Orthopaedic Research Center

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WHAT'S INSIDE

New Scientific Support for Long Used Intra-articular Therapies

New scientific support for long-used intra-articular therapies from a recent study has shown positive effects in the CSU equine osteoarthritis model with the intra-articular medications polysulfate glycosaminoglycans (PSGAG) and hyaluronan or hyaluronic acid (HA). The study, from the Gail Holmes Equine Orthopaedic Research Center at Colorado State University, was published in *American Journal of Veterinary Research.* "These study results clearly indicate that both drugs are viable therapeutic options for horses with osteoarthritis," reported first author David Frisbie regarding his paper with Drs. Christopher Kawcak, Wayne McIlwraith, and Natasha Werpy as co-authors. While both PSGAG (Adequan™) and HA have long been used in horses, it is only with this study that there has been clear scientific documentation of value in a clinically relevant model. "Before this study the best science we had was clinical impressions with long-term use by veterinarians and one clinical study published in Norway. This study supports the impressions of experienced practitioners, but did clarify and differ with some commonly held beliefs," said Dr. McIlwraith.

There were no adverse treatment-related events. Synovial effusion

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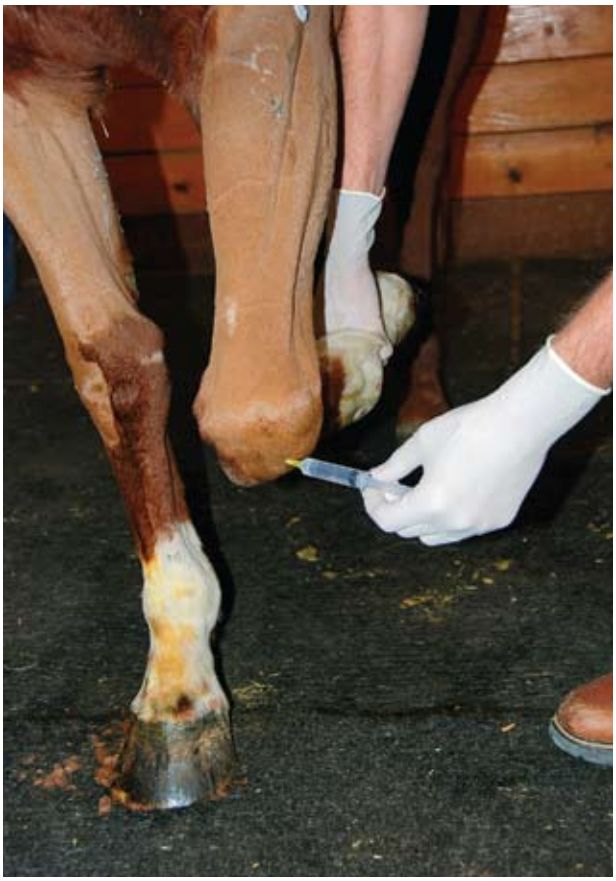

Researchers have confirmed that two joint injection drugs positively impact the structure of joint cartilage.

Letter from Dr. McIlwraith

It is probably safe to say that all research institutions at universities have had a tough year; in fact, all veterinary schools have had major challenges because of the economic recession. While Colorado State University has weathered the situation relatively well, the endowed corpuses which provide salaries for our four endowed chairs within the Orthopaedic Research Center have certainly suffered greatly in the last 18 months. Fortunately, we had enough donated money uncommitted to endowments to make up for salary needs.

The other good news is that we have continued to receive good external research funding which is based on the ability of our faculty and staff to compete for these dollars. We have received three "top-ups" from NIH with stimulus package grants. The cartilage healing program grant with MIT, on which Dave Frisbie is PI on the subcontract, has received an additional infusion to increase the number of horses that we could evaluate; the K08 training grant of Dr. Laurie Goodrich received an additional grant of slightly more than $100,000 to do a dose titration for gene therapy; and a new grant involving stem cell therapy for cartilage healing headed up by Dr. Connie Chu at the University of Pittsburgh with Drs. Goodrich, McIlwraith, and Kisiday involved in an equine subcontract was also recently funded.

It has been a productive year in research. We have got some exciting results that will make differences and have been able to add good staff and graduate students to our program. We will continue to have challenges as it appears that the budget shortfall will be even greater for the fiscal year of July 1, 2010-June 30, 2011, but we will cope. Everyone has been very productive and, as always, I wish to express particular appreciation to our donors for supporting our work.

Best wishes,

Wayne McIlwraith
Director

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New Scientific Support

(continued from Page 1)

(increased fluid in the joint with OA) and parameters of inflammation in the synovial membrane were significantly reduced with PSGAG compared with controls. On examination of the articular cartilage there was significantly less fibrillation (early OA change) in the articular cartilage seen with HA treatment compared with controls, and there was a trend for a decrease in cartilage fibrillation with PSGAG. These results indicated that PSGAG and HA had beneficial disease-modifying effects and were both viable options for osteoarthritis.

Clinically, previous reports have suggested that HA might work better in acute synovitis and PSGAG in osteoarthritis, but the findings in the present study question this. It would seem that HA provides long-term disease-modifying effects to the cartilage and this fits with clinical experience in man, where it is often necessary to have to go six months follow-up to see any benefit with intra-articular HA. It also explains why a dramatic decrease in synovitis was not typically noted when HA was used alone and why many veterinarians use HA in combination with intra-articular corticosteroids to get symptomatic relief at the same time the HA is offering a long-term disease-modifying effect. On the other hand with PSGAG, it would appear to be more effective in reducing acute inflammation and this supports our experience with the beneficial effects of intra-articular Adequan™ after arthroscopic surgery when there is considerable cartilage loss and subchondral bone exposure, which leads to a persistent synovial inflammation. This study clarifies a number of issues and allows us to make better recommendations on specific use on these two important joint medications.

Gene Therapy Vectors to Reduce Joint Inflammation

Can we effectively reduce inflammation in the joint long term using gene therapy vectors? Adenoassociated viral vectors appear to be promising in the field of gene therapy. They gain entrance into cells very effectively, without causing any harmful effects on the cells, and produce proteins of interest for long periods of time. The long-term protein production is important in musculoskeletal repair since tissues such as cartilage and bone have extended healing times.

Dr. Laurie Goodrich has been testing several different types (serotypes) of these vectors to determine which gain entrance into the cells of joints the most effectively. The serotype determines how the body's immune system will recognize the vector. Figure 1, at right, reveals excellent production of green fluorescent protein (GFP) in synovial cells that line the joint capsule. Various doses (number of viral particles) also determine how many viral particles are needed to produce high amounts of protein. These results have guided us in developing a gene therapy program to elevate a protein called Interleukin Receptor Antagonist Protein (IRAP or IL1-RA). The equine protein was originally cloned by Dr. Rick Howard et al. at the Orthopaedic Research Center during his surgical residency.

The DNA that encodes for the protein was then put into an adenovirus vector AdIRAP and injected into the middle carpal joints of horses that had induced osteoarthritis in the chip fragment model. Dr. Dave Frisbie et al. were able to obtain elevated levels of the protein and decrease joint degeneration in the joints in which the AdIRAP therapy was used. Dr. Goodrich is currently working on developing an AAVIRAP vector (adenoassociated virus with the DNA sequence that encodes for IRAP) with the aim of producing high levels of this protein for long periods of time without causing any inflammation associated with the gene therapy vector.

Adenoassociated viral vectors appear to be promising in the field of gene therapy.

Investigators at the ORC are collaborating with Dr. Jude Samulski, Director of the Gene Therapy Center at the University of North Carolina and world renowned virologist, to develop the AAVIRAP vector. Initial results of vector development have been promising as we have been able to achieve extremely high levels of the protein in cell culture. Once the vector is fully developed and validated, Drs. Goodrich, Frisbie, and Wayne McIlwraith plan to test it in the osteochondral chip fragment model in the horse.

These efforts in vector development have been funded by a National Institutes of Health training grant in which Dr. Goodrich was principle investigator and Dr. McIlwraith and Dr. Samulski (UNC) are co-mentors. A paper on the development of these vectors entitled *Ex vivo serotype-specific transduction of equine joint tissue by self-complementary AAV vectors* is accepted and in press in the journal *Human Gene Therapy*. Authors are: Drs. Goodrich, Vivian Choi, Beth Duda Carbone, McIlwraith, and Samulski.

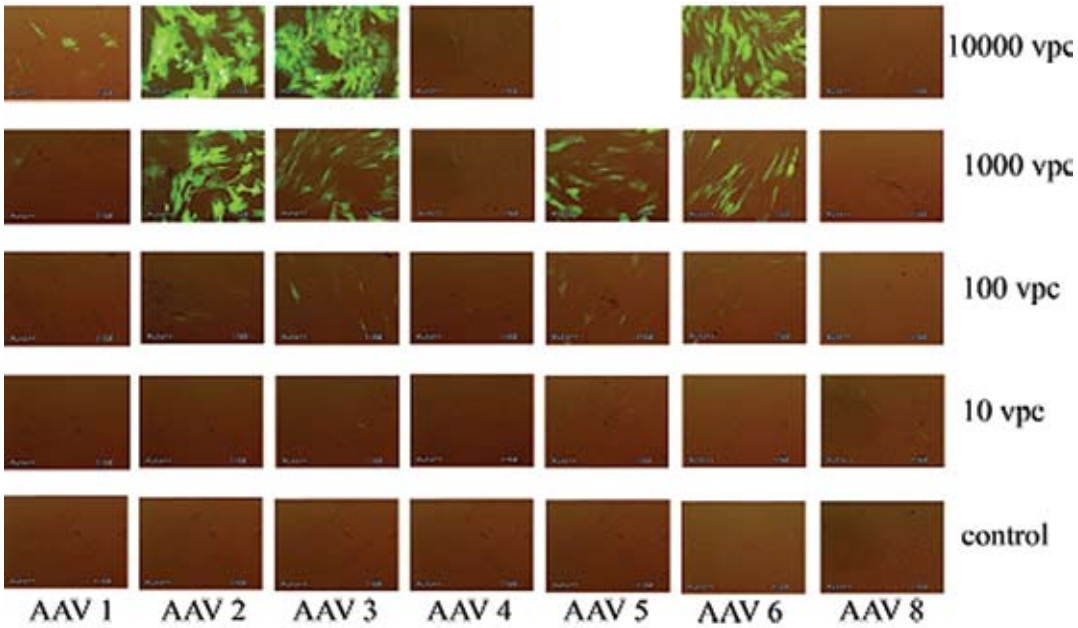

Figure 1. Initial screening of AAV vectors using green fluorescent protein (GFP) attached to the vector to indicate expression of the desired protein in synoviocytes in monolayer cell culture at day 7. scAAV2, 3, 5, and 6 had the greatest amount of GFP expression. Relative GFP expression provides an indication of the protein production of each vector when used in vivo. Viral dosage of 10,000 viral particles per cell had the highest percentage transduction. Bottom labels indicate serotypes and right column labels indicate viral particles per cell (vpc). Magnification 40X

Exploring MSC Therapies to Stimulate Healing

At the Orthopaedic Research Center, we continue to maintain a strong research focus in the use of bone marrow-derived mesenchymal stem cells (MSCs) for the treatment of orthopaedic tissues. As previously reported in the Fall 2008 issue of *Arthros*, our motivation for exploring MSC therapies is based on an extensive body of literature that has demonstrated that MSCs are capable of multilineage differentiation, which has resulted in widespread enthusiasm that the treatment of cartilage, bone, meniscus, tendon, and ligament may be possible. Given our access to clinical cases, we have administered intra-articular injections of MSCs for joint conditions that had not responded to conventional treatment modalities. Recent long-term follow-up on 40 cases revealed 72 percent returned to work, a result that has supported the continued investigation of this exciting new treatment modality.

We have been pleased to find that our present clinical strategy of minimally invasive, intra-articular injections of MSCs has stimulated healing in the majority of cases. However, given the novelty of this treatment modality, data that would define an optimal dose for a given condition has yet to be collected. In advance of such data, we hypothesize that certain conditions will require a high dose of adherent MSCs, and that techniques for concentrating MSCs at the damaged tissue will be necessary to induce healing.

The challenge of localized MSC delivery was recently explored by Benjamin Hale, a master's student in the department of Biomedical Engineering at CSU. For his project, Hale investigated the potential of fibrin, a hydrogel material widely used in medical and tissue engineering applications, as a delivery carrier for MSCs. Motivation for the use of fibrin hydrogels for MSC delivery was based on reports of MSC migration out of fibrin hydrogel into the pores of natural coral, and onto subcutaneous tissue in rats and human skin, over a period of weeks. With the goal of rapid delivery of MSCs within a period of hours, Hale designed his studies to determine whether the dilution of standard fibrin concentrations would result in a more open scaffold that would be conducive to MSC migration.

MSC migration was evaluated by casting MSC-seeded fibrin hydrogels onto tissue culture surfaces that support the attachment of outwardly migrating MSCs. After 24 hours, the MSCs that had escaped from the hydrogel and onto the tissue culture surface were collected and counted. Fibrin hydrogels were created using fibrinogen prepared from autologous plasma using a technique that creates a 20-fold concentration of fibrinogen, or from commercially-available fibrinogen solutions. Both preparations are used in equine medicine. MSC migration was quantified from these stock preparations (referred to as 100 percent), as well as from hydrogels that were created after diluting the fibrinogen concentration to 75 percent, 50 percent, and 25 percent of the stock preparations.

In autologous preparations, carrier-to-surface migration was observed for all hydrogels. Migration from 100 percent, 75 percent, and 50 percent hydrogels was not significantly different, while 25 percent hydrogels encouraged approximately a 4-fold increase in migration. In hydrogels created from commercial fibrinogen, few MSCs migrated from the undiluted condition. Sequential dilutions resulted in significant increases in migration, with migration in 25 percent approximately 5- and 2.5-fold higher than 75 percent and 50 percent hydrogels, respectively. In all cases, migrating MSCs displayed a linear, low-surface-area morphology that has been associated with a strong ability to undergo differentiation, thereby suggesting that the hydrogel delivery system did not change the therapeutic potential of the cells.

This work confirms our hypothesis that a carrier with reduced fibrin content is more conducive to MSC migration, and suggests that a diluted fibrin carrier will be best-suited to the application of rapid, localized delivery of MSCs. We consider this to be the first step toward a new method of cell transplantation for treating orthopaedic tissue defects that may require a high seeding density of MSCs to stimulate repair. Ongoing work will include the evaluation of dilute fibrin carriers in tissue explants and animal models to determine in situ attachment levels and the resulting therapeutic benefit.

Ben Hale examines fibrin hydrogels for MSC outgrowth.

Biomarkers and Prediction of Injury in Thoroughbreds

by Dave Frisbie, Orthopaedic Research Center, Colorado State University

The following manuscript will describe the history of serum biomarker analysis at the Orthopaedic Research Center at Colorado State University. Investigators at the ORC first started their research work in biomarkers in the early 1990s with a study that assessed clinical cases of osteochondral fragmentation in the knee compared to a controlled population of horses that were found to be free of lameness and osteochondral fragmentation. In this study, the retrospective use of serum biomarkers to discriminate these two populations is part of the design. Eight horses were used as control and 26 clinical cases were considered the injured population. Serum markers 846, CPII, and keratan sulfate, as well as the degree of arthroscopic articular cartilage damage, were recorded for each horse. The results of the study showed that serum levels of 846 and CPII were significantly elevated in horses that had osteochondral fragmentation. Serum CPII and 846 levels were also found to have a quadratic relationship to cartilage damage assessed arthroscopically. Finally, through the use of step-wise model selection it was found that serum CPII and 846 provided the best overall prediction of which group, injured or uninjured, the horse fell into. Using discriminate analysis, the overall error rate was 20.6 percent, which was felt to be an acceptable level for this pilot study.

From here the investigators undertook a randomized blinded experimental study where non-exercising horses underwent controlled exercise and then they either had a sham surgery or induction of OA. The goal of this study was to evaluate the synovial fluid and serum markers induced by exercise as well as the increases induced from experimental osteoarthritis. The hope of this study was that biomarkers that increased with exercise could be differentiated from those that increased with pathology (OA). Outcome parameters that were measured were glycosaminoglycan (GAG), 846, CPII, 2-3/4CEQ, C1- 2C, osteocalcin, and CTX1. The results of this study indicated that many of the biomarkers were significantly increased with exercise alone as well as a continued elevation with superimposition of experimental osteoarthritis that could be differentiated from the increase seen with exercise.

The main goal was to be able to assess the predictive value of serum biomarkers prior to an injury occurring.

Next, the investigators undertook a longitudinal clinical prospective study in racing Thoroughbred horses to determine the mean biomarker levels prior to and after injury. Levels were compared to those horses that were in the study and did not incur an injury. The main goal was to be able to assess the predictive value of serum biomarkers prior to an injury occurring. The design of this study included 238 Thoroughbred racehorses that began their 2- or 3-year-old race season. The exit criteria were defined as a horse that was out of training for more than 30 days or had completed 10 months of the study and had not sustained an injury. All horses had to complete at least two months in the study to be considered. The injury criteria that were analyzed were only horses with a solitary musculoskeletal injury. More specifically a musculoskeletal injury was defined as intra-articular fragmentation, tendinitis or ligamentous injury, stress fractures, or dorsal metacarpal disease. All horses entering the study had a monthly lameness examination as well as serum collected for analysis of the seven biomarkers as previously mentioned in the experimental OA study. The analyses that were performed looked at the mean values both at entry into the study as well as after injury and the longitudinal data throughout the study. A specific study to look at the prediction of injury using pre-injury longitudinal data was also undertaken. The results of this study yielded 59 injured horses and 71 uninjured control horses that met the previously defined criteria. Sixteen horses were diagnosed with a solitary intra-articular fragmentation, 17 tendinitis/ligamentous injuries, seven stress fractures, and 19 with dorsal metacarpal disease. The baseline marker levels, where uninjured or control horses were compared to the injured group, yielded a significant difference in only one biomarker. When endpoint samples, or post-injury and/or the final sample of control horses were compared, no significant differences were seen. When longitudinal samples were compared leading up to injury, significant changes were seen for all of the injury types. These changes were typically three to six months prior to the time of injury or exit from the study. Using these data, standard discriminate analysis was undertaken as well as logistic regression. These techniques were found to be only 50percent to 60 percent accurate in predicting which group, injured or uninjured, that the horse was in. This level of accuracy was felt by the investigators to be unacceptable and more sophisticated statistical methods were applied.

Kallie Meek, a master's graduate student in the Department of Statistics at CSU, was enlisted to help assess novel methods for analyzing these types of data. As part of her master's thesis, she was able to describe a local alignment kernel that improved the ability to analyze the data. This data was then analyzed using discriminate analysis, logistic regression, and support vector machines. The results of her work using the local alignment kernel, which is a methodology analogous to provide

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Mimicking Osteoarthritis and Test Treatments in a Dish

It is well documented that traumatic joint injury leads to the progression of osteoarthritis in equine athletes. OA is a painful and debilitating disease that reduces the animal's quality of life, and can have a significant economic impact on the performance industry by dramatically reducing the number of years a horse can perform. It is our goal to determine the early molecular responses of joint tissue to injury so that we can develop therapies to prevent or treat the progression of OA after injury. It is important to have a highly controlled and reproducible model of cartilage injury when investigating responses on a molecular level. Dr. Christina Lee, a postdoctoral fellow at the ORC working with Drs. Dave Frisbie and Wayne McIlwraith, is developing an *in vitro* model of injury-induced OA using adult equine tissue where mechanical load is applied to rapidly compress cartilage explants to 60 percent of the total thickness. This model of injurious load has demonstrated success at inducing histologic changes in cartilage that mimic injury-induced OA observed clinically. After compression and at least 28 days in culture, the explants display the hallmarks of OA, such as surface crack formation, chondrocyte cell death, and chondrocyte cluster formation. We have expanded this *in vitro* model by including additional joint tissues, such as synovial cells (synoviocytes), to more effectively mimic the joint environment. Synoviocyte responses to cartilage injury are of particular interest as these are the ideal target cells for applications of gene therapy. The data from our co-culture experiments indicate synoviocytes protect cartilage from the effects of injury. For example, in injured cartilage that was co-cultured with synoviocytes, there is a reduction in the size and number of chondrocyte clusters formed and the typical increases in chondrocyte gene expression observed with injury are subdued. We are currently using this co-culture model of cartilage injury to investigate a detailed list of genes for expression in both synoviocytes and injured cartilage. With this *in vitro* work, we aim to develop and begin screening molecular-based therapies, including gene therapy, to alter the progression of OA in response to injury and avoid the use of testing in live horses until we have defined what the best options are.

Effects of Joint Surface Geometry on Joint Disease

Catastrophic injury continues to plague horse racing and has stimulated congressional oversight of the industry. Musculoskeletal injuries are the most common reason for euthanasia in racehorses. In the United Kingdom, distal limb fractures of the lateral condyle of the third metacarpal bone are the most common, and in the United States, only sesamoid fractures are more frequent than condylar fractures (Figure 1). Pathologic studies have shown that condylar fractures can be chronic in nature due to chronic repetitive loading and inappropriate bone remodeling. Consequently, methods to diagnose fatigue fractures prior to a complete catastrophic event are necessary. However, most imaging techniques today fail to identify preclinical injuries.

One form of imaging analysis that may be useful is that which assesses the geometrical properties of the joint. In human studies, small geometric changes in the knees, such as lateral condyles with distal and posterior flattening, have been correlated directly with osteoarthritis. Based on this information, the goal of this study was to determine differences in geometric properties between fractured and non-fractured joints of the same horse and compare those results to horses that were euthanized for non-musculoskeletal problems. The hypothesis was that horses that had sustained condylar fractures will have significant changes in the lateral-to-medial dimensions and curvature of the third metacarpal condyles compared to horses that did not fracture. This project is a collaborative venture between Drs. Tim Parkin and Kenton Morgan in the UK with Drs. Kawcak, Puttlitz, and McIlwraith at CSU and is part of a research project for Katrina Easton, who is a D.V.M./Ph.D. student at the ORC.

Computed tomographic scans were obtained from a large epidemiologic study in the United Kingdom. A total of 192 condyles underwent computed tomographic imaging. Fifty-one condyles were from the fractured limb of the horse (FX), 61 condyles were from the contralateral non-fractured limb from the fractured horse (NFX), and 80 condyles were from horses that were euthanized for non-musculoskeletal reasons (CTL). A custom-designed software package was used to reconstruct the condyle images into a three-dimensional model.

The lateral-to-medial width ratio was significantly different between FX and CTL condyles in almost all locations (Figure 2). In particular, the magnitude of difference was greatest in the palmar aspect of the joint. The lateral-to-medial ratio was significantly lower in NFX condyles compared to CTL condyles in two of the dorsal sites only and there was significantly smaller lateral-to-medial ratio in the FX condyles compared to the NFX condyles over the entire palmar aspect of the condyles. Curvature in fractured cases was significantly higher in the palmar lateral parasagittal groove compared to NFX and CTL samples. For surface area ratio, the ratio of lateral

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Can We Use Gene Therapy to Turn Bone Marrow-Derived Mesenchymal Stem Cells into Bone to Help Fracture Repair?

Gene therapy vectors were utilized in a project studying the effects of genetic modification of bone marrow-derived mesenchymal stem cells. Adenoviral vectors were used to deliver DNA to cells in an attempt to cause an "overproduction" of two different proteins, Bone Morphogenetic Protein 2 and 7 (BMP 2 and 7). Each protein is believed to be important in healing bone by causing these cells to turn from cells that are young, immature components of bone to cells that become part of the bone callus. Many studies have found that the addition of these proteins can augment bone healing; however, the proteins have a short half-life and the benefits of adding the proteins themselves are short-lived. In addition, supplementing the fracture bed with these proteins is expensive and cost-prohibitive in the horse.

Gene therapy is a technique by which viral vectors (segments of viruses that cannot replicate but can gain entrance into cells) are utilized to deliver DNA to cells to initiate the cells to produce the protein of interest. Further, it is thought that the combination of BMP-2 and BMP-7 has better healing potential than either protein alone. A study was performed to determine (1) if elevated levels of protein could be produced by genetic modification of bone marrow-derived mesenchymal stem cells (BMDMSCs) in culture, (2) if increasing these important proteins through gene therapy would have an effect on osteogenesis (the ability of these cells to turn into bone), and (3) if the combination of these gene therapy vectors (AdBMP2 and AdBMP7) was better than either vector alone in causing osteogenesis.

These effects were studied in both equine and human BMDMSCs. Results of the project revealed that extremely high levels of BMP 2 and 7 can be achieved when these cells are genetically modified. Furthermore, when the cells are genetically engineered to overproduce this protein, they produce elevated levels of alkaline phosphatase (Alk Phos), a marker for bone production (Figure 1). Our results revealed that the combination of genes did not have greater "bone-enhancing" properties than either alone and that cells modified with AdBMP2 actually had the greatest potential of turning to bone over cells that were modified with AdBMP7 or both vectors together. Both the horse and the human cells had augmented bone characteristics using this gene therapeutic approach. The addition of dexamethasone to the media (fluid supplementing the cells in culture) further seemed to augment this effect, especially in the equine cells.

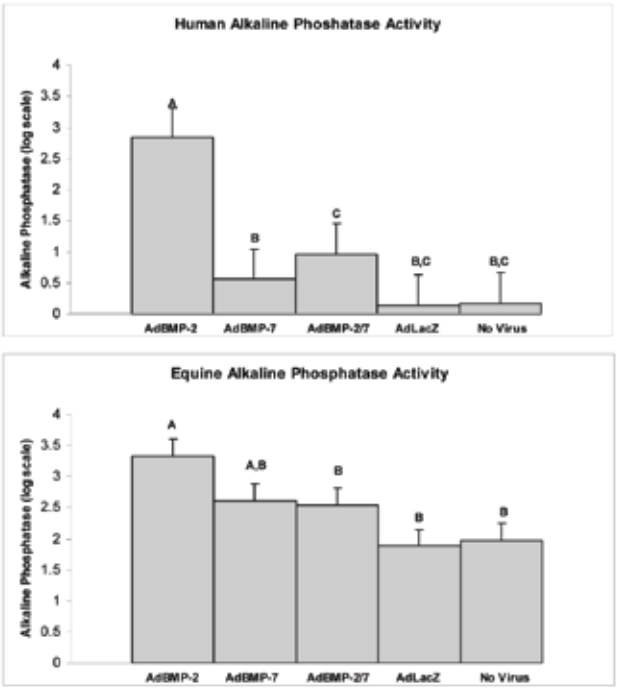

Figure 1. Alkaline phosphatase activity for genetically modified human (top) and equine (bottom) BMDMSCs for each of the five different treatment groups. AdBMP-2 elicits the greatest effect on ALP production. Different letters indicate significant ($P<0.05$) differences among groups.

The results of this study have been submitted to the *Journal of Orthopedic Research*. The authors are: Dr. Ryan Carpenter, Dr. Laurie Goodrich, Dr. Dave Frisbie, Dr. John Kisiday, Dr. Wayne McIlwraith, Dr. Chris Centeno (Centeno-Schultz Clinic, Denver, Colo.), Dr. Chisa Hidaka (Hospital for Special Surgery, New York, N.Y.), and Beth Carbone. Dr. Goodrich is currently developing gene therapy vectors using adenoassociated viruses to deliver BMPs to determine if these vectors will further enhance bone production. The ultimate goal is to test these vectors in an equine model of fracture healing for clinical applications in the horse. This study was funded by the College Research Council grant in which Dr. Goodrich was principle investigator and Dr. Carpenter (surgery resident) and collaborator Dr. Hidaka were co-investigators. The research was also presented at the American College of Veterinary Surgeons meeting and the Veterinary Orthopedic Society meeting in 2008 by Dr. Carpenter. Dr. Carpenter received the resident award for best research presentation at VOS and second-place award for best research presentation at ACVS for this paper. Dr. Carpenter completed his surgical residency in July 2009.

New Graduate Students

Kaydence Cowley, B.S., M.S.

Kaydence Cowley joined the Orthopaedic Research Center in May 2009 as a Ph.D. student under Dr. Dave Frisbie and Dr. John Kisiday. Her dissertation project is to develop a clinically relevant *in vitro* model of tendon injury utilizing tissue explants in order to understand the biological mechanism of healing and repair. Cowley completed an undergraduate degree in mechanical engineering from Lafayette College in Easton, Pa., and a master's degree in bioengineering from the University of California Riverside. She did previous work in orthopaedic repair and injury at the Colorado Health Science Center and has presented work at the Orthopaedic Research Society, Biomedical Engineering Society, and Biophysical Society Conference. She is very excited to once again be living in her native state, and is looking forward to taking advantage of the seasons, the mountains, and the amazing research facilities Fort Collins has to offer.

Daniel Hemphill, B.S.

Daniel Hemphill graduated with a B.S. in chemical engineering in 2008 from CSU and started his Ph.D. He decided to work with Dr. Laurie Goodrich doing gene therapy research after completing lab rotations through the school of biomedical engineering. In his free time Hemphill is an avid mogul skier and enjoys trail running and the occasional bouldering or rock climbing. He ran on the CSU cross country and track teams for three years as an undergraduate. Hemphill enjoys anything that gives him an excuse to spend time outdoors.

Valerie Moorman, D.V.M.

Valerie Moorman is originally from Durham, N.C. and graduated from North Carolina State University in 2004 with a D.V.M. She completed a large-animal medicine and surgery internship at Auburn University in 2004-2005, and then stayed on 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 on a research project using 2-D kinematic analysis. In 2006, she began an equine surgical residency and combined master's degree at Oklahoma State University, which she completed in 2009. In July 2009, she accepted a position at Colorado State University as an after-hours large-animal emergency clinician and Ph.D. student at the Orthopaedic Research Center. She is interested in equine sports medicine and surgery, as well as lameness and imaging. In her free time, Moorman enjoys sailing, hiking, and riding hunter-jumpers.

Storm Cat Career Development Award

Drs. McIlwraith, Kawcak, and Haussler present Dr. King with the Storm Cat Career Development Award on behalf of Grayson-Jockey Club Research Foundation.

Dr. Melissa King was awarded the Storm Cat Career Development Award by the Grayson-Jockey Club Research Foundation for 2009. Dr. King is working with Wayne McIlwraith, Chris Kawcak, and Kevin Haussler on a study of the role of underwater treadmill exercise in diminishing the development of osteoarthritis.

The Storm Cat Research Career Advancement Award Program from the Grayson-Jockey Club Research Foundation is a competitive program intended to promote development of promising investigators by providing a one-year salary supplement of $15,000. This program is restricted to one award per year and is known as the Storm Cat Career Development Award, named in honor of the famous Thoroughbred stallion at Overbrook Farm in Lexington, Ky.

Dr. King graduated from Colorado State Veterinary School in 1997. After graduating she did a one-year internship at Rood & Riddle Equine Hospital in Lexington, Ky. Upon completion of her internship, Dr. King returned to northern Colorado to begin her career as an equine ambulatory clinician focusing on equine lameness. After practicing for 10 years, Dr. King sold her practice to pursue a Ph.D.

Colorado State Bestows Highest Honor of University Distinguished Professor on Three Professors

Dr. Wayne McIlwraith

Adapted from CSU Press Release dated April 29, 2009

FORT COLLINS – Colorado State University President Tony Frank presented the title of University Distinguished Professor – the highest recognition awarded for outstanding accomplishments in research and scholarship – on three professors at the annual "Celebrate Colorado State" event.

Collectively, these world-renowned professors have made great strides in diverse fields of science, but share a common goal to improve global health and environment issues. They have garnered more than $100 million in research grants, taught hundreds of students who now make contributions around the world, and have influenced scientific thought in their field. They are:

Dr. Wayne McIlwraith, professor of Veterinary Medicine and director of Colorado State University's Gail E. Holmes Equine Orthopaedic Research Center;

Ian Orme, professor and researcher of Microbiology, Immunology and Pathology and co-founder of the Colorado State University's Mycobacteria Research Laboratory; and Diana Wall, professor of Biology and director of Colorado State University's School of Global Environmental Sustainability.

"The designation of University Distinguished Professor is the highest honor Colorado State University bestows to faculty," said CSU President Frank. "Professors Wall, McIlwraith, and Orme have all led pioneering research that has transformed their fields of study, and all three are known around the world as among the most distinctive, innovative, and accomplished thinkers in their disciplines. Each has contributed significantly to the quality of research and education at Colorado State, and this honor is a fitting tribute to the stature they've attained as scholars and faculty leaders." Only 1 percent of CSU faculty are honored with the rank of University Distinguished Professor and it is a lifetime award which carries into retirement as an Emeritus Professor. To obtain the rank, faculty members are nominated through an extensive review process and must be approved by the current University Distinguished Professors.

Current Colorado State University Distinguished Professors include Barry Beaty, Patrick Brennan, and Edward Hoover, Department of Microbiology, Immunology and Pathology; George Seidel, Department of Biomedical Sciences; Bernard E. Rollin, Department of Philosophy; Robert Williams, Department of Chemistry; Graeme Stephens and Thomas Vonder Haar, Department of Atmospheric Science; Gary Smith, Department of Animal Sciences; Stephen Withrow, Department of Clinical Sciences; Jan Leach, Department of Bioagricultural Sciences and Pest Management; Karolin Luger, Department of Biochemistry and Molecular Biology; Jorge Rocca, Department of Electrical and Computer Engineering; and John Sofos, Department of Animal Sciences.

"Drs. McIlwraith, Orme, and Wall are each outstanding members of our faculty and tremendous researchers," said CSU Interim Provost Rick Miranda. "It is an honor to name each of them as a University Distinguished Professor, and to have them as key leaders of our University, serving as an inspiration to other faculty and to our students."

Dr. McIlwraith is director of Colorado State University's Gail E. Holmes Equine Orthopaedic Research Center. The center, which is part of the College of Veterinary Medicine and Biomedical Sciences, treats orthopaedic injuries of the world's finest horses and investigates orthopaedic treatments and preventive medicine. Many of the innovations at the Equine Orthopaedic Research Center also can be applied to human medicine.

Dr. McIlwraith joined Colorado State in 1979 in the Department of Clinical Sciences in the College of Veterinary Medicine and Biomedical Sciences as an equine surgeon with a research focus in orthopaedics. Milestones include his appointment as director of the university's equine sciences program in 1994, which accompanied a major expansion of the orthopaedic research program. In 2001, he became the director of the orthopaedic research program. The program has helped make the university the world's leading center for comparative orthopaedic research.

Dr. McIlwraith's history of accomplishment as a researcher, clinician, and educator can be seen in his leadership and development of numerous programs at Colorado State including the musculoskeletal research program, a Program of Research and Scholarly Excellence.

Dr. McIlwraith, who was born in New Zealand, also is a Diplomate, American College of Veterinary Surgeons, and Diplomate, European College of Veterinary Surgeons.

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Biomarkers and Prediction of Injury *(continued from Page 5)*

alignment of disparate DNA sequences, also allows the degree of alignment to be quantified. Specifically, the number of months each horse was in the study and the specific results of each biomarker per month were analyzed for each horse. There were 130 horses; every horse was then compared to every other horse to create a 130 X 130 matrix of comparisons. The results showed that the alignment scores were significantly higher for uninjured horses when compared to other uninjured horses, while uninjured horses compared to injured horses had lower alignment scores. Using the local alignment kernel, discriminate analysis was now repeated on the data and found to be 73.1 percent accurate with an average apparent error rate (APER) of 26.9 percent. Logistic regression had a 72.9 percent accuracy rate with a 27.1 percent APER. Finally, support vector machines had the best accuracy at 73.9 percent and lowest APER at 26.2 percent.

The conclusion of this statistical exercise showed that a local alignment kernel was a very useful tool in dealing with this type of data. Discriminate analysis and logistic regression require step-wise selection of variables that are significant to model prediction and are very sensitive to multidimensional comparisons. Therefore, they do not yield themselves to this type of data analysis when large numbers are present. Support data machines provided the best accuracy in this data set and the lowest error rate, with no multidimensional sensitivity, and thus appeared to be the best method for prediction models.

With this information in hand, the investigators launched another longitudinal clinical prospective musculoskeletal disease study, this time using a population of reining horses. This population was selected, because unlike the Thoroughbreds, a varied exercise protocol would be implemented by different trainers.

Although similar to the Thoroughbred racehorses they would have set target or show dates when the horses would be performing and thus defined endpoints that could be used for monitoring. In this particular study, the addition of molecular markers was undertaken based on previous work at the ORC. The design of this study and the preliminary outcome will be presented in another manuscript.

In conclusion, work completed thus far at the ORC has shown promising results for serum biomarker levels and their ability to predict injury prior to its occurrence. This work has been advanced by more sophisticated statistical modeling that takes into consideration specific issues and challenges that are encountered with this type of data. The commercial application of these data is currently being undertaken.

ORTHOPAEDIC RESEARCH CENTER

Arthros

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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.

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Joint Surface Geometry *(continued from page 6)*

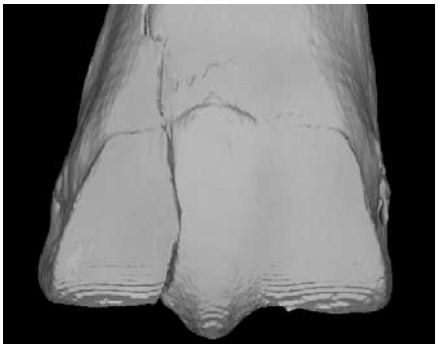

Figure 1. A CT image of the distal cannon bone within the fetlock of a horse that suffered from a condylar fracture.

to medial surface area was significantly lower in FX condyles compared to NFX and CTL.

The results of this study show that condylar width varies significantly between fractured condyles and non-fractured condyles. In particular, the lateral condylar width is significantly smaller in fractured condyles compared to non-fractured and control condyles in the palmar aspect of the joint. This difference in geometry may lead to excessive stress and fatigue in the lateral condyle of fractured horses. In addition, the ratio of curvature was higher, particularly in the parasagittal groove of the lateral condyle. This indicates that the condyle was rounder in FX horses compared to NFX and CTL horses. The areas surrounding the parasagittal groove had significantly less curvature in the palmar aspect compared to the dorsal aspect of the condyle in fractured cases compared to controls. Therefore this may possibly increase the stress that may lead to fracture. The difference in surface area measurement leads to the conclusion that the lateral condyles are relatively smaller in fracture cases compared to controls and non-fractured cases.

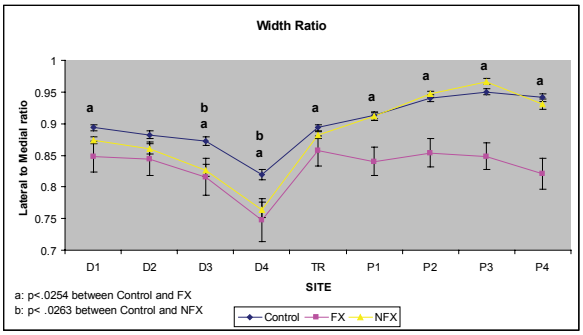

Figure 2. Means and standard errors for lateral to medial ratio in the third metacarpal condyle of CTL, FX, and NFX condyles. D1 – D4 represents measurements taken in the dorsal aspect of the condyle, TR represents the transverse ridge, and P1 – P4 represents measurements made in the palmar aspect of the condyle.

In the future, these data will be placed into a finite element model that is currently being developed in order to demonstrate the amount of stress that the joint is truly undergoing. There is a concern that some of these geometrical abnormalities may be developmental in nature and future efforts will be made to determine the influence of limb conformation and shoeing on condylar geometry. The goal of this future work will be to identify factors that may lead to condylar fractures.