ORTHOPAEDIC RESEARCH CENTER

ARTHROS

CSU GAIL HOLMES EQUINE ORTHOPAEDIC RESEARCH CENTER | FALL 2016

NUMBER 21

$20 MILLION GIFT WILL HELP MAKE RESEARCH INSTITUTE A REALITY | by Coleman Cornelius

Philanthropists John and Leslie Malone pledged $42.5 million for the new Institute for Biologic Translational Therapies. They challenged CSU to raise $32.5 million to match the equivalent amount that they directed toward facility construction.

AN ANONYMOUS RACEHORSE BREEDER has donated $20 million to Colorado State University to build a state-of-the-art regenerative medicine research facility, fulfilling the matching challenge from lead donors and fellow horse aficionados, John and Leslie Malone, for the $65 million building. In December 2014, the Malones pledged a record-breaking $42.5 million for the planned facility, prompted by their interest in stem cell therapy and its effectiveness in treating equine joint problems. The Malones raise world-class dressage horses and Thoroughbred racehorses.

CSU TRANSLATIONAL THERAPIES INSTITUTE CLOSER TO REALITY

The generous donations allow construction of the CSU Institute for Biologic Translational Therapies, which promises to tap the body's healing powers for innovative treatments that improve animal and human health. Groundbreaking will occur later this year; an exact date has not been set. *Continued*

ORTHOPAEDIC RESEARCH CENTER

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Colorado State University

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Dr. Wayne McIlwraith at the end of arthroscopic surgery. With Drs. David Frisbie and Chris Kawcak, he has led planning for the CSU Institute of Biologic Translational Therapies.

Philanthropists John and Leslie Malone pledged $42.5 million for the regenerative research facility. They challenged CSU to raise $32.5 million to match the equivalent amount that they directed to facility construction.

"We are deeply grateful for another tremendous gift to help establish the Institute for Biologic Translational Therapies," CSU President Tony Frank said. "This support, combined with the transformational gift from John and Leslie Malone, will advance Colorado State's work in a new era of veterinary and translational medicine."

On Feb. 13, CSU publicly announced its first $1 billion campaign to generate philanthropic support for teaching, research, outreach, and veterinary clinical services. The University is more than halfway to fulfilling that lofty goal, with help from the sizeable gifts for regenerative research.

The Institute for Biologic Translational Therapies will develop next-generation remedies based on living cells and their products. These include patient-derived stem cells to treat musculoskeletal disease and other ailments.

CSU FACULTY CREATE VISION FOR INSTITUTE

Faculty with the Orthopaedic Research Center, in the College of Veterinary Medicine and Biomedical Sciences, developed the vision for the institute as part of their focus on equine musculoskeletal problems; other CSU faculty with interests in regenerative medicine then became involved.

Planning of the new research institute has been led by Drs. Wayne McIlwraith, David Frisbie, and Chris Kawcak. Dr. McIlwraith is a University Distinguished Professor, Barbara Cox Anthony University Chair in Orthopaedic Research, and international pioneer of arthroscopic surgery and joint disease research in horses. Dr. McIlwraith is founding director of CSU's Orthopaedic Research Center and has worked with other faculty in the center to pursue regenerative treatments to augment surgery and to hasten recovery from injury and joint disease. These include stem cell and gene therapy, specialized tissue replacement, and use of novel proteins.

Colorado State veterinarians are experts at investigating medical treatments for animal patients, then providing knowledge gained to boost human medical advancements; the progression is known as translational medicine and is successful because of similarities in animal and human physiology and disease.

GENEROUS GIFTS MAKE PROJECT A REALITY

Dr. McIlwraith and his veterinary colleagues have treated joint problems in horses owned by the Malones and by the anonymous donor.

"We are very thankful for these supporters who have seen the potential for regenerative therapies in the successful treatment of equine athletes," Dr. McIlwraith said. "Our new Institute for Biologic Translational Therapies will propel our work by investigating regenerative therapies for a wide range of disease."

"This institute will be distinguished for working from laboratory inception to commercialization under one roof," he said. "We will work from the outset of each project with the goal of translating therapeutic knowledge from animals to people."

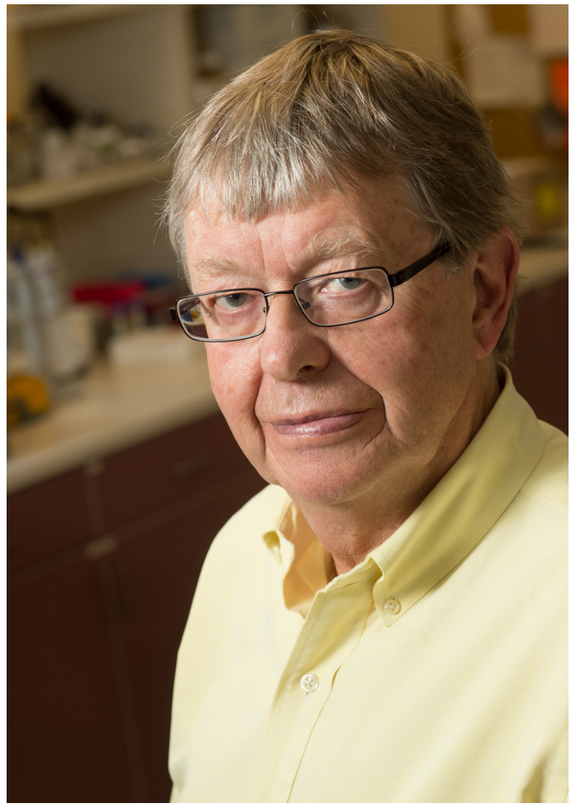
The alliance between veterinarians and physicians is familiar to Dr. McIlwraith and colleagues, who have teamed with medical experts, including those at the Steadman Clinic, the preeminent orthopaedic and sports-injury center in Vail. They also have collaborated on research funded by the National Institutes of Health at Massachusetts Institute of Technology, the University of Pittsburgh, and Indiana University. More recently, CSU orthopaedic faculty have established new partnerships with Rush University Medical Center and Stanford University.

The Malones' earlier gift of $42.5 million provides $32.5 million for construction of a building featuring laboratories, specialized surgical suites, and conference space for veterinarians and physicians, as well as $10 million for institute operations over the first five years.

The lead gift required $32.5 million in matching donations, amounting to $65 million for building construction. The challenge has been fulfilled in just over a year with the $20 million gift from the anonymous donor and $12.5 million from other donors and the University. To contribute to the building fund, visit https://advancing.colostate.edu/ibttbuilding ■

Architectural diagram of proposed Institute for Biologic Translational Therapies.

LETTER FROM DR. WAYNE MCILWRAITH



Dr. Wayne McIlwraith, Barbara Cox Anthony University Chair, Director of the Orthopaedic Research Center, and University Distinguished Professor, at Colorado State University's Orthopaedic Research Center. June 15, 2016

WELCOME TO THE FALL EDITION OF *ARTHROS,* which reviews 2015 events of significance and those extending into early 2016. The newsletter highlights some important news as well as research findings from the past year. Most notable is the $20 million gift from an anonymous donor that allows us to complete funding of the new Institute for Biologic Translational Therapies building and is truly transformational for our Orthopaedic Research Program. There is a separate article on this. We are so grateful to this donor (whose identity will be eventually revealed) for the generosity and support of our program. The person responsible for this gift is a long-term client of mine who I look forward to being able to acknowledge in the future. The fact that we are able to respond to the challenge from John and Leslie Malone and provide the funding for the other half of the IBTT, with them putting forward matching dollars, is testimony to the quality of our people, our program, and the productivity we have had.

We are now moving forward quickly with completion of final designs of the building and selection of the company to build the IBTT. We are hoping to break ground early next year. Dr. Dave Frisbie has been appointed interim director of operations to lead the strategic plan for this effort.

Our partnership with Rush Orthopaedics has already brought considerable escalation of human-orientated research. Further details of our research in the last year will be available in our 2015 Orthopaedic Research Center Report. If you'd like a copy, please contact Paula Vanderlinden.

ARTHROS also details new developments in the faculty, staff, and facilities. A third graduate from the residency program in Equine Sports Medicine and Rehabilitation, Dr. Josh Donnell, passed the examination and is now a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation, becoming the third resident in our program to become a Diplomate of ACVSMR.

We have also welcomed new people, including Dr. Sherry Johnson who joined the Equine Sports Medicine and Rehabilitation Services residency program in July 2015 following completion of a one-year equine diagnostic imaging internship. Dr. Jodie Daglish moved into the positon of the equine diagnostic imaging internship, which precedes doing the ACVSMR residency. Holly Stewart and Katie Trella, both postdoctoral fellows, joined the ORC in 2016. Suzy Tabbaa joined us also as a postdoctoral fellow and was successful in gaining a National Sciences Foundation grant, "Improving Performance of Osteochondral Graphs for Cartilage Repair" with Drs. Dave Frisbie and Bob Sah. Dr. Bob Sah, Professor of Bioengineering in the University of California, San Diego, Jacobs School of Engineering, and a longtime collaborator, has recently joined our program in a part-time appointment and adds great strength to our program and to the IBTT. His role will be profiled in detail in the next issue.

Last but not least, thank you to all our donors for their contributions which are critical to our continued productivity and fulfillment of our mission.

Best wishes,

Wayne McIlwraith, Director

EVALUATION OF ARTICULAR CARTILAGE MATRIX USING CONTRAST-ENHANCED COMPUTED TOMOGRAPHY IN THE HORSE

OSTEOARTHRITIS IS THE DEGENERATION OF ARTICULAR cartilage and is a significant problem in the horse, leading to decreased athleticism, early retirement, and potentially necessitating euthanasia. OA results in significant costs, both monetary and in the loss of use of the horse (>$700 million per year, based on an estimate from the USDA in 2000). OA also largely affects humans with estimates of 50 percent of adults over age 65 developing OA, and costs hundreds of billions of dollars per year. The diagnosis of early OA and articular cartilage injury is challenging. Although magnetic resonance imaging is widely considered the best imaging modality to detect cartilage injury, routine MRI does not have the capacity to detect the early degenerative changes in cartilage that occur in OA. On a molecular level, one of the earliest markers of OA is the depletion of glycosaminoglycans from within the cartilage tissue.

The main objectives of this work is to improve the early detection of osteoarthritis and articular cartilage injury and thus develop strategies to slow or stop the progression of OA. We aim to use new diagnostic imaging techniques to detect this loss of GAGs from articular cartilage.

Through collaboration with ORC researchers and Dr. Mark Grinstaff at Boston University (CSU affiliate faculty), we have evaluated a cationic (or positively charged) contrast medium (CA4+) developed by Dr. Grinstaff. Once injected into the joint, this novel contrast medium is attracted to the negatively charged GAGs in cartilage. Conversely, all commercially available contrast media are anionic (negatively charged) and thus do not penetrate cartilage as well as CA4+ (Figure 1). Once the injected contrast equilibrates within the cartilage, the amount of CA4+ in cartilage can be quantified using computed tomography (cationic CT)(Figure 2).

To evaluate the potential of cationic CT imaging in a joint with degenerative articular cartilage, cartilage defects were created in one stifle of one horse while the opposite stifle remained as a control. Cationic CT imaging seven weeks later revealed that full thickness cartilage defects were readily detectible with this technique (Figure 3). Articular cartilage/bone plugs were collected postmortem. The GAG content and equilibrium compressive modulus (E, mechanical property) of cartilage was directly determined from each of these plugs. These values were then compared to the measurements made on the cationic CT images (recorded on the Hounsfield unit scale). Low-quality cartilage tissue (low GAG or E) could be successfully predicted from the values measured on cationic CT images. Excitingly, some of the cartilage on visual inspection appeared normal and thus supports the potential of cationic CT to detect early microscopic changes in articular

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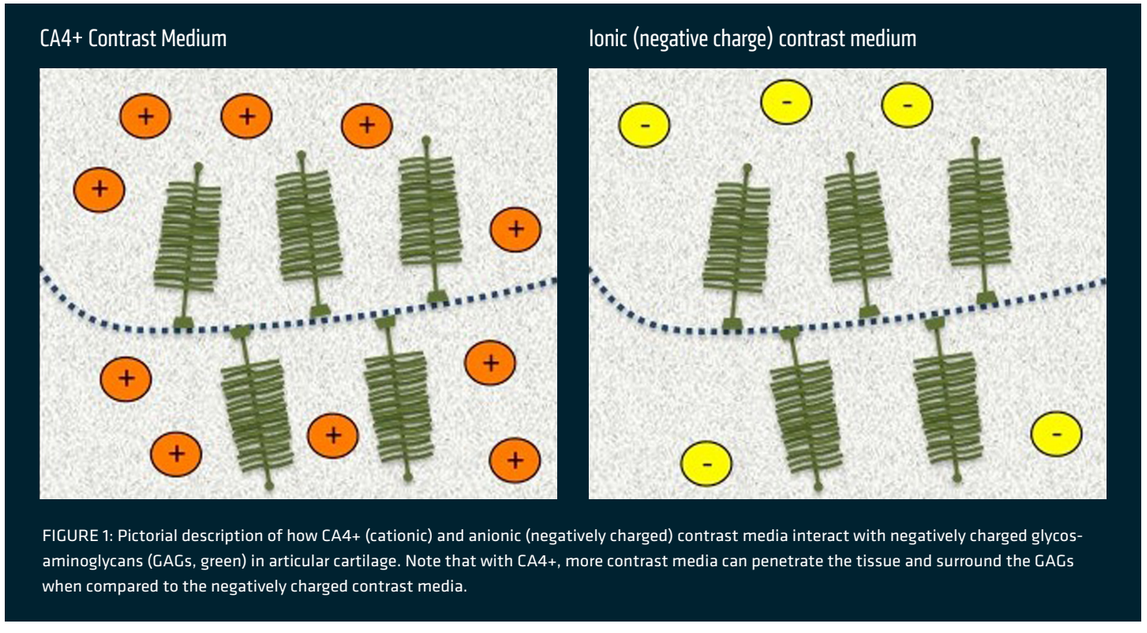

FIGURE 1: Pictorial description of how CA4+ (cationic) and anionic (negatively charged) contrast media interact with negatively charged glycosaminoglycans (GAGs, green) in articular cartilage. Note that with CA4+, more contrast media can penetrate the tissue and surround the GAGs when compared to the negatively charged contrast media.

ARTICULAR CARTILAGE MATRIX *continued from Page 5*

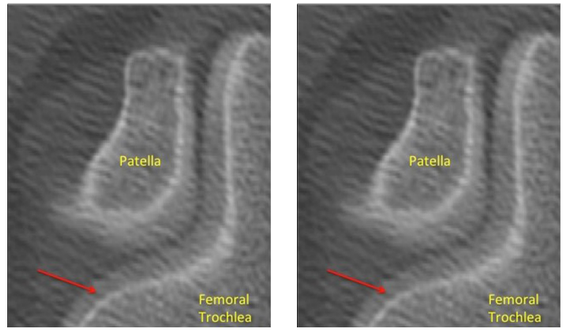

FIGURE 2: Computed tomography scans of the equine stifle using two different contrast media. The left image shows the femoropatellar joint compartment injected with CA4+ and the right image following injection of an anionic, negatively charged contrast media. The red arrow points to the articular cartilage in both images. In the left image, note how the cartilage has more of a light gray hue, reflecting penetration of the CA4+ contrast media. In comparison, the right image shows most of the negatively charged contrast media being retained in the joint space not entering the cartilage; although the anionic media outlines the cartilage, it does not penetrate cartilage and limits evaluation on computed tomography.

cartilage before macroscopic changes have occurred.

To expand upon this preliminary work, we have obtained funding through the Grayson-Jockey Club and the College Research Council at CSU to further investigate the cationic CT technique. The next study being performed is in the use of an impact device to simulate post-traumatic osteoarthritis in the equine stifle and to evaluate the subsequent degeneration of articular cartilage with cationic CT imaging.

The plan is to follow the progression of OA with cationic CT imaging. Multiple cationic CT imaging examinations will be performed biweekly after OA has been initiated. This study will help determine how early the degeneration of articular cartilage can be detected with cationic CT as well as determine any alterations in how OA progresses over time. Future directions are to evaluate cationic CT as a potential monitoring technique and to compare cationic CT to other quantitative MRI techniques available in research settings. The impact of this work will benefit the early detection of OA in horses, but there is also a translational application to humans with OA. If the long-term objectives of the cationic CT technique are achieved, then this technique may be explored in the evaluation of humans with OA. Potentially, by using a technique for *in vivo* preclinical research studies, cationic CT also has the potential to decrease the number of research horses needed in longitudinal studies that would normally require the sacrifice of the horse. ■

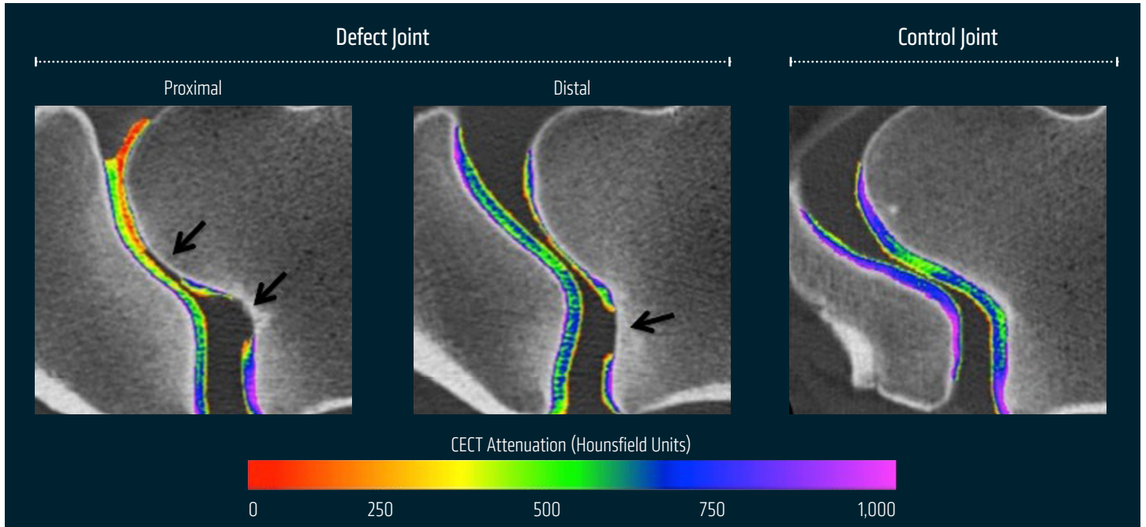

FIGURE 3: Cationic computed tomography scans of the equine stifle seven weeks following the creation of articular cartilage defects on the femoral trochlea of one horse while the opposite limb was maintained as a control. The articular cartilage in these three images has been segmented from the surrounding tissues and quantified on the Hounsfield unit scale. This is shown as a range of color values with blue/purple reflecting more penetration and higher amounts of CA4+, while yellow/orange hues demonstrate less CA4+. The black arrows point to the full thickness defects easily detected, and comparison of the color values in the defect joint demonstrates less CA4+ in cartilage and therefore fewer GAGs present than in the control joint.

FUNCTIONAL ASSESSMENT OF THE EQUINE PATIENT | Melissa King, D.V.M., Ph.D., Diplomate ACVSMR

A COMPREHENSIVE FUNCTIONAL ASSESSMENT of the horse should be divided into two categories: static (standing) and dynamic (moving) evaluations. Dynamic evaluation involves functional joint motion both passive (the joint range of motion the individual person can achieve) and active (the joint range of motion required for the horse to successfully complete a given task) as well as gait analysis.

Equine static assessment involves evaluating for structural correctness, symmetry, and muscling. A horse's conformation should be assessed with a thorough understanding of the individual's discipline and the demands placed on the musculoskeletal system. Examination should include assessing the horse in a standing balanced position and while moving. A well-balanced or symmetrically built horse that is structurally correct is more likely to remain sound upon being subjected to the repetitive cyclic loading naturally incurred with training and performance. Poor conformation leads to a greater risk of injury as well as limited function. The horse's body should be proportional with the neck, shoulder, back, and hip approximately equal lengths. The topline aspect of the neck should be twice as long as the underline (2:1 ratio). An imbalance in the neck ratio often results in a neck that ties in low, which will limit neck flexibility and the horse may travel more on the forehand. For every 10 cm increase in length of underline aspect of the neck, the odds of developing fetlock effusion increases by a factor of 5.1. While the topline aspect of the back should be half as long as the abdominal side (1:2 ratio). A horse that has a longer back region will have limited ability to get his hindlimbs underneath the body, limiting power and impulsion, often distributing more weight onto the forelimbs.

FORELIMB CONFORMATION

When looking at the horse from the side or the "lateral view," the slope of the shoulder should be approximately a 40° to 55° angle. A steeper shoulder angle results in a shorter, jarring stride. A study evaluating racehorses demonstrated that horses with a steeper shoulder angle were at an increased risk of developing carpal (knee) osteochondral chip fragments. Horses that are described as back at the knee (concave appearance to the dorsal aspect of carpus) are often predisposed to carpal injuries, especially racehorses that have a tendency to hyperextend the carpus during fatigue. Over at the knee, or "buckling," describes a convex dorsal surface of the carpus. Over at the knee conformation may be sign of lameness as a way to relieve pain – for example proximal suspensory injuries often present with some form of carpal flexion or buckling during stance.

Continued

FIGURE 1: Conformational assessment of the forelimbs. The horse in the image above presents with bilateral carpal valgus and toed out conformation – right front more so than left front.

TEN KEY ORTHOPAEDIC RESEARCH CENTER DISCOVERIES

The following is reprinted from *Colorado State Magazine*, for CSU alumni and friends that reviewed 10 key discoveries during the evolution of the Orthopaedic Research Center.

1. Led development of the techniques for equine arthroscopic surgery and produced a textbook on the subject.
2. Advanced understanding of microdamage associated with exercise as the cause of traumatic arthritis and fractures.
3. Developed fluid and imaging biomarkers, allowing earlier diagnosis of osteoarthritis and pre-fracture injury.
4. Created the first dedicated equine MRI facility.
5. Validated conventional and novel therapies for traumatic osteoarthritis in the horse.
6. Developed a special gene therapy in the horse and demonstrated for the first time that it would mitigate disease in any species, including humans.
7. Developed novel biologic therapies, including the use of stem cells, for treatment of musculoskeletal conditions.
8. Developed articular cartilage repair models in a horse joint that are now accepted by both the FDA and NIH as important translational models in human orthopaedics.
9. Pioneered work in bone marrow-derived stem cells, including clinical demonstration of enhanced results with a variety of injuries as well as articular cartilage repair.
10. First to demonstrate scientifically that underwater treadmill therapy reduces osteoarthritis and enhances coordination in the equine athlete.

CURRENT RESEARCH SPONSORS

AlloSource
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Equine Orthopaedic Research Foundation
Grayson-Jockey Club Research Foundation
LifeNet Health Foundation
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National Science Foundation
Nexvet
New Zealand Equine Research Foundation
Steadman Philippon Research Institute
United States Equestrian Foundation
Xalud Therapeutics Inc.

FUNCTIONAL ASSESSMENT

Continued from Page 7

HINDLIMB CONFORMATION

The balance of the hindlimbs is critical to a horse's athletic ability. The length and slope of the croup should be similar to the topline length of the back and slope of the shoulder. A horse with a steep croup will have a decreased length stride when bringing the hindlimb forward and reduced range of motion, which combines to limit the horse's ability to produce power. The steeper croup angle shifts the center of gravity in a caudal direction increasing the risk of developing hindlimb lameness issues. A lateral hock angle of less than 150° is often referred to as having a sickle hock appearance. Sickle hock conformation concentrates load in the back aspect of the hock, predisposing horses to injury of the long plantar ligament of the tarsus (curb) and to distal hock joint pain. Horses that are straight behind are also more prone to hindlimb lameness issues, as the hock and stifle angles are in a more extended position. Horses with a hock angle greater than 170° often develop upward fixation of the patella, suspensory strains, and distal hock joint osteoarthritis.

Understanding conformation allows the clinician to interpret the impact the conformation faults may have on future performance and where the limitations may occur. For example, a horse that toes in will asymmetrically load the lateral (outside) aspect of the lower limb, increasing strain on the lateral branch of the suspensory ligament. In comparison to a horse that toes out, increased load will be placed on the inside aspect of the lower limb. (Figure 1) Furthermore, assessment of conformation in light of the injury can provide a realistic prognosis. Horses with straight hock conformation that have suffered a proximal suspensory injury are at an increased likelihood of developing chronic suspensory ligament desmitis. A thorough conformational assessment provides the client with a reliable prediction of athletic potential and soundness. ■

HIGH-ENERGY FOCUSED SHOCK WAVE THERAPY ACCELERATES BONE HEALING

A BLINDED, PROSPECTIVE, RANDOMIZED CANINE CLINICAL TRIAL

This is a summary of an article published in the *Journal of Veterinary and Comparative Orthopaedics and Traumatology* by Drs. Nina Kieves, Chris Mackay, Kelly Adducci, Sangeeta Rao, Clara Goh, and Felix Duerr.

Shock wave therapy is a treatment that has been used for many purposes in people including bone healing, tendon problems and to dissolve kidney stones by applying the treatment through the skin (which is why it is also called extracorporeal SWT – meaning outside the body). A shock wave is similar to a sound wave and other waves in that it carries energy. Shock waves are known to carry a large amount of energy, and this energy is rapidly released when the shock wave hits its target. Energy is easily released at the interface between firm and soft tissues (such as kidney stones or bones in the body) and, as such, bone poses the ideal target for SWT. While there is evidence that SWT accelerates bone healing in people, there is little data to support this claim in dogs. Therefore, the purpose of this study was to evaluate whether SWT is capable of accelerating bone healing in dogs.

To study this question, we selected a clinical model that requires creation of a bone fracture: tibial plateau leveling osteotomy or TPLO. TPLO is a procedure commonly performed to treat canine cruciate disease, which is a rupture of the stabilizing ligament of the knee (the equivalent to ACL tears in people). During this procedure the top part of the shin bone is rotated to stabilize the knee – this requires performing a bone cut and applying a plate. This procedure is well standardized and therefore is an ideal model to evaluate methods of enhancing bone healing in a clinical setting.

For the study, we enrolled 42 clinical patients (healthy dogs between 2-9 years of age) that presented to CSU's James L. Voss Veterinary Teaching Hospital for treatment of cranial cruciate ligament disease. If owners elected to pursue TPLO, they were offered the option to enroll in this study. The animals were then randomly assigned to receive either SWT or sham treatment (i.e., no treatment). SWT was applied with a VersaTron 4Paws device (PulseVet Technologies) immediately postoperatively and at the time of suture removal (approximately two weeks post-operatively). The first SWT treatment was applied under general anesthesia, and the second SWT treatment was applied under sedation. A total of 1,000 shocks were applied at each treatment. Sham treatment was performed as described above, but the SWT device was not activated. Three radiologists who were unaware of whether a dog received SWT or sham treatment evaluated X-rays that were performed at eight weeks postoperatively with two bone healing scores. When these scores were compared, we found that healing scores were significantly higher eight weeks postoperatively for the SWT group compared to the sham treatment group for both scoring systems.

These findings confirmed that two treatments of SWT are capable of accelerating bone healing in dogs. However, a few questions remain unanswered. For example, we don't know whether one application of SWT may provide similar results, and it is unknown which of the two treatments produced the beneficial effects in our study. We also don't know the ideal number of shocks required to enhance fracture healing. We selected a fairly low number of shocks compared to previous reports, as clients are charged per shock and, hence, treatment cost rises with the number of shocks applied. We are hopeful that we may be able to answer some of these questions with future studies using a similar study design. ■

This study was funded by PulseVet Technologies.

EVALUATION OF ARTICULAR CARTILAGE PROGENITOR CELLS FOR THE REPAIR OF ARTICULAR DEFECTS IN THE HORSE

This is a summary from a paper recently published in the *Journal of Bone and Joint Surgery* and was a collaboration between Drs. David Frisbie, Myra Barrett and Wayne McIlwraith at the ORC and Drs. Helen McCarthy and Charles Archer of the University of Cardiff.

FOCAL CHONDRAL defects are identified in more than one-half of the arthroscopic procedures performed in the human knee, and similar numbers are seen in clinical cases of equine stifle arthroscopy. Despite a vast amount of research, a recent systematic review of Level I and II studies involving 421 human patients treated with autologous chondrocyte implantation, osteochondral autografts, matrix-induced autologous chondrocyte implantation, or microfracture indicated that no technique consistently yielded superior long-term results. This confirms that improved cartilage repair techniques are desired. Drs. Helen McCarthy and Charles Archer had previously isolated novel chondroprogenitor cells from the superficial layer of human articular cartilage and, more recently, in collaboration with the ORC, from equine articular cartilage. Equine articular cartilage chondroprogenitor cells demonstrate a multipotent differentiation capacity similar to that of bone marrow-derived MSCs and also exhibit delayed senescence and retain their chondrogenic potential following extensive in vitro expansion. The purpose of this study was to evaluate both autologous and allogenic cartilage progenitor cells implanted in a fibrin matrix to heal critically sized articular defects over a 12-month period in the horse.

The superficial chondroprogenitor cells were cultured from the superficial zone cartilage after 14 days of expansion and were cryopreserved in liquid nitrogen. There were four groups evaluated: (1) autologous chondroprogenitor cells transplanted in fibrin, (2) allogenic chondroprogenitor cells transplanted in fibrin, (3) fibrin alone, and (4) empty defects. On second-look arthroscopy cartilage and bone attachment, as well as, firmness of the repair tissue was inferior in the control group compared with other treatment groups, with the autologous cell group having the best results when averaged over the study period. The greatest defect filling by repair tissue was in the autologous group. On histologic examination, the repair tissue at 12 months was predominantly fibrocartilage in the autologous group, whereas mostly nonchondrocytic cells with some fibrocartilage was seen in the other groups (Figure A and Figure B).

Reconstitution of the subchondral bone in the autologous group was also superior as was the safranin-O staining (this stains the glycosaminoglycan in the articular cartilage). The radiographic outcomes were improved with autologous cell treatment compared with allogenic cell treatment or fibrin treatment alone. It was noted also that there was less subchondral bone inflammation in the autologous group compared to the allogenic group. Immunogenicity of autologous compared with allogenic cells in horses is still an ongoing area of debate, but it would appear from this study that, at least with superficial chondroprogenitor cells, it is a potential problem.

Overall this study showed support for autologous chondroprogenitor cells from the articular cartilage promoting cartilage repair when placed in fibrin. This is certainly a positive advance compared to another study where bone marrow-derived stem cells in fibrin and PRP produced bone in four out of 12 of the defects (which is not a positive response when you are trying to promote the growth of articular cartilage). ■

Acknowledgments

This project was principally funded by discretionary dollars donated to our Orthopaedic Research Center (Herbert Allen and James Kennedy, James M. Cox Foundation).

Figure A

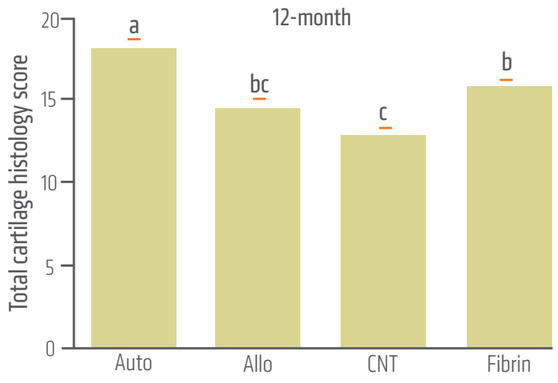

Figure B

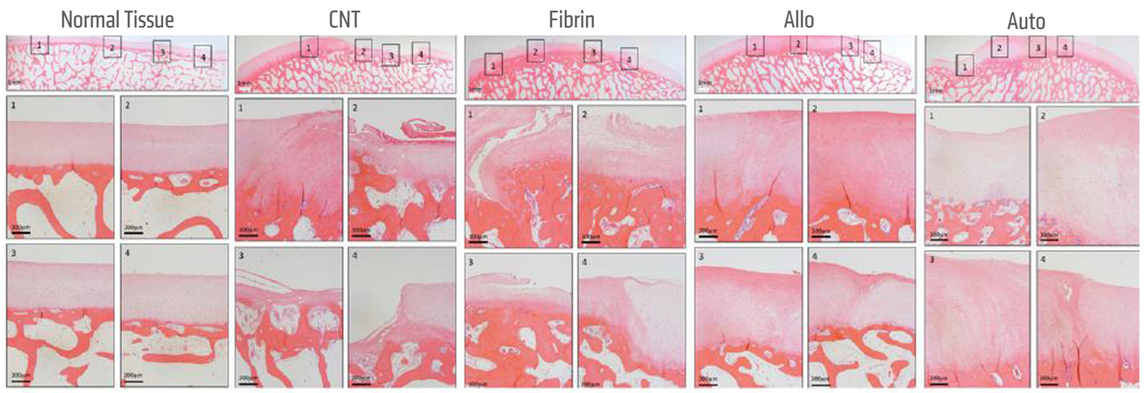

Figure A and B: Cartilage histology at 12 months. Figure A: Mean total histology score (and standard error). Groups labeled with the same letter did not differ significantly from each other. Figure B: Photomicrographs of a representative histologic section from each treatment group as well as a normal reference (hematoxylin and eosin).

Comparison of subjective lameness evaluation, force platforms, and an inertial-sensor system to identify mild lameness in an equine osteoarthritis model

THIS IS A SUMMARY OF AN ARTICLE by Drs. Josh Donnell, David Frisbie, Melissa King, Laurie Goodrich, and Kevin Haussler that was published in ***The Veterinary Journal***, 2015.

When mild lameness exists, agreement between clinicians is often controversial due to its subjective nature. The goal of the study was to compare subjective and objective methods to identify the presence of mild lameness using an established model of osteoarthritis.

A study was done on 16 horses that had osteoarthritis in the carpal joint. Subjective lameness evaluations (blinded and unblinded), force plate examination, and the use of an inertial sensor were used to detect forelimb lameness at four time points (days 0, 15, 42, and 71 after induction of osteoarthritis). The limbs identified as lame by each method were compared as well as compared to the osteoarthritis limb at each time point. Independent of time, blinded subjective evaluations (54 percent) and the use of the inertial-sensor system (60 percent) identified a higher percentage of horses as lame in the osteoarthritic limb compared to the force plate (40 percent) (Figure 1).

Blinded subjective evaluation and inertial sensor agreed on which forelimb was lame more often (50 percent) than blinded subjective evaluation with force plate (38 percent). Presence of lameness related to the osteoarthritis was supported by an increase in the frequency of horses considered lame by both subjective evaluations, the inertial sensor, and a decreased (3.6 percent) in mean (among all horses) of peak vertical force from baseline to osteoarthritis induction. Percentage of horses identified as lame in the osteoarthritic limb, independent of time, was highest by inertial sensor (60 percent) followed by blinded subjective evaluation (51 percent) and the FP (42 percent).

The conclusions of the study were that the sole use of a force plate platform or inertial-sensor system for detection of mild lameness is not recommended, and the current assumption that force platform data is the gold standard for objective lameness detection should be reconsidered. Recognition of subtle lameness related to osteoarthritis was more easily identified by subjective evaluation or inertial-sensor system than by force platforms. The take-home message is that subjective lameness evaluation is still more reliable in the detection of mild forelimb lameness compared to force platforms and an inertial-sensor system.

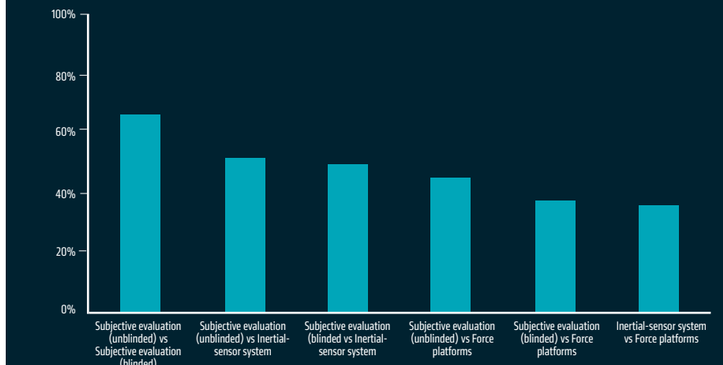

FIGURE 1: Overall percentage agreement between lameness detection methods for identifying the same forelimb as lame independent of time point.

PRCA Photo by Larry Smith. Photo courtesy of PRCA.

Cutting-Edge Clinical Research Knowledge Enables Barrel Racing Horse to go from Severe Stifle Injury to NFR Winner

IN EARLY 2014, LISA LOCKHART WAS DRIVING HOME, trailering her horse, Louie, a barrel horse that had just won a major rodeo, when she stopped to let him take a break from the trailer. While Louie was in a turnout stretching his legs, he badly injured his stifle (knee), much to Lisa's dismay. Lisa took Louie to Littleton Equine Medical Center to see Dr. Terry Swanson, where it was determined that Louie had a serious stifle injury and would need surgery. His prognosis for returning to a high level of athletic activity was unknown, but Lisa realized stifle injuries were notorious for ending high-level athletics.

Despite the prognosis, Lisa was willing to go forward with the surgery and, in speaking of her horse, she said, "Louie has been a very special horse for a long time, not only in the arena but outside the arena too. He's got a magnetic personality, which makes it twice as sweet to have the success in the arena and to enjoy him outside of the arena as well."

Dr. Swanson reached out to Dr. David Frisbie at the Orthopaedic Research Center and asked for his involvement in Louie's surgery as well as recommendations regarding stem cell treatment that could complement the surgical procedure. With his work on stem cell treatments for similar injuries, Dr. Frisbie was able to recommend the use of stem cells that could potentially better the prognosis for Louie to return to barrel racing.

The surgery was performed in April 2014, and Louie was treated with stem cells in June. Lisa then followed all the recommendations for bringing Louie back slowly and, as a result, they did not compete until the Nationals Final Rodeo at the Thomas and Mack Center in Las Vegas, Nev., where Louie and Lisa took home the average title and came in second in the over world standings! Lisa said, "It was a remarkable recovery to be able to have him back just in time for the rodeo."

"LOUIE HAS BEEN A VERY SPECIAL HORSE FOR A LONG TIME, NOT ONLY IN THE ARENA BUT OUTSIDE THE ARENA TOO. HE'S GOT A MAGNETIC PERSONALITY, WHICH MAKES IT TWICE AS SWEET TO HAVE THE SUCCESS IN THE ARENA AND TO ENJOY HIM OUTSIDE OF THE ARENA AS WELL." – LISA LOCKHART

In 2015, Louie and Lisa continued to compete and place well at such renowned rodeos as The Calgary Stampede as well as The American, which he won back-to-back years, breaking history. Louie and Lisa have captured the hearts of the veterinary teams that have helped them get back to work, and the results of the surgery and stem cell treatment are positive reinforcement that the research being done at the Orthopaedic Research Center has great clinical implications. Interestingly enough, Lisa also had to have knee surgery this year and found herself asking her surgeon if she could get the stem cell treatment like her horse did! Lisa gave a shout out "to the talented group that put us back together and gave us another chance to do what we love doing. Louie loves his job, and he would not have been able to do it without Dr. Frisbie and Dr. Swanson and them putting their heart and soul into getting him better!"

This story is a great success on many fronts; one of which is the collaboration between private practice and the University amplifying the benefits in applying cutting-edge research to get an elite athlete back to full potential, as well as emphasizing how research in veterinary medicine can potentially pave the way for better treatments not yet realized in human medicine. ■

Smooth Talkin Style NCHA 2015 Horse of the Year

Lloyd Cox, Gail Holmes, Bobby & Dottie Hill. Photo courtesy of NCHA.

Smooth Talkin Style, trained and shown by Lloyd Cox for Gail Holmes and Hill Ranches (Bobby and Dottie Hill), was named 2015 Horse of the Year, 2015 Breeder's Invitational Derby Open Champion, and 2015 NCHA Super Stakes Open Co-Champion. Congratulations to Gail as well as Bobby and Dottie Hill and Lloyd Cox. Gail has been the co-chair of our Advisory Board since 1996, and it is great to see her success in the sport she has been devoted to for a long time.

Lindy Burch – Bet Shes Smooth

Lindy Burch is Reserve Champion at the NCHA Open Futurity with Bet Shes Smooth

Another member of our Advisory Board, Lindy Burch, also had a very successful year in 2015. Most notable was Bet Shes Smooth, shown by Lindy for Oxbow Ranch, Weatherford, Texas, scoring 222 points to claim the Reserve Champion's title at the 2015 NCHA Open Futurity. Stevie Rey Von won the event with 225 points. Lindy had previously won the World Championship Futurity Open Division title in 1980 aboard Mis Royal Mahogany and was the first woman to win it. She was 29 at the time and living in California. Also during 2015, Lindy was NCHA Superstakes Champion on Adelle. Lindy is also in the NCHA and Cowgirl Hall of Fame.

CRC GRANT AND DONORS RICHARD AND EILEEN GREENBERG HELP LAUNCH PROJECT FOR OFF-THE-SHELF STEM CELL TREATMENT FOR FRACTURE REPAIR IN THE HORSE

Long bone fracture repair in horses is fraught with difficulty. Primary problems include mal-union and non-union, implant loosening, and that surgeons are constrained to work at mechanical limits of fixators and implants. Secondary problems include support limb laminitis, infection, and propensity to refracture. One of the aims of the ORC has been to decrease the frequency of fracture-related complications in the horse, and therefore decrease fracture-associated mortality. Fracture treatment with recombinant bone morphogenic protein-2 (rhBMP-2) has been shown to induce potent bone formation. RhBMP-2 was FDA-approved in 2002 to promote anterior lumbar vertebral body fusion (Infuse®, Amplify™) in humans; however, success has been controversial due to unforeseen side effects such as ectopic bone formation and, further, due to high costs.

Genetically modifying the cells offers an attractive alternative to recombinant protein therapy. Sustained low-level growth factor secretion of BMP-2 potentiates continuous bone formation without the unwanted side effects of rhBMP-2. Graduate student Alyssa Ball is working to optimize the amount of BMP-2 secreted from cryopreserved mesenchymal stem cells. By doing so, she will acquire the preliminary data for the possible novel development of an "off-the-shelf" stem cell treatment for fracture repair in the horse. Richard and Eileen Greenberg have been instrumental in the progression of this project through University giving, and we extend our deepest thanks for their support. ■

Eileen Greenberg, Alyssa Ball, and Richard Greenberg

VISITING SPEAKERS

We had a number of renowned speakers visit us this past year. These visitors all presented excellent seminars on their work to our group.

CHELSEA BAHNEY, PH.D.

Dr. Chelsea Bahney is an assistant professor, University of California San Francisco Department of Orthopaedic Surgery, UC Berkeley Department of Bioengineering & Material Science. Her seminar title was "A new model for endochondral ossification: basic and translational implications on bone regeneration."

Dr. Bahney's laboratory develops translational therapies for the treatment of musculoskeletal diseases and injuries. Specifically, the focus is on improved tissue regeneration by recapitulating the normal sequences of development and repair. Dr. Bahney's background is a degree in chemical engineering from the University of Colorado Boulder, a Ph.D. in stem cell and developmental biology from Oregon Health and Science University, and a postdoctoral fellowship in orthopaedic surgery at the University of California, San Francisco. Current aims are to use biologically modified polymers to promote a sequence of biological milestones that parallel native repair by endochondral ossification.

PHILIPPE BENOIT, D.V.M.

Dr. Philippe Benoit is a French veterinarian who graduated from Alfort Veterinary School in Paris, France, in 1989, and then obtained an M.S. in nutrition and exercise physiology in 1991. He established an equine clinic in Les Breviaires next to Versailles and now also has a part-time presence in the U.S. His primary interest is in sport horses, especially jumpers. His practice focuses on ultrasound imaging, orthopaedics, and sports medicine. He was team veterinarian for the French equestrian team between 1992 and 1999 and has been a consultant for other foreign teams since 2000. Recently, Dr. Benoit became a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation. Dr. Benoit's topic was "My thoughts on diagnosing and treating the equine athlete" and, in addition to his talk, he gave a clinic on his techniques on diagnosing and treating the equine athlete.

Arthros

GAIL HOLMES EQUINE ORTHOPAEDIC RESEARCH CENTER

DIRECTOR

Dr. Wayne McIlwraith

EDITOR

Paula Vanderlinden
Lindsie Talkington

GRAPHIC DESIGN

Communications and Creative Services, Colorado State University

OUR PURPOSE:
To find solutions to musculoskeletal problems, especially joint injuries and arthritis, in horses and humans.

OUR PHILOSOPHY:
To offer the best treatment of clinical cases possible, with continued and critical assessment of our results; to use these results to change our treatments; to point our research toward prevention of problems we cannot treat effectively or that cause permanent clinical damage.

OUR GOALS:
To find new methods to heal joints already damaged; to use state-of-the-art research techniques to find ways to prevent the occurrence of joint diseases and musculoskeltal injuries; to find methods of early treatment to prevent permanent damage when joint disease does occur.

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www.csuequineortho.com

MARK W. GRINSTAFF, PH.D.

Dr. Mark Grinstaff is the Distinguished Professor of Translational Research and a professor of biomedical engineering, chemistry and materials science, and engineering and medicine at Boston University. Dr. Grinstaff received his Ph.D. from the University of Illinois and was an NIH postdoctoral fellow at the California Institute of Technology. The title of his talk was "Contrast-enhanced computed tomography as a quantitative tool for monitoring cartilage composition and structure in preclinical models of osteoarthritis." Dr. Grinstaff is currently collaborating with us at the ORC in this technique as we investigate its applicability in the horse (also a major project for Dr. Brad Nelson's Ph.D.). Dr. Grinstaff has received a number of significant awards and is a Fellow of the National Academy of Inventors, including being the co-founder of four companies that are commercializing his ideas. He has three products being sold and used in the clinic.

CHARLES HO, PH.D., M.D.

Dr. Charles Ho is the director of imaging research for the Steadman Clinic and Steadman Philippon Research Institute. The title of his talk was "Applications and directions of musculoskeletal MRI in human orthopaedic sports medicine." His talk addressed the diagnosis and treatment of common injuries in human sports medicine. In addition to his duties in Vail, Dr. Ho is responsible for imaging interpretation at the NFL combine for both the Denver Broncos and the San Francisco 49ers. His talk was particularly illuminating on the comparisons in both imaging screening of athletes as well as the clinical problems that occur in them.

VISITING SPEAKERS

MARK HURTIG, PROFESSOR, D.V.M., M.V.SC., DIPLOMATE ACVS

Dr. Mark Hurtig is professor and director of the Comparative Orthopaedic Research Laboratory at the University of Guelph in Canada, which was established by the Canadian Arthritis Network as a strategic research resource laboratory. The focus of the laboratory is translational studies to facilitate regulatory approval of new therapies for both human and veterinary osteoarthritis, including disease modifying therapies and cartilage repair techniques. The title of Dr. Hurtig's talk was "Slow release platforms for intra-articular medication."

DICK MANSMANN, V.M.D., PH.D., HON ACVIM-LA

Dr. Dick Mansmann was a 1968 V.M.D. graduate of the University of Pennsylvania School of Veterinary Medicine and received his Ph.D. in 1974 from UC Davis School of Veterinary Medicine. He has had a long-standing interest in equine sports medicine. Dr. Mansmann retired from North Carolina State University and reopened his private practice in 2010, the same year he was made an honorary Diplomate of the American College of Veterinary Internal Medicine. His topic was "The relationship between hind feet break over, gluteal area pain, and performance? If you can measure it, you might be able to prove it."

SUZANNE TABBAA, PH.D. BIOENGINEERING, CLEMSON UNIVERSITY

Dr. Suzanne Tabbaa gave a seminar on "Development of fiber technology for bone regeneration with technology transfer considerations" as part of an interview for a postdoctoral research position at the ORC (see new staff additions on Page 18).

ASHLEE WATTS, D.V.M., PH.D., DACVS

Dr. Ashlee Watts is an assistant professor in the Department of Large Animal Clinical Sciences at Texas A&M University where her research emphasis includes techniques for improved stem cell isolation and expansion for autologous therapy; techniques for the optimization of stem cells for tendon, cartilage, bone. and anti-osteoarthritis therapy; and investigation into the basic mechanisms of successful stem cell therapy. Dr. Watts received her D.V.M. from Colorado State University and worked at the ORC when she was a student. She did her surgical residency and Ph.D. at Cornell University before going to Texas A&M. Dr. Watts presented a seminar on "A randomized, double-blind, placebo-controlled, clinical trial in 45 performance horses with hock lameness."

2015 SUPPORTERS

With grateful acknowledgment we thank those who are so critical to the continued success of our program. More specifics on our donor contributions will be included in the 2015 Annual Report.

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GRADUATE STUDENTS AND STAFF

JODIE DAGLISH, B.V.SC., MRCVS

Dr. Jodie Daglish joined the Equine Sports Medicine and Rehabilitation Service residency program July 1, 2016, having finished a one-year equine diagnostic imaging internship with Dr. Myra Barrett at CSU. Dr. Daglish graduated from Bristol University in the U.K. before completing a two-year equine internship at Newmarket Equine Hospital. Following this, Dr. Daglish worked for 18 months in a busy equine practice, specializing in eventing and racing, before moving to the U.S. to pursue her interests in equine sports medicine, undertaking a year with the Equine Sports Medicine Service at University of California, Davis, before joining the program at CSU.

SHERRY JOHNSON, D.V.M.

Dr. Sherry Johnson joined the Equine Sports Medicine and Rehabilitation Service's residency program in July 2015, following completion of a one-year equine dagnostic imaging internship with Dr. Myra Barrett. Dr. Johnson graduated from Iowa State University's College of Veterinary Medicine, and then completed an equine internship in Ocala, Fla., prior to continuing her sports medicine training at CSU.

LYNN PEZZANITE, D.V.M.

Dr. Lynn Pezzanite joined the CSU Equine Surgery Service residency program in July 2015 following completion of a one-year rotating equine internship at Littleton Equine Medical Center. Dr. Pezzanite graduated from Cornell University College of Veterinary Medicine in 2014. She previously worked in the Comparative Orthopedics and Regenerative Medicine Laboratory of Dr. Lisa Fortier at Cornell University. Her research interests include regenerative medicine and diagnostic imaging.

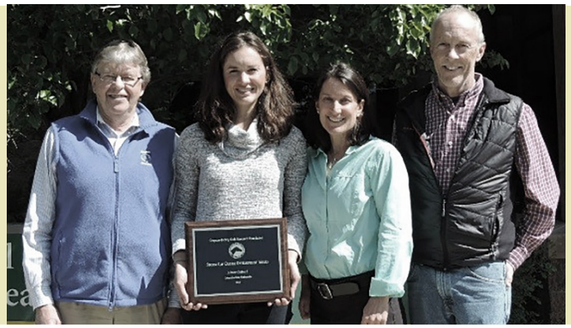
LINDSIE TALKINGTON

Lindsie Talkington came to work for the ORC in 2015 as a veterinary transcriptionist. Along with medical transcription, she has previously worked as a veterinary technician and also as a medical assistant.

SUZANNE TABBAA, PH.D.

Dr. Suzy Tabbaa joined the ORC in the fall of 2015 as a postdoctoral researcher working jointly between the ORC and the Cartilage Tissue Engineering Laboratory at University of California San Diego under the guidance of Dr. Dave Frisbie and Dr. Bob Sah. Dr Tabbaa completed her doctorate in bioengineering from Clemson University and worked as a senior commercialization technology analyst at the Clemson University Research Foundation prior to joining the ORC team. Her two main projects at CSU are an improved cartilage repair strategy using the technology she discussed in a talk she gave as part of her interview, as well as correlating the equine induced osteoarthritis model formerly developed at CSU to human OA. Her postdoctoral work includes determining the commercial potential of the research projects and developing commercialization strategies for translation to the clinic.

2015 AWARDS



STORM CAT CAREER DEVELOPMENT AWARD

THE GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION Career Development Awards program was initiated in 2006 with the first Storm Cat Award. This was named for the famous stallion that stood at Overbrook Farm, the family home of foundation board member Lucy Young Hamilton. Mrs. Hamilton personally underwrites the $15,000 stipends to assist in specific research by young candidates who pursue career paths in equine research. The 2015 Storm Cat Career Development winner is Aimee Colbath of Colorado State University. ■

CVMBS COOPERATIVE VETERINARY SCIENTIST RESEARCH TRAINING FELLOWSHIP

DR. BRAD NELSON, who is currently a Ph.D. candidate at the ORC, received the inaugural College of Veterinary Medicine and Biomedical Sciences Cooperative Veterinary Scientist Research Training Fellowship. This program, begun to support promising and dedicated young veterinarians in the CVMBS toward careers in biomedical research, is designed as a partnership between the College and sponsoring research programs, in this case, the ORC. Dr. Nelson was chosen based on his application, interview, the vote of the NIH T32 Selection Committee, and the support of the CVMBS associate dean for research (Dr. Sue VandeWoude), the CVMBS dean (Dr. Mark Stetter), and especially the strong support of his mentors, Drs. Chris Kawcak, Wayne McIlwraith, and Laurie Goodrich. The fellowship took effect July 1, 2015, and provides stipend support at NIH postdoctoral level for up to three years devoted specifically (minimum of 85 percent time/effort) to his Ph.D. research and career development as a scientist. The stipend for the first year of support is provided entirely by the CVMBS, with subsequent years shared by the College and the ORC. This fellowship is intended to be a signature program demonstrating and adding to the CVMBS commitment to the training of elite-level veterinary medical scientists to conduct translational research and lead the profession. Congratulations to Dr. Nelson on being the first recipient! ■

AAOS/ORS YOUNG INVESTIGATOR AWARD

LIVIA CAMARGO-GARBIN, a Brazilian veterinarian pursuing a Ph.D. at the ORC, was selected to participate in the AAOS/ORS Biologic Treatments of Orthopaedic Injuries research symposium Nov. 5-7, 2015, in Rosemont, Ill., as a Young Investigator by Co-Chairs Robert LaPrade, M.D., Ph.D.; Constance Chu, M.D.; Jason Dragoo, M.D.; and Jason Koh, M.D. Camargo-Garbin not only had a poster presentation but was also invited to give a 10-minute podium presentation on her work on PRP. ■

AAEP FOUNDATION PAST PRESIDENTS' RESEARCH FELLOW

COLORADO STATE UNIVERSITY DOCTORAL CANDIDATE Aimee Colbath, V.M.D., M.S., received the 2015 AAEP Foundation Past Presidents' Research Fellow for her research into the use of bone marrow-derived mesenchymal stem cells, a promising therapy for musculoskeletal disease in the horse that is widely used but not fully understood. Dr. Colbath was recognized Dec. 7, 2015, during the Frank J. Milne State-of-the-Art Lecture at the AAEP's 61st Annual Convention in Las Vegas, Nev. The $5,000 fellowship was founded in 2006 and is awarded annually to a doctoral or residency student who has made significant progress in the field of equine health care research. Dr. Colbath also received a $500 stipend to support her travel to Las Vegas. "We are delighted to honor Dr. Colbath, and thank her for continuing her education and career toward equine research," said AAEP Foundation Chairman Jeff Berk, V.M.D. "If our knowledge of horse health is going to continue to advance, we must encourage and financially support more veterinarians to become researchers like Dr. Colbath."

Dr. Colbath's research focuses on the immune properties of BMDMSCs and equine induced pluripotent stem cells. If the immune properties of allogeneic BMDMSCs are equivalent to those of autogenous BMDMSCs, these cells may provide a more readily available, potentially less expensive and more consistent biologic therapy for musculoskeletal disease in the horse. In contrast, if allogeneic cells or iPSCs are found to be immunogeneic, then clinicians should be wary of their use.

Dr. Colbath received her veterinary degree from the University of Pennsylvania in 2010. She completed her equine surgery and lameness residency and her master's in clinical sciences at Colorado State in 2015. ■

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