytokines, horse, satellite cells

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#### 0384 Use of mesenchymal stem cells in bone repair.

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Equine bone fractures are often catastrophic, potentially fatal, and costly to repair. Traditional methods of healing fractures have limited success, long recovery periods, and a high rate of re-injury. Current research in the equine industry has demonstrated that stem cell therapy is a promising novel therapy to improve fracture healing and reduce the incidence of re-injury; however reports of success in horses have been variable and limited. Stem cells can be derived from embryonic, fetal and adult tissue. However, based on the ease of collection, opportunity for autologous cells, and proven success in other models, adipose or bone marrow derived mesenchymal stem cells (MSC) are often used in equine therapies. Methods for isolation, proliferation, and differentiation of MSC are well established in rodent and human models but are not well characterized in horses. There is recent evidence that equine bone marrow MSC are able to proliferate in culture for several passages in the presence of autologous and fetal bovine serum which is important for expansion of cells. Mesenchymal stem cells are able to differentiate into osteoblasts, the bone forming

cells, and this complex process is regulated by a number of transcription factors including, runt-related transcription factor 2 (*Runx2*) and osterix (*Osx*). However, it has not been well established if equine MSC are regulated in a similar manner. In the presence of L-ascorbic acid-2-phosphate, glycerol-2-phosphate, and dexamethasone, equine bone marrow MSC are able to differentiate into osteoblasts in culture as demonstrated by increased alkaline phosphatase activity and mineralization ( $P < 0.05$ ). In addition, similar to rodent and human models, in equine bone marrow MSC, *Runx2* expression increased threefold ( $P < 0.001$ ) during early differentiation and *Osx* expression increased ninefold ( $P < 0.05$ ) during late differentiation. Further, expression of a novel transcription factor, *T-box3*, which is required for proliferation of mouse osteoblast cells and inhibits differentiation of osteoblasts, was reduced fourfold ( $P < 0.01$ ) during differentiation of equine bone marrow MSC. These data demonstrate that equine bone marrow MSC may be regulated similar to rodent and human cells during osteoblast differentiation. Stem cell therapy is promising in equine bone repair, however additional research is need to identify optimal methods for reintroduction and potential manipulations to improve their ability to form new bone.

**Key Words:** bone, equine, mesenchymal stem cells