Over the past several decades the diagnostic capabilities and treatments of musculoskeletal injuries have rapidly advanced. Imaging technologies developed and produced for human medicine are now readily available, applicable and in widespread use in veterinary medicine. Digital radiography and digital ultrasound systems are routinely utilized by many mobile equine practitioners and have dramatically improved the diagnostic value of these examinations by providing superior images within seconds. Advanced imaging techniques, such as digital fluoroscopy, nuclear scintigraphy, computed tomography (CT), and magnetic resonance imaging (MR), are now routinely used in horses at diagnostic imaging and surgical referral centers. Radiography and ultrasound when used together are great screening tools and provide complementary information about the bone and soft tissues of an area, respectively. However, when these imaging tools provide equivocal information about a region advanced modalities such as CT and MR are indicated which require general anesthesia to position the limb of the horse within the gantry. Because general anesthesia carries an increased risk of injury when the horse recovers, the clinician must be willing to accept the risk of anesthesia in order to gain a more accurate diagnosis.

Ultrasound remains a practical, inexpensive, non-invasive and readily accessible imaging technique that allows real time evaluation of the soft tissues of the horse. The ability to visualize these structures in real time makes ultrasound the ideal tool to accurately guide interventional regenerative therapies and is currently utilized by many equine practitioners. However, ultrasound often has a limited field of view and the ability to visualize a lesion is directly related to the operator, the angle of incidence, the equipment and the physical and physiological status of the tissue. If a lesion(s) can be diagnosed with standard ultrasonographic imaging then it is typically not necessary to pursue advanced imaging and expose the patient to the associated anesthetic risks. However, due to the inherent limitations of ultrasound, it is important to remember that a negative ultrasound study does not rule out that an abnormality(s) does in fact exist and may warrant MR examination of this area. MRI provides unparalleled resolution and wide fields of view of the distal limb of the horse. In addition, MRI can provide physiologic information about soft tissues and bones of the distal limb of the horse, such as the presence of inflammation or edema, through diagnostic weighting. MRI is particularly useful in anatomical areas where conventional imaging modalities have limitations, such as the foot, palmar/plantar soft tissues, and joints of the distal limbs. Indeed the use of MRI in equine lameness evaluations has allowed us to identify soft tissue and bone injuries not previously recognized and to treat injuries more precisely and accurately. While MRI is now considered the gold standard in assessing lameness’s of the distal limb of the horse it is expensive and requires general anesthesia. At present, interventional techniques performed during the MR examination are impractical in the horse and would prolong anesthesia time increasing risk of injury to the patient.
We are currently investigating the accuracy and applications of recently developed MR/US fusion technology for use in the horse at our clinic at NC State College of veterinary medicine. Fusion of these modalities allows matching of the exact CT or MR slice with the ultrasound image. Through a magnetic GPS tracking device attached to the ultrasound probe, the ultrasound machine determines the spatial location of the ultrasound probe and displays the corresponding representative slice of the previously performed CT or MR examination. As the ultrasound probe is moved the computer determines the change in location and orientation, and alters the CT or MR image accordingly. Accurate visual verification and calibration of fused images is crucial in this process and needs to be documented as the fusion accuracy plays a critical role in determining the overall precision and effectiveness of this technology. This technology enables precise determination of the location of lesions only visible with advanced imaging for ultrasound guided interventional therapies. Integrating CT or MR images into the ultrasound data set facilitates image analysis and surgical intervention. Many uncertainties regarding intra-operative lesion localization and delivery of therapeutics can be removed by precisely mapping the pre-operative and planning data into the coordinate system of the live ultrasound during the actual procedure. Procedure time and risk may be greatly decreased with this technique.

This presentation will discuss our experiences with this innovative technology in clinical cases of musculoskeletal injuries in the horse. In addition, the results of a pilot study evaluating the accuracy of MR and CT/US fusion through placement of fiducial markers compared with registration using anatomical landmarks of the distal limb will be presented. MR/US fusion was generally performed to guide an interventional procedure (injection of PRP/stem cells or to perform a tendon or ligament splitting) in cases that had lesions diagnosed with MR but undetectable on the standard US exam. Some horses had large lesions that were only partially visible with US and required approaching the injury from multiple directions. MR/US fusion also was utilized when it was considered necessary to increase the precision and accuracy in anatomical locations where it would be dangerous to approach a lesion blindly. The ultrasound vendor, Biosound Esaote (Genoa, Italy), has allowed us the use of a MyLab Twice ultrasound system which features the software program Virtual Navigator now commercially available on some of its ultrasound systems.

It is unfortunate that most comparisons of US and MR imaging put these modalities at odds with one another rather than emphasizing their complementary roles in assessing and treating musculoskeletal pathology. There is clearly considerable overlap between these two imaging modalities and the use of one modality should not preclude the use of the other.
Stem Cells
Stem cells are broadly defined as undifferentiated cells that are capable of self-renewal and differentiation into specific lineages, i.e. of the 3 germ layers which are the ectoderm, endoderm, and mesoderm. Stem cells are classified both by potency and type (tissue source). Multipotent stem cells are able to give rise to more than 1 cell type but are generally restricted to 1 germ layer (for example, give rise to cartilage and adipose tissue which are both mesoderm in origin), while pluripotent stem cells are to give rise to all cell types within the body from all 3 germ layers. Types of multipotent stem cells include adult-derived mesenchymal stem cells (MSCs), which are discussed below, and hematopoietic stem cells. Types of pluripotent stem cells include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), which are both discussed below.

Adult-derived mesenchymal stem cells
Adult-derived mesenchymal stem cells (MSCs) can be obtained from bone marrow, fat, umbilical cord blood, muscle, and many other tissues including cartilage, trabecular bone and tendon. Two techniques are commonly used for the treatment of equine musculoskeletal injuries with MSCs. One relies on a cultured cell population derived from bone marrow while the other utilizes a mixed cell population derived from adipose tissue. Each technique has its strengths and weaknesses and both in vivo and clinical evidence will be discussed in the lecture.1-7 The potential for allogeneic stem cell transplantation will also be discussed.

1) Bone marrow derived MSCs (BM-MSCs): BM-MSCs are chosen as they appear to perform superiorly to MSCs recovered from other tissues (including tendon) in terms of differentiation into known cell types. Furthermore BM-MSCs have received the most attention scientifically and hence are the best characterized. Bone marrow is collected from the sternum (or the tuber coxae) under standing sedation, followed by isolation and expansion of the nucleated adherent cell population (containing the MSCs) in the laboratory. A 3 week culture period is then needed to expand these selected cells until in excess of $10^6$ cells are available for implantation either under standing sedation into a tendon or ligament lesion using ultrasound guidance or under general anesthesia into a cartilage defect using arthroscopic guidance. Intra-articular injection of BM-MSCs has also been investigated, particularly for the treatment of meniscal injuries.6

2) Adipose-derived stromal vascular fraction (AD-SVF): This technique is based on data which suggested that adipose-derived MSCs exhibited a similar degree of multi-potentiality to BM-MSCs although in many studies they perform less well than BM-MSCs in differentiation assays. The technique that is used clinically most often is comprised of a mixture of cells derived from the adipose tissue (taken surgically from the tail head) after digestion and centrifugation - there is no culture step. This has the advantage of supplying large numbers of cells (but with only an estimated 20-40% being MSCs) in a short period of time (48 hours).8 The cells are implanted as outlined above. While the isolation and characterization of cultured
MSCs derived from adipose tissue (AT-MSCs) has been well described in the literature, this technique is used less frequently in the clinical setting than AD-SVF most likely due to the highly publicized commercial availability and short turnaround time of AD-SVF.7

Embryonic stem cells and induced pluripotent stem cells
1) Embryonic stem cells (ESCs): True ESCs are derived from the inner cell mass of the pre-implantation blastocyst and have an indefinite replicative life span. As described above, they are able to give rise to all cell types of the body and are able to form teratomas when implanted into immune compromised (SCID) mice. To date, no one has been able to culture such cells from the horse; however there are equine ESC-like cells described in the literature as well as equine fetal-derived ESC-like cells. These equine ESC-like cells have some pluripotent characteristics but are unable to form teratomas in SCID mice.9-11

2) Induced pluripotent stem cells (iPSCs): iPSCs are derived from adult somatic cells which have been reprogrammed using pluripotency genes back to an ESC state. Like true ESCs, equine iPS cells have an indefinite replicative life span and are able to form teratomas in SCID mice.12

All of these cells have tremendous promise for the treatment of musculoskeletal injuries in horses but are in the very early stages of investigation. An in vivo study using equine fetal-derived ESC-like cells will be discussed in the lecture.11

Platelet Rich Plasma
Platelet rich plasma (PRP) is defined as plasma with a 2 or more fold increase in platelet concentration above baseline levels or >1.1x10^6 platelets/μl. PRP is generated primarily by centrifugation or gravity filtration. There are differences in the volume of autologous blood required, time and speed of centrifugation, addition of an activating agent, leukocyte concentration, method of delivery, and qualitative/quantitative differences with respect to final PRP volume and final platelet and growth factor concentrations between the available systems. Overall, the final PRP platelet concentration is 2-8 times over baseline. It is important to recognize and understand that there are obvious differences between types of platelet concentrates that are being used, the general term/abbreviation PRP will be used herein.

The concept that PRP would improve tendon/ligament or joint disease is based on the physiologic role of platelets in wound healing. Through a modulation of the inflammatory response, promotion of local angiogenesis, attraction of fibroblasts and local stem cells to the site of injury and an induction of autocrine growth factor production by uninjured adjacent cells, platelets and their products are instrumental in normal tissue repair and regeneration. Work in our laboratory suggests that white blood cells in PRP increase tissue catabolism and decrease matrix synthesis. Data from our laboratory group indicates a positive correlation between white blood cells, predominantly neutrophils, and both IL-1 and tumor necrosis factor-α (TNF-α). These data suggest that the optimal PRP preparation would be one with the lowest white blood cell content to maximize the benefits of platelet - derived growth factors while minimizing the inflammatory and catabolic effects of white blood cells. There is presently no head-to-head comparison amongst the various PRP products, but the practitioner should ask the
manufacturer not only what the platelet content is in the PRP preparation, but what the white blood cell content is as well.

Once isolated, the PRP can be injected into a tendon/ligament lesion or joint with or without an activating (clotting) agent. The addition of bovine thrombin to the PRP sample just prior to or during injection is used in some systems to activate platelets resulting in initiation of the clotting cascade. Clotted PRP serves as a fibrin matrix which serves as a scaffold for tissue repair and a reservoir for retention and slow release of growth factors. There are now numerous equine studies in the literature investigating the efficacy of PRP for tendon and ligament repair.13-17

**Bone Marrow Aspirate Concentrate**

Bone marrow aspirate concentrate (BMAC) is generated through centrifugation of bone marrow aspirate. The advantage of BMAC over PRP is that it contains MSCs, which have demonstrated utility for repair of tendons, ligaments, and cartilage. It is important to realize, however, that the number of MSCs in BMAC is dramatically lower than that of a cultured cell population of BM-MSCs. Like PRP, BMAC is a fully autologous biologic that can be generated patient-side and when clotted, forms a scaffold. Also, like PRP, BMAC contains platelets and therefore is a rich source of growth factors.

In an equine model of 15mm diameter, full thickness cartilage defects, BMAC resulted in significantly improved cartilage repair compared to microfracture using short-term arthroscopic inspection and longer-term macroscopic, histological and quantitative magnetic resonance imaging analyses.18 Differences between BMAC and microfracture observed arthroscopically at 12 weeks persisted at 8 month evaluation.18 In particular, repair tissue in BMAC-treated defects was much better integrated into surrounding normal cartilage, the tissue was thicker, and had a smoother surface. Like PRP, BMAC is being used as a primary intra-articular joint injection, but no clinical data has been reported on its use.

**Autologous conditioned serum/IRAP**

Autologous conditioned serum (ACS) was probably the first biologic to be tested in horses. ACS is generated through the same process as IRAP, but for primarily legal reasons, it is called ACS. It is thought to act by blocking the receptor to the inflammatory cytokine interleukin-1 (IL-1). When injected intra-articularly into horses with surgically created synovitis/early arthritis, ACS resulted in decreased synovial hyperplasia and lameness compared to placebo treated groups.19 There is a newer generation of ACS termed IRAP II which boasts increased IRAP levels and is presently being tested by the equine group at Colorado State University.20

**References:**


