

**Equine Research Committee
2021 Report
San Antonio, Texas**

1. To approve the following proposals for funding from the General Research Fund in the amount of \$271,170.67:

“The Effect of Administration of Platelet-rich Plasma and Triamcinolone Acetonide in Combination on Inflamed Equine Osteochondral and Synovial Explants in Co-culture” at Auburn University in the amount of \$13,050

Young Investigator Award: Sophie Boorman, Equine Surgery Resident

Osteoarthritis (OA) is a painful, progressive, degenerative disease of articular tissue that limits the athletic career and welfare of the horse. Currently treatment of OA is limited to alleviating pain without modifying the disease. In equine practice, corticosteroids are injected into arthritic joints to suppress inflammation, however cell-based research has indicated that steroids are harmful to cartilage cells. One notable exception is triamcinolone acetonide (TA). Platelet-rich plasma (PRP), an autologous blood product, is steadily gaining popularity in the treatment of joint disease, as it is anti-inflammatory (and can therefore alleviate pain) and encourages cartilage regeneration and repair. The effect of combining PRP with various steroids has been examined, however no studies to date have considered PRP administered in combination with a cartilage-friendly steroid, TA. The researchers hypothesize that concurrent treatment with TA and PRP would reduce cartilage cell and synoviocyte cell death (the two key cells in the equine joint) and inflammation to a greater degree than TA or PRP treatment alone in a cell-based model of OA. These results will be directly applicable to the treatment of equine OA, a debilitating condition that greatly affects the Quarter Horse industry.

Board of Trustees Action: Approved

“Comparing the Immunomodulatory Potency of Equine Culture-expanded Articular Chondrocytes to Mesenchymal Stromal Cells” at Colorado State University in the amount of \$20,000

Young Investigator Award: Bethany Liebig, Graduate Research Assistant

Osteoarthritis (OA) is a painful degenerative joint disease for which there is no cure and can occur at any age. Failure of conventional therapies to treat OA has led to the exploration of injecting mesenchymal stromal cells (MSCs). Unfortunately, intra-articular (IA) injections of MSCs have not yet proven to be an effective therapy for OA. While most studies to date have used bone marrow MSC, researchers postulate that healing can be improved using chondrocytes from adult equine articular cartilage. Importantly, this approach is supported by numerous reports that culture-expanded chondrocytes (CECs) can suppress the immune response in the joint, which can lower inflammation that drives the progression of OA. Given the early stage in their investigation of CECs for OA, this proposal seeks to establish the immunomodulatory potency of CECs. In this study bone marrow MSCs will be analyzed. Therefore, these data are expected to provide important information that could advance IA therapeutics use to treat equine OA.

Board of Trustees Action: Approved

“Effect of Donor Age on the Functionality of Equine Articular Chondroprogenitor Cells” at Colorado State University in the amount of \$20,000

Young Investigator Award: Parvathy Thampi PhD

Joint disease/injury and the associated cartilage changes are a significant clinical problem in horses. Currently available treatment options including surgical and therapeutic approaches have limited long-term success. Stem cell-based therapies are increasingly being adopted. However, the resulting repair tissue is often fibrocartilage, which lacks the unique biomechanical properties of the normal cartilage tissue and deteriorates over time. Articular chondroprogenitors (ACPs) are cells that exist in the surface zone of articular cartilage that can be expanded long-term in cell culture without loss of their ability to make stable cartilage. Researchers hypothesize that donor age does not influence the functionality of equine ACPs. They aim to test this by examining the effect of donor age on the chondrogenic potential of ACPs – ACPs isolated from the surface of articular cartilage from three different age groups (1-3 years, 6-10 years and over 13 years) will be compared for their cartilage-forming ability in culture. This study is expected to have a significant impact on the equine industry by addressing an important question that would help guide the successful clinical use of ACPs as a therapy cell for cartilage repair in horses.

Board of Trustees Action: Approved

“Prevention of Persistent Breeding-induced Endometritis in the Mare” at Colorado State University in the amount of \$52,965

Principal Investigator: Patrick McCue DVM, PhD

Persistent breeding-induced endometritis is the most common reproductive abnormality encountered and is associated with a reduction in fertility, increased costs and risk of bacterial infection. The research goal is to critically evaluate current treatments as well as novel treatments to prevent this syndrome. Mares will be divided into two groups and will receive one of four treatment protocols: a saline placebo or dexamethasone administered four hours prior to insemination, or a uterine lavage performed four hours after insemination, or a combination of dexamethasone plus uterine lavage. Mares in group two will also receive one of four different treatment protocols: a saline placebo or firocoxib four hours prior to insemination, or intrauterine infusion of either alpha 2 macroglobulin or stem cell-derived extracellular vesicles 24 hours prior to insemination. Mares will be inseminated with one billion dead spermatozoa and uterine samples will be collected and evaluated for markers of inflammation. A safe, effective preventive strategy is needed to benefit breeding programs through the equine industry.

Board of Trustees Action: Approved

“Efficacy of Xenogen-free MSCs in Equine Joint Disease” at Texas A&M University in the amount of \$60,851

Principal Investigator: Ashlee Watts DVM, PhD

Stem cell therapy has excited scientists, orthopedists and veterinarians as a possible cure for osteoarthritis and other musculoskeletal injury, yet all approved stem cell therapies have eventually been taken off the market due to lack of repeatable effectiveness. This laboratory has determined that the main reason for stem cell destruction is fetal bovine serum. The objective is therefore to use a randomized and placebo controlled clinical trial to test the effectiveness of stem cells prepared with their

new method to treat osteoarthritis, against the industry standard of stem cells supplemented with fetal bovine serum as the control treatment.

Board of Trustees Action: Approved

“Determination of the Time Required to Achieve Peak Concentration of Amikacin in the Radiocarpal Joint during Intravenous Regional Limb Perfusion using 10% DMSO” at University of California, Davis in the amount of \$19,214

Principal Investigator: Isabelle Kilcoyne MVB

For the equine veterinarian, wounds account for a large portion of the caseload of clinical practice. Traumatic wounds are one of the most common medical conditions to affect horses, necessitating veterinary intervention and due to the limited soft tissue coverage of the distal limb involvement of an underlying synovial structure is not uncommon. These wounds are commonly contaminated with bacteria either from the resident microbes of the skin or from microbes that are encountered in the horses' environment. Intravenous regional limb perfusion (IVRLP) with antibiotics is a simple procedure, which allows effective levels of antibiotics to be administered locally by placement of a tourniquet resulting in minimal systemic effects. Standardization and reduction of the tourniquet time required to perform these perfusions would allow treatment of these conditions in a more time efficient manner. Dimethylsulfoxide (DMSO) is a potent anti-inflammatory that acts by limiting free radical production. The objective of this study is to determine if utilizing a 10% DMSO solution will increase the concentration of amikacin attainable in the joints of the distal limb during IVRLP and will help determine if use of the 10% DMSO solution will reduce the time to mean peak concentration of amikacin sulfate in the joint.

Board of Trustees Action: Approved

“Fecal Biomarker Validation for Detection of Intestinal Inflammation in Horses” at University of Illinois in the amount of \$16,500

Young Investigator Award: Rebecca Bishop, Graduate Student

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed by equine veterinarians for a variety of painful conditions. However, NSAID use can result in undesirable side effects, including stomach ulcers and the development of colitis (inflammation of the large colon). Unfortunately, clinical signs related to these side effects are often non-specific; these include mild signs of colic, weight loss, and/or poor performance. While ulcers can be easily diagnosed with gastroscopy, there are few reliable tests to diagnose large colon inflammation. Researchers propose that fecal inflammatory markers have the potential to fill this need. Fecal inflammatory biomarkers are non-invasive, reflect inflammation specific to the intestine, and may allow diagnosis of inflammation in parts of the intestine that cannot be assessed by other techniques such as endoscopy or ultrasonography. Myeloperoxidase (MPO) and calprotectin (CP) are two fecal inflammatory biomarkers that are used to detect colonic inflammation in humans. There are commercially available tests to measure MPO and CP in horse blood, but these tests have not yet been evaluated for use with horse feces. They evaluate the detection limit, reliability, and accuracy of the tests when used with feces, and make a preliminary assessment of their usefulness in clinical patients.

Board of Trustees Action: Approved

“Effect of Day of Estrus Cycle at TVA on Rates of in vitro Oocyte Maturation and Blastocyst Production after ICSI” at University of Pennsylvania in the amount of \$68,590.67

Principal Investigator: Katrin Hinrichs DVM, PhD

In vitro embryo production via intracytoplasmic sperm injection (ICSI) has become an important technique for equine reproductive management and has been used to produce embryos from mares in strict competition schedules, as it requires minimal downtime on the part of the mare. Many clinical practices offer transvaginal ultrasound-guided follicle aspiration (TVA) to recover oocytes from mares for use in ICSI at any time of the estrous cycle as well as any time of the year. However, on average only about 50% of the recovered oocytes will mature to be able to be fertilized by ICSI, and only about 25% of the oocytes subjected to ICSI will produce transferrable embryos. The status of the follicle and so the quality of the oocyte are known to differ at different times of the follicular wave, and this could directly affect results obtained with ICSI. This leads to the question “when is the best time of the cycle to do TVA to recover oocytes for ICSI?” The purpose of this study is to determine when in the estrous cycle maximum oocyte recovery, maturation, and blastocyst production rates are obtained, in order to more efficiently and successfully utilize oocyte recovery and ICSI as an equine breeding management tool.

Board of Trustees Action: Approved

2. To approve the following proposal for funding from the Emergent Research Fund in the amount of \$42,888:

“Effects of Phenylbutazone, Flunixin Meglumine, and Ketoprofen on Renal Effects of Furosemide in Horses” at Michigan State University in the amount of \$42,888

Principal Investigator: Harold Schott DVM, PhD

Exercise-induced pulmonary hemorrhage (EIPH) has been demonstrated to affect performance with severity of EIPH linked to the decrease in performance. To lessen EIPH, furosemide is commonly administered to horses four hours before racing. This treatment has been shown to both enhance performance and limit EIPH.

Phenylbutazone, flunixin meglumine, and ketoprofen are non-steroidal anti-inflammatory drugs (NSAIDs) that are frequently administered to racehorses 24-36 hours prior to racing. Current medication testing programs have established thresholds for blood concentrations of these NSAIDs to ensure that they are not administered at excessive doses or closer to race starts. When phenylbutazone is administered 30-90 minutes before furosemide administration, urine output is diminished by 30%. Because loss of body fluid after furosemide administration is considered the essential response to both limit EIPH and improve racing performance, any treatment that reduces the diuretic response to furosemide could also lessen the potential benefits of furosemide. Currently, the duration of NSAID inhibition of furosemide’s diuretic effect is unknown and effects of other NSAIDs have not been studied. Thus, the purpose of the experiments outlined in this proposal is to determine the effects that administration of phenylbutazone, flunixin meglumine, or ketoprofen have on the diuretic effect of furosemide.

Board of Trustees Action: Approved

3. To approve the following proposal for funding from the Refrigerator Fund for Equine Head Trauma in the amount of \$41,584:

“The Effect of Hyperosmolar Fluid Administration on Intracranial Pressure” at University of Georgia in the amount of \$41,584

Principal Investigator: Clare Ryan DVM, PhD

Traumatic brain injury is a common, and often deadly, component of neurologic disease related to trauma. It can cause severe medical issues or death due to increased intracranial pressure (increased pressure in and around the brain). Because such serious consequences occur, fast and effective treatment for increased intracranial pressure is needed. Currently, people and horses with increased intracranial pressure from brain injury are treated with special hyperosmolar intravenous fluids (fluids with more dissolved particles than blood, and that go through a catheter into a vein). The two most common of these fluids are hypertonic saline, a concentrated salt solution, and mannitol, a concentrated sugar solution. However, the effect of these fluids on intracranial pressure has not been proven in horses. The dose appropriate for treating horses with increased intracranial pressure is also unknown. In this study, researchers will determine the effects of two hyperosmolar fluids (hypertonic saline and mannitol) by measuring how giving the fluids to healthy horses changes their intracranial pressure while they are under general anesthesia. The results of this study will help equine veterinarians know if these medications can reduce intracranial pressure, and what dose is likely to be effective. This study will also help provide a foundation for future studies, with the ultimate goal of improving outcomes for horses with traumatic brain injury.

Board of Trustees Action: Approved

The committee discussed various agenda items including the potential for an AQHF equine research internship program to support youth and young adult engagement opportunities within the horse industry and AQHA.