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Imaging Biomarkers of Orthopaedic Disease

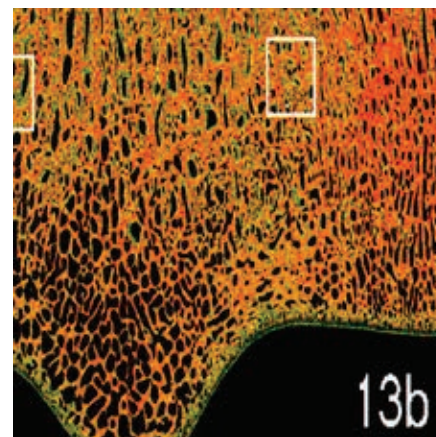
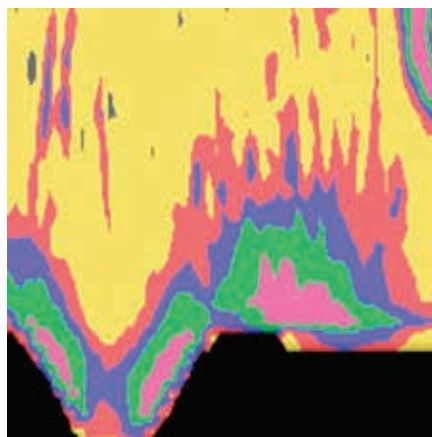


Fig. 1: CT scan (left) and microscopic image (right) of density and bone changes in the joint

Although there have been significant strides in developing advanced imaging techniques such as computed tomography and magnetic resonance imaging in equine medicine, there are still limitations on what the human eye can detect. When a problem is detected on any current imaging technique, the disease process has already physically changed the tissues, and it is unlikely that these tissues will revert back to normal. However, physiologic changes can occur in the tissues prior to an irreversible state (Fig.1), and it is the goal of the researchers in the Orthopaedic Research Center, led by Dr. Chris Kawcak, to identify methods to detect those changes.

Can joint shape be a predictor of disease?

The fetlock joint in the horse is commonly affected by a wide variety of diseases. Fractures, (which sometimes

can be catastrophic, necessitating euthanasia of the horse), osteoarthritis, and pain in general, are common disease entities in the fetlock joint.

Recently, investigators at the ORC identified another possible cause of joint disease: joint shape. Researchers determined that joint shape is oftentimes different among different animals. Therefore, the researchers hypothesize that joint shape may be important in predisposing an animal to joint disease. To address this hypothesis, Dr. Kawcak is now collaborating with Dr. Todd Bredbenner at the Southwest Research Institute in Texas to use sophisticated three-dimensional shape and density modeling techniques (Fig. 2) to investigate this hypothesis. The goal of this research is to identify a means of characterizing joint shape and its influence on joint disease.

Letter from Dr. McIlwraith

Welcome to the 2012 edition of *Arthros*. Our newsletter highlights some of our important findings from last year, including some new information on imaging biomarkers of orthopaedic disease and analysis of shape in joints contributing to osteoarthritis and fracture in the horse; the use of bone marrow-derived mesenchymal stem cells with cranial cruciate ligament disease in dogs; optimization of a new viral vector to treat osteoarthritis in the horse more effectively; demonstration of enhancement of tears of the meniscus using bone marrow-derived mesenchymal stem cells in fibrin glue; clarification of any differences between mesenchymal stem cells acquired from a sternal bone marrow aspirate compared to a pelvic bone marrow aspirate; and development of an inertial measurement unit system to be ultimately used in the evaluation of equine distal limb motion as well as force plate analysis at fast activity (force plate measurement is limited to the trot).

Further details of our research productivity for the last two years is available in our 2010-2011 Orthopaedic Research Center and Orthopaedic Bioengineering Research Laboratory Report. If you would like a copy, please contact Katie Briggs at the ORC.

The newsletter also contains information on new staff and a new resident, Josh Donnell. Particularly notable is the acquisition of Katie Briggs to help support fundraising activities, publish newsletters, and maintain the ORC website. Thanks to our donors, as well as our research funding agencies, we've been able to continue with all our programs and research efforts. We will continue to justify your investment in what we do here.

Best wishes,



Wayne McIlwraith
Director



Imaging Biomarkers

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In preliminary studies, the investigators have identified that horses with a condylar fracture had abnormal condylar widths and roundness in their fractured and unfractured limbs compared with horses that had no fracture. The investigators are currently applying SSDM to these data in order to address the problem.

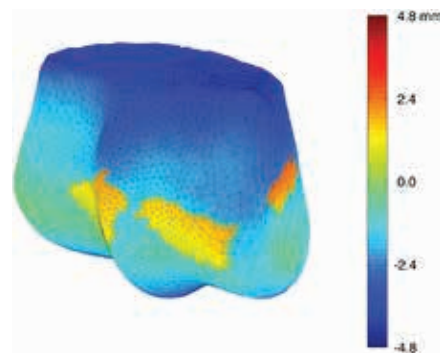


Fig. 2: An example of SSDM of the fetlock joint

SSDM and shape changes

In preliminary studies using SSDM, ORC researchers have found that shape can change even in adult horses. As an example, when SSDM was applied to horses before and after a cast was applied to a single forelimb, researchers found that not only did significant density changes occur, but also that shape changes occurred after the cast was removed. This finding is significant, considering that the joints of adult horses can change depending on the situation.

Based on these preliminary data, the ORC researchers will investigate growth variability during development. In foals, the time from birth to 1 year of age is when the most rapid growth occurs, and consequently, when the joint shape is most influenced. Because of the high prevalence of abnormal conformation in young horses, it makes sense that the joint shape could be influenced by abnormal conformation. Therefore, ORC researchers will further study the effect of conformation on joint shape in young horses.

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www.csuequineortho.com.

Regenerative Therapy for Dogs

The effect of bone marrow-derived mesenchymal stem cells in preventing the development of cranial cruciate ligament disease in dogs

Drs. John Kisiday and Kevin Haussler are collaborating with Dr. Clara Goh of the Small Animal Orthopaedic section at Colorado State University on a project to investigate the therapeutic value of bone marrow-derived mesenchymal stem cells in preventing the development of cranial cruciate ligament rupture in dogs.

Rupture of the CCL, analogous to the anterior cruciate ligament in humans, is the most common cause of lameness in dogs. Management of canine CCL disease is a major orthopaedic dilemma as it is thought that degeneration weakens the ligamentous structure over time, increasing its susceptibility to complete rupture during seemingly normal activities. A disease-modifying therapy that slows or prevents CCL degeneration, thereby preserving the mechanical functionality of the ligament, would be a major advancement in the treatment of this disease.

Multiple applications for stem cells

The goal of this project is to determine if intra-articular injection of autologous bone marrow-derived mesenchymal stem cells can act as a disease-modifying agent for the CCL and prevent the development of complete rupture. While the disease-modifying properties of MSCs have been intensely researched over the past 20 years, relatively few studies have considered intra-articular MSC therapies, and none has evaluated the effect of MSCs on damaged or degenerative ligaments in naturally occurring disease.

This project is being conducted with client-owned dogs with unilateral CCL rupture and a contralateral, intact CCL that is at future risk for rupture due to increased weight bearing and compensatory gait mechanisms associated with CCL rupture. Dr. Goh will aspirate a small volume of bone marrow from which MSCs will be isolated and culture-expanded by Dr. Kisiday in the cell culture laboratory in the Orthopaedic Research Center.

At a follow-up examination, Dr. Goh will inject 5 million MSCs into the at-risk joint in half of the subjects, while the remaining subjects will receive placebo injections. The subjects will be evaluated over the subsequent 18 months with physical examinations and force plate gait analysis at the ORC (Fig. 1 and Fig. 2). From this study, researchers will determine whether the novel approach of administering intra-articular injections of MSCs is promising for preventing the development of CCL degeneration and rupture.



Fig. 1: (top) A dog trots across the force platforms to measure the amount of weight applied to each of the four limbs. Dogs with cruciate ligament injuries will have reduced weight bearing on the affected hind limb.

Fig. 2: Video capture on a computer screen of the same dog as it trots across the force platforms. Video records help to demonstrate how the dog is moving, and to identify which limbs strike the individual force platforms for data analysis.

Reducing Back Problems in Horses

The first study to develop and validate a method that induces and quantifies local saddle pressures

Equine back pain is a common clinical condition that negatively affects a horse's performance. The use of a saddle is, of course, intended to protect a horse's back and to distribute a rider's weight over as large of an area as possible. However, if a saddle doesn't fit the horse properly, increased local pressures occur which are often implicated as primary causes of back pain, soft tissue trauma, and poor performance. Unfortunately, after centuries of applying saddles to ridden horses, no studies exist that evaluate the effect of quantifiable increased saddle pressure on the development of back pain and associated changes in spinal motion and lameness.

This study is being conducted by Drs. Kevin K. Haussler, Melissa King, and Kirk McGilvray. The purpose is to develop an instrumented saddle and to collect pressure data underneath the saddle in areas of known induced pressures; this data can then be used to induce back pain at common sites of poor saddle fit. The instrumented saddle (Fig. 1) incorporates a force transducer placed under a weighted saddle to simultaneously induce and measure localized saddle pressures during simulated ridden exercise on a high-speed treadmill (Fig. 2). Stereophotogrammetric techniques allow analysis of spinal and limb movements during induced saddle pressures at the cranial, middle, and caudal sections of the saddle.

Clinical signs of back pain, including heat, swelling, and muscle hypertonicity, are monitored with pressure algometry and detailed evaluation in areas along the trunk and under the saddle. Force plate analysis is used to assess the presence of limb lameness due to the induced saddle pressures. To date, the researchers have finalized saddle



Fig. 1 (above): The instrumented saddle showing induced pressure on the right caudal portion of the saddle. The gap on the right side between the tree (dorsally) and the moveable saddle section (ventrally) represents how the different portions of the saddle are used to induce increased local saddle pressures.

Fig. 2 (below): A horse with the weighted, instrumented saddle on the high-speed treadmill with cameras in the foreground that measure spinal and limb movements via attached reflective markers.



continued on Page 5

Hope for Restoring a Torn Meniscus

Healing of meniscal lacerations using bone marrow-derived mesenchymal stem cells and fibrin glue

When a human or equine athlete suffers a severely torn meniscus, often the treatment plan involves removal of either part of or the entire meniscus (meniscectomy). The problem with either option is it can result in long-term negative effects, including osteoarthritis and joint disability. However, if the meniscus can be treated or repaired instead of removed, the patient may recover and avoid continued dysfunction of the knee or stifle.

This research project considers promising treatment options that use fibrin glue created from a patient's own blood that can be used as a carrier to deliver mesenchymal stem cells to the specific site of an injury. An experimental model for optimizing various versions of this delivery system *in vivo* was tested in this study.

Harvested equine meniscal sections were reapposed with fibrin glue, or fibrin glue and equine bone marrow-derived mesenchymal stem cells. These constructs were then

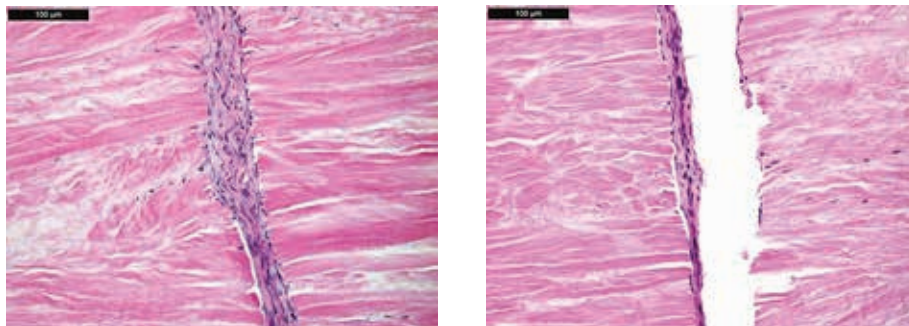


Fig. 1a: (left) A cell-treated (Group 1) meniscal section with cellular repopulation of the acellular meniscal tissue and evidence of bridging between the two meniscal sections, especially in the upper aspect of the image. Fig. 1b: (right) A non-cell-treated section (Group 0) with separation of the repair tissue from the meniscal fibers on the right side (indicative of a less robust attachment). While this detachment occurred during processing, a lack of bonding can be seen on the left side of the repair tissue as well.

implanted subcutaneously in nude (immunologically impaired) mice. After harvesting of the constructs, BMSC that contained constructs showed significantly increased vascularization, and histology showed subjectively decreased thickness of repair tissue and increased total bonding (Fig. 1a) compared to fibrin-alone constructs (Fig. 1b).

The BMSC model allowed direct comparison of different meniscal treatment groups while using a small number of animals. This *in vivo* model could be valuable in the future to optimize fibrin and cellular treatments for meniscal lesions in the horse and potentially humans as well.

Back Problems

continued from Page 4

construction and preliminary evaluation to document changes in pain thresholds, limb lameness, and behavioral changes associated with induced saddle pressures.

Investigators can use the instrumented saddle in future randomized clinical trials to assess the immediate and longer-

term clinical features and adaptations to known sites of induced saddle pressures. The information gathered will add significantly to our current knowledge of proper saddle fitting and compensatory mechanisms due to localized back pain. The long-term goal is to reduce the prevalence of back problems in horses.

The ORC has strong collaborative research efforts with the Orthopaedic Bioengineering Research Laboratory, which assisted with force transducer instrumentation and the computer programming needed for this project.

Mesenchymal Stem Cells Acquired from the Sternum and Ilium Are Not Significantly Different

The Orthopaedic Research Center has been a pioneer in the advancement of bone marrow-derived mesenchymal stem cells over the past decade. MSCs are thought to increase healing by promoting regeneration of tissues such as cartilage, bone, and tendon.

When a horse is injured and stem cell therapy is indicated, the clinical treatment process is usually the same. The therapy begins with a bone marrow draw. The marrow is then sent through a series of centrifugations, and the plastic adherent cells are cultured and passaged three times. These cells are then clinically inserted into the lesion of the injured horse.

What can differ in this process is the location the doctors choose to draw the marrow from. Clinicians can acquire

the bone marrow from the initial draw from either the sternum or the wing of the ilium. Currently, the harvest location is completely dependent on clinician preference. PVM student Karla Penman, working with Drs. Laurie Goodrich, John Kisiday, and Wayne McIlwraith, compared the differentiation potential of cells acquired from both locations.

Bone marrow was harvested from the sternum and ilium of horses in 5 mL aspirates. Cells from each site were grown to the third passage (the cell lineage that would be used clinically to treat an injured horse) and differentiated into osteocytes, chondrocytes, and adipocytes.

Osteogenic cells were stained with alizarin red and alkaline phosphatase to highlight bony changes in monolayer growth (Fig. 1). Chondrogenic cells were

cultured in agarose gel wells (Fig. 2) to simulate the 3-D matrix promoting cartilage development. These cells were stained with a toluidine blue to highlight glycosaminoglycan production as well as a “live-dead” stain to ensure cellular viability.

The MSCs’ differentiation capacity were quantified with various ELISAs and gene expression assays. Samples from the sternum and ilium were not found to be different in their potential to differentiate *in vitro* and, furthermore, the gene transduction potential for future gene therapeutic applications was not different between sites.

The primary objective of this study was to determine if we would derive better bone healing from one site over another. We did not observe any difference across aspirate locations, indicating that practitioners can confidently draw bone marrow from either location and be assured that the cells will be capable of differentiation.

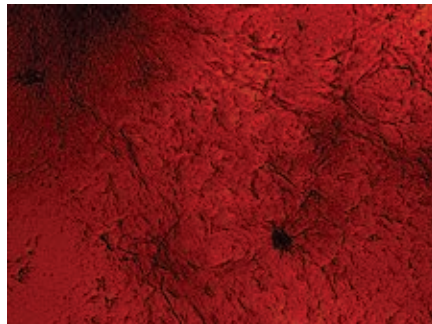
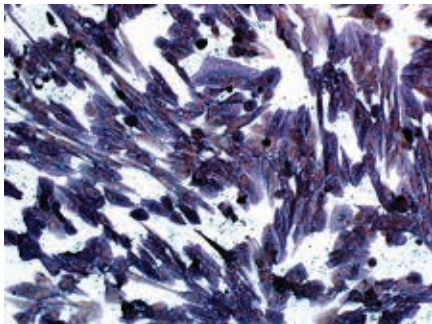


Fig. 1: Osteogenic Staining of MSCs

MSCs grown to become osteocytes (bone cells). Alkaline phosphatase and Alizarin red stains both highlight bony changes such as calcium deposition.

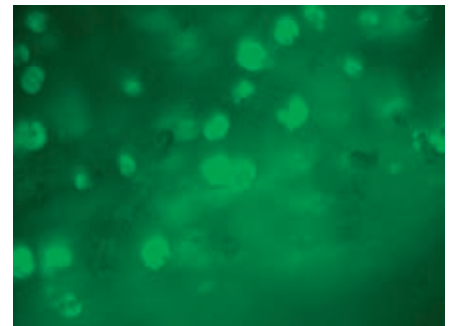
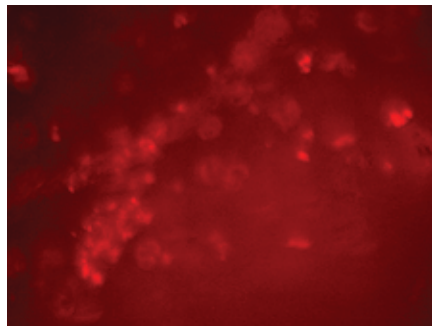
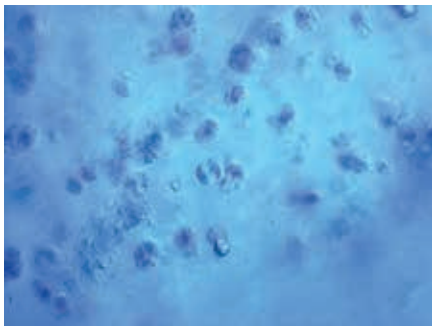


Fig. 2: Chondrogenic MSCs (Location B, Ba, Bb)

MSCs cultured to become cartilage were grown in agarose gel plugs and sliced for staining. Toluidine blue stain highlights the glycosaminoglycan matrix around cells that are confirmed to be alive when they fluoresce green. Notice how the cells with the largest “halo” are also live cells.

Early Detection of Equine Injuries

Development of an inertial measurement unit system for the evaluation of equine distal limb motion

Lameness is documented as one of the leading causes of wastage in all types of performance horses. Many catastrophic injuries are thought to be fatigue related, and these may show subtle abnormalities or lameness prior to the injury. In addition, early lameness is not always appreciable at all gaits and during every stride, making these injuries more difficult to identify.

Because catastrophic injuries give the equine industry a poor public perception, early detection and prevention of these injuries is critical. Our goal with this research is to develop diagnostic techniques, such as motion analysis, for early detection of equine injury.

Sensor development

There has been a trend by equine musculoskeletal researchers to develop horse-mounted motion analysis systems that can be used while horses are ridden. These sensors have been used on the body and the distal limbs of horses. The development of small, lightweight sensors, such as the inertial measurement unit, can allow the evaluation of equine distal limb motion in all three planes: cranial-caudal, medial-lateral, and vertical. Our objective is to develop a hoof-mounted system (Figure) that can collect meaningful kinematic data at all gaits, including the gallop.

In our first investigation involving Drs. Valerie Moorman (PhD student), Chris Kawcak, and Wayne McIlwraith, in collaboration with Drs. Raul Reiser (CSU Department of Health and Exercise Science), and Mick Peterson and Christie Mahaffey (University of Maine), we compared an IMU to 3-D optical



Figure: Research horse instrumented with an Inertial Measurement Unit on the right front hoof and laptop computer attached to the surcingle for real-time data collection.

kinematics in five normal horses at the walk and trot over-ground. We found that the equine IMU could accurately detect normal sagittal plane motion, as compared to a 3-D optical kinematics system at both gaits.

In a second investigation, we examined the specific kinematic changes that occur with experimentally induced single forelimb lameness. We used a rapidly reversible, sole pressure model of lameness and induced mild to moderate degrees of lameness, which were not visible at the walk but could be detected at the trot.

Using 3-D optical kinematic data, we determined that with induction of very mild lameness, we could detect differences in hoof orientation between

the lame and non-lame forelimbs. We are currently analyzing the kinematic data from the IMU to determine if the same kinematic changes can be detected.

Full speed ahead

One long-term goal for the IMU is to be able to evaluate the galloping horse. The current IMU that we have been evaluating has appropriate specifications to withstand the accelerations of the horse at high speed, and thus should be able to be used in this manner.

We believe that the IMU will be a valuable tool to evaluate equine distal limb motion and promote equine musculoskeletal health with its ability to be used on animals exercising at high speed.

Managing Osteoarthritis Symptoms

Optimization of scAAVIL-1ra vectors to deliver high levels of therapeutic protein into the joint for treatment of osteoarthritis

Osteoarthritis is a progressive and debilitating joint disease for which there is no cure. In the equine industry, lameness due to joint injury and disease is the most prevalent cause of diminished athletic performance and wastage in equine athletes.

Over the last two decades, researchers have explored the use of pharmaceuticals to control symptoms of OA, as well as to slow the progression and degeneration of the joint in an effort to bypass the need for joint replacement. Although drugs such as hyaluronan and polysulfated glycosaminoglycan have been somewhat disease modifying, they have not alleviated symptoms as effectively as originally forecasted. On the other hand, corticosteroids definitely lower symptoms but some have deleterious effects as well and their use – in racing at least – is controversial.

Maximizing protein expression

Gene transfer using viral vectors is a promising approach in delivering therapeutic genes to joints by direct *in vivo* injection. Many viral vectors have been utilized for intra-articular gene therapy. While these vectors have been shown to produce high levels of the targeted protein *in vivo*, they have also been shown to cause significant acute inflammation in the injected joint, transduce targeted cells inefficiently, and have a sharp decrease in protein levels after.

Self-complementary AAV, however, has been shown to induce a dramatic increase in viral transduction in multiple cell types, causing little to no immune reaction. It has also been shown to

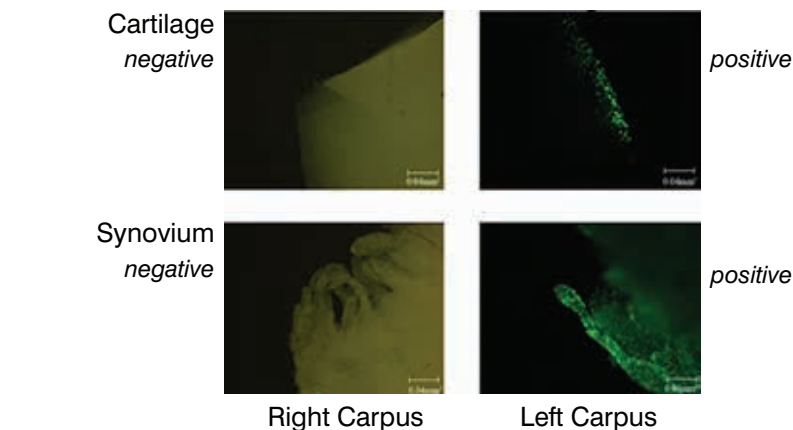


Figure: GFP Expression in injected carpal joints. Positives reflect green fluorescent marker gene uptake in the joint.

produce significant levels of targeted protein for long periods of time. Regardless of promising results seen in culture, protein expression in cell culture may not always represent protein expression in animals due to a number of biological factors. We optimized variables of a scAAV vector expressing equine IL-1ra to explore the application of scAAV gene therapy in an equine model.

An initial pilot study consisting of two horses was performed over a six-month period to assess dosing and serotype administration of a scAAV that would produce equine IL-1ra. Both fore metacarpophalangeal and mid-carpal joints were dosed with either saline (negative control), scAAVGFP (positive control), or scAAVIL-1ra. Viral constructs were given at a high dose (5×10^{13} viral particles per joint) and a low dose (5×10^{12} viral particles per joint).

Lameness and physical examinations were conducted, and serum and synovial fluid samples were collected at Day 0

to establish baseline levels. Serum and synovial fluid samples were collected two weeks post-injection and every week thereafter for 3 or 28 weeks. Tissue samples were collected for histology, biodistribution, and functional assays.

Promising outcomes with scAAV

The results of this study reveal the first successful demonstration of dramatic increases in the therapeutic protein, IL-1ra (Figure), for extended periods of time in large animal (equine) joints. The results also revealed that a repeat dose of a viral vector can be utilized, causing a “booster effect” and extending the duration of protein production of the gene of interest. These data are important, as they suggest that scAAV gene therapy will be effective for extended periods of time without causing intra-articular toxicity in the equine model, which is commonly used to mimic OA in humans.

New Staff and Graduate Students

Dr. Josh Donnell



Josh joined the ORC as an Equine Sports Medicine and Rehabilitation resident in July 2012. He is originally from Canyon, Texas, where he received a bachelor's degree in animal science from West Texas A&M University. Josh graduated from Texas A&M College of Veterinary Medicine in May 2010, and was an intern at Goulburn Valley Equine Hospital in Shepparton, VIC, Australia. He then worked for a year at La Mesa Equine Lameness Center and Equine Sports Medicine in Pilot Point, Texas.

Dr. Valerie Moorman



Valerie is currently completing her PhD and is investigating hoof-mounted inertial measurement units to examine real-time kinematics in both normal and lame horses. She has also recently been appointed as the ORC staff veterinarian. She graduated from North Carolina State University College of Veterinary Medicine in 2004 with a DVM. She then completed a large-animal medicine and surgery internship at Auburn University in 2004-2005, and continued as a clinical instructor in Auburn's large-animal

ambulatory service. During this time, she worked with Dr. Robert Gillette and the sports medicine service examining the effect of hoof wedges on distal limb motion in the sagittal plane using 2-D optical kinematic analysis. In 2006, she began an equine surgical residency and combined master's degree at Oklahoma State University, which she completed in July 2009. In July 2009, she accepted a position at Colorado State University as an after-hours large-animal emergency clinician and PhD student at the ORC. In March 2010, she became board certified as a Diplomate of the American College of Veterinary Surgeons.

Julia Carter



Originally from Prineville, Ore., Julia graduated from Lamar Community College with an associate's degree in horse training and management, and then earned a certified veterinary technician degree and license from Bel-Rea Institute in 1996. After graduation, she worked at CSU in Small Animal Anesthesia and Critical Care as a student hourly and at Heska Corp. in the medical technical support and service departments for medical instrumentation. Julia then worked at Jorgensen Laboratories as a veterinary product manager in quality control and marketing, and joined the ORC as the clinical/MRI technician in early 2012.

Amanda Mills



Amanda graduated from CSU with a BS in equine science and interned for eight months at Hagyard Equine Medical Institute in Lexington, Ky. After graduating, she worked for a breeding season at the Four Sixes Ranch in Guthrie, Texas. She next moved to the LSU Veterinary Teaching Hospital as the equine internal medicine nurse and equine nursing supervisor. Amanda joined the Equine Sports Medicine service in May 2012 as the client care coordinator. Her responsibilities with ESM include communicating with clients, scheduling appointments, and nursing assistance in the field.

Katie Briggs



Katie received her BA in technical journalism at CSU, and has worked in a variety of editing and writing positions in publishing and marketing since graduating in 1991. She worked part time as Dr. Wayne McIlwraith's assistant in 2007, and joined the ORC full time as outreach coordinator in early 2012. Her responsibilities include supporting fundraising activities, publishing newsletters, and maintaining the ORC website.

Presentations and Speakers

Corticosteroids in Racehorses

Dr. Wayne McIlwraith addressed the appropriate use of corticosteroids in the racehorse at the Welfare and Safety of the Racehorse Summit IV, held Oct. 16-17 at Keeneland Race Course in Lexington, Ky. Dr. McIlwraith cautioned that although corticosteroids can lead to a decrease

in inflammation, musculoskeletal pain, and other joint and balance issues, users must select the drug with care because not all corticosteroids are the same.

You can find an article about the seminar at www.bloodhorse.com.

Visiting Speakers 2011-2012

We're proud to have hosted two speakers this year. Both Jos Malda and Troy Flanagan visited the ORC to present their seminars.

Dr. Jos Malda

Dr. Jos Malda, PhD, Assistant Professor, Department of Orthopaedics, University Medical Centre, Utrecht University, The Netherlands, visited in



May and presented, "Approaches to Restore the Native Articular Cartilage Complexity." His research focuses on physiological osteochondral grafts with a controlled architecture that mimic the topographical organization of cells in the native tissue.

Dr. Troy Flanagan

Dr. Troy Flanagan, PhD, High Performance Director, US Ski and Snowboard Association, shared how his work has helped to fine-tune Olympic athletes. "Using Science and Technology to Enhance Performance," detailed how his lab at the USSA is collecting data to take the human body to the superhuman level through advancements in everything from the uniform fabric the athletes wear on the outside to the nutrition that fuels them from the inside.



Arthros

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Arthros is an annual Colorado State University Equine Orthopaedic Research Center publication.

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 musculoskeletal injuries; to find methods of early treatment to prevent permanent damage when joint disease does occur.

Laurie Goodrich Receives Elastikon™ Award from the Grayson-Jockey Club Research Foundation

Congratulations to Dr. Laurie Goodrich for receiving the fifth annual Elastikon™ Research Award, which provides funding for her research project on osteoarthritis, a malady common to horses. This funding will allow her to study the use of gene therapy in an attempt to produce beneficial protein that will allow cartilage to heal.

The Elastikon™ Award is supported in part by a donation to Grayson-Jockey Club Research Foundation from Johnson & Johnson's Consumer Products Division, which manufactures Elastikon™ tape and other equine products.



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Colorado State University's

14th Annual Stallion Auction to benefit Equine Research

Jan. 9-12, 2013

Online bidding opens at 11 a.m. Jan. 9, 2013, and closes at 11 a.m. Jan. 12, 2013 (all times MST).

Bid Online!

Go to www.csuequineortho.com and click on the Stallion Auction link (under the ORC Support heading). Bids do not include chute fees, booking fees, or associated reproductive and veterinary costs.

How You Are Helping

Proceeds will go to the outstanding equine research programs at CSU's Orthopaedic Research Center and Equine Reproduction Laboratory. With your bid, you can help these programs improve the musculoskeletal and reproductive health of horses.

Questions? Call (970) 297-4165 or e-mail Paula.Vanderlinden@ColoState.edu. Stallions and minimum bids listed at www.csuequineortho.com.

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