



# ORTHOPAEDIC RESEARCH CENTER

*including the  
Orthopaedic  
Bioengineering  
Research Laboratory*

**2010-2011  
Report**



**Colorado State University**

## Preface

It is my pleasure to present our 2010-2011 report from the Orthopaedic Research Center and the Orthopaedic Bioengineering Research Laboratory at Colorado State University. Our principal focus continues to be solving the significant problems in equine musculoskeletal disease as can be seen in this report but we also continue to investigate questions relevant to human joint disease and techniques and devices for human osteoarthritis and articular cartilage repair when the technique can also benefit the horse. There have been a number of notable projects in this regard. The studies, led by Dr. Dave Frisbie at the ORC in partnership with Dr. Alan Grodzinsky at MIT on an NIH Program Grant in cartilage repair, have been completed and results are still be analyzed. We have three other ongoing NIH grants in partnership with collaborators. One is with Dr. Connie Chu at the University of Pittsburgh looking at stem cells in fibrin/PRP and articular cartilage repair (Dr. Laurie Goodrich is the PI on the sub-contract at CSU and other collaborators include Dr. Lisa Fortier of Cornell University and Dr. Bob Sah of the University of California, San Diego). This was a 12-month study that has been completed and results are still being analyzed with the other collaborators. The work with Dr. Jude Samulski at the University of North Carolina on Dr. Laurie Goodrich's NIH KO8 grant on gene therapy (co-mentored by Drs. Samulski and McIlwraith) is progressing well and we have some exciting results with regard to long term protein production with the AAV vector. The third NIH grant is with Dr. Steve Trippel at the University of Indiana in which Drs. Frisbie and McIlwraith are co-PIs on a sub-contract of an NIH grant involving gene therapy and articular cartilage repair and the equine study will commence in the summer of 2012.

There have been some significant publications from the horse-human collaborative projects in leading journals in the last two years. At the same time, many projects addressing equine specific problems have been completed and published and all of this is detailed in this report. We handled the challenges created by the economic recession well in the 2010-2011 period. This would not have

been possible without a number of our donors stepping up to provide supplemental funding. I'm particularly grateful to Mr. Jim Kennedy for providing supplemental operating funds and continuing the legacy of his mother, Barbara Cox Anthony. I'm also grateful to Abigail Kawanakoa for continued support over and above the Endowed Chair she donated three years ago; and Herbert Allen for continuing to provide considerable support for investigation of cutting edge therapies.

We have added an Equine Sports Medicine Ambulatory clinical arm to the Orthopaedic Research Center and also initiated two residencies in Sports Medicine and Rehabilitation. This follows on from the accreditation of a new specialty college, the American College of Veterinary Sports Medicine and Rehabilitation. Drs. Haussler, Frisbie and Kawcak, as well as myself, are Foundation Diplomates and felt we had the appropriate ability and responsibility to train residents in this new specialty. Dr. Dora Ferris is in her second year and Dr. Erin Contino is in her first year. I would like to extend special thanks to Gail Holmes for providing funding for the three year program for Erin Contino.

Accomplishments at the ORC over the past two years are detailed in this report. Validation and clarification of the benefits of mesenchymal stem therapy, platelet-rich plasma (PRP), as well as autologous conditioned serum (IRAP II) are all in this report. These accomplishments could not be achieved without our team of faculty and staff as well as the excellent support of equine funding agencies (Grayson-Jockey Club Research Foundation, American Quarter Horse Association, and United States Equestrian Federation), corporate funding, and individual donors. With this help we continue to achieve our goals and also make new ones as new clinical questions arise.

Best wishes,



Wayne McIlwraith

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## Mission

**To investigate the pathogenesis, diagnosis, treatment, and prevention of musculoskeletal disease and injury for the betterment of both animals and humans.**





## **Research Focuses of the Orthopaedic Research Center**

### **Musculoskeletal Tissue Healing**

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Until now, we have principally addressed articular cartilage healing and will continue to do so, but we have enlarged the focus to include tendons, ligaments, and menisci.

### **Early Diagnosis of Bone and Joint Disease**

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This includes the development of novel imaging techniques (present and future), body fluid markers, and also molecular monitoring. The uses of these early diagnostic techniques include:

- a. evaluation of the pathogenesis of bone and joint disease
- b. early detection of disease processes
- c. monitoring of therapy, with the long-term goal of preventing severe arthritis or failure

### **Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease**

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These investigations use both molecular tools such as reverse transcriptase PCR for evaluation of tissues in various stages of the disease, biomechanical and modeling studies, and imaging techniques including magnetic resonance imaging (MRI) and computed tomography (CT) to monitor early events in bone disease.

### **Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse**

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These include evaluation of biologic inhibitors of critical mediators in joint disease, novel protein therapies including PRP, gene therapy techniques, and mesenchymal stem cell therapies.

### **Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease**

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These include objective assessment of integrative therapies including manipulation and acupuncture for management of musculoskeletal disease and pain, as well as rehabilitative techniques of swimming, underwater treadmilling, and hyperbaric therapy.



## **Research Focuses at the Orthopaedic Bioengineering Research Laboratory**

The Orthopaedic Bioengineering Research Laboratory (OBRL) is an interdisciplinary research and educational effort bringing together engineers, clinicians, biologists, and scientists all over campus. The goal of the laboratory is to provide an environment for undergraduate and graduate education in Biomedical Engineering while advancing treatment and/or prevention of muscular, neuromuscular, or skeletal injury and/or disease. The primary research focuses include:

### **Computational Simulation of Orthopaedic Conditions and Treatments**

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- a. Finite element analysis
- b. Cadaver and animal experiments to validate and augment the computational models

### **Biomaterials Development**

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- a. Enhancing wear resistance of polymeric orthopaedic implant bearing materials
- b. Biopolymer derivative synthesis and characterization
- c. Bioactive and osteoinductive bone graft materials

### **Engineering and Growth Factor Therapy for Cartilage and Bone Repair**

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- a. *In vitro* cell culture assessment
- b. Animal models to evaluate repair
- c. *In vitro* micro-assessment of mechanics of regenerated and normal tissue
- d. Development and assessment of biomaterial carriers

### **Retrieval Analysis for Failure Assessment, Design Improvement, and Tissue Interface**

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- a. Orthopaedic implants
- b. Allograft bone composites
- c. Synthetic bone graft materials

### **Biocompatibility and Biomaterial/Tissue Interface**

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- a. Interface biomechanics
- b. Tissue response to biomaterials

### **Comparative Orthopaedics and Animal Models**

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- a. Animal model development and validation
- b. Comparison of human and other animal disease mechanisms and treatment efficacy



## **Research Focuses at the Orthopaedic Bioengineering Research Laboratory**

*Continued*

### **Biomechanical Analysis**

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Equipment available include; minibionix MTS machine, standard MTS, spine tester, biaxial tester

- a. Range of motion/kinematics
- b. Materials testing for shear strength
- c. Tension and compression analysis

### **Hard Tissue Structural Analysis**

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- a. MicroComputedTomography ( $\mu$ CT) - High resolution imaging of bone to determine bone volume and morphology.
- b. Non-decalcified hard tissue histology
- c. Histomorphometric analysis

## Musculoskeletal Research Program

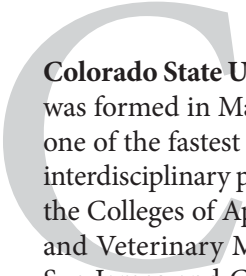
The **Musculoskeletal Research Program** has been designated as a Program of Research and Scholarly Excellence at Colorado State University (initially designated in 2004 and renewed in 2008).

The Musculoskeletal Research Program covers all orthopaedic research at Colorado State University and includes:

1. Orthopaedic Research Center
2. Orthopaedic Bioengineering Research Laboratory
3. Small Ruminant Orthopaedic Research Laboratory
4. Orthopaedic Oncology



## School of Biomedical Engineering



**Colorado State University's School of Biomedical Engineering (SBME)** was formed in March 2007 to address society's needs in bioengineering, one of the fastest emerging areas of scientific discovery. The SBME is an interdisciplinary program built on strong faculty and research programs in the Colleges of Applied Human Sciences, Engineering, Natural Sciences, and Veterinary Medicine and Biomedical Sciences. In particular, Drs. Sue James and Christian Puttlitz of the Orthopaedic Bioengineering Research Laboratory are co-coordinators of the program, and Drs. Wayne McIlwraith, Chris Kawcak, David Frisbie, Kevin Haussler, Laurie Goodrich, and John Kisiday of the Orthopaedic Research Center are core faculty members of the program in biomedical engineering research, which is rapidly expanding to all areas of human health. New technologies being developed at CSU are enabling people to continue active and healthy lifestyles. SBME students have the opportunity to collaborate with faculty from these four colleges and eleven departments, including the highly ranked Professional Veterinary Medicine program.

SBME now offers Master of Engineering, Master of Science, and Ph.D. degrees. The M.S. and Ph.D. programs focus on three main research areas: biomechanics and biomaterials; molecular, cellular, and tissue engineering; and medical diagnostics, devices, and imaging. Within these three areas, students participate in cutting-edge research from therapies and imaging modalities for fighting cancer to improving equipment used in open heart surgery. In order to allow flexibility to explore the multiple research possibilities, fully funded (stipend and tuition) lab rotation fellowships are available for first-year Ph.D. students.



**C. Wayne McIlwraith**, B.V.Sc. (Dist.), M.S., Ph.D., D.Sc. (Purdue), Dr. med. vet. (hc) (Vienna), D.Sc. (hc) (Massey), L.Dr. (Turin), Dvetmed (hc)(London), FRCVS, Diplomate ACVS, Diplomate ECVS, University Distinguished Professor, Director of the Orthopaedic Research Center, Barbara Cox Anthony University Chair in Orthopaedics; Department of Clinical Sciences

*Research Interests:* Equine orthopaedic surgery and joint disease (arthritis), biomarkers and cartilage repair research.

Dr. McIlwraith has been Director of the ORC since its inception, advancing the Orthopaedic Research Center's reputation through research and publications, scientific presentations at key meetings throughout the world, and also through his fundraising efforts. He is a Past-President of the American College of Veterinary Surgeons, the American Association of Equine Practitioners, and the Veterinary Orthopedic Society, and a recognized leader in the field of equine orthopaedic research and surgery. He consults worldwide as a specialist equine surgeon and has received national and international honors for his contributions to joint research and clinical orthopaedics. Dr. McIlwraith is the author of four textbooks: *Techniques in Large Animal Surgery* (two editions), *Equine Surgery: Advanced Techniques* (two editions), *Arthroscopic Surgery in the Horse* (three editions) and *Joint Disease in the Horse*. He has authored or co-authored over 300 refereed publications and textbook chapters, and has presented more than 500 seminars both nationally and internationally to equine practitioners, veterinary specialty meetings, and human orthopaedic meetings.

*Honors include:* Colorado State University AAEP Faculty Award for Excellence in Teaching Equine Medicine and Surgery, 1981-82; Colorado State University Alumni Outstanding Faculty Award, 1983; DLT Smith Visiting Scientist, University of Saskatchewan, 1992; Inducted into the George H. Glover Gallery of Distinguished Faculty and Alumni, CSU, 1993; Awarded the Tierklinik Hochmoor Prize at Equitana, 10th Equine Veterinary Conference, Essen, Germany, 1993, for international contributions to Equine Orthopaedics; the Schering-Plough Award from World Equine Veterinary Association for Equine Applied Research for outstanding research work in equine locomotor disorders in Yokohama, Japan, 1995; Jacques Jenny Lecturer, Veterinary Orthopaedic Society, 1997; John Hickman Award for Equine Orthopaedics for leading work in arthroscopic surgery and equine joint disease research, British Equine Veterinary Association and Equine Veterinary Journal, Harrogate, England, 1997; Dr. med. vet. (honoris causa), University of Vienna, 1995; D.Sc., Purdue University, 2001; D.Sc. (hc), Massey University, 2003, Laurea Dr. (hc), Turin University 2004; Inducted into UK Equine Research Hall of Fame 2005; Frank Milne Lecturer (Lifetime Contribution Award), AAEP 2005; Founders Award for Lifetime Achievement, ACVS, 2006; Elastikon Equine Research Award, Johnson & Johnson and Grayson-Jockey Club Research Foundation, 2008-2009; Colorado State University Scholarship Impact Award 2007, University Distinguished Professor, Colorado State University 2009; Distinguished Life Member, AAEP, 2009; CSU Graduate School Ceremony, Grand Marshall, 2010; Dr. med. vet. (honoris causa), Royal Veterinary College, University of London, 2010; Life Member, New Zealand Equine Veterinary Association, 2011.



## Faculty

College of Veterinary Medicine and Biomedical Sciences



**Gary M. Baxter, VMD, M.S., Diplomate ACVS, Professor, Assistant Department Head, Department of Clinical Sciences**

*Research Interests:* Initial research focused on the cause and treatment of equine laminitis.

Dr. Baxter has most recently been involved with research evaluating the use of corticosteroids to treat horses with joint disease, the value of oral nutraceuticals as a preventative for osteoarthritis, and the use of the diode laser for surgical arthrodesis of the distal hock joints in horses with osteoarthritis (bone spavin). He has recently obtained funding to evaluate the efficacy of urinary bladder matrix (UBM; ACell) in a model of superficial digital flexor tendonitis in young horses.

Dr. Baxter has a national reputation as an equine surgeon and is actively involved in the American College of Veterinary Surgeons and American Association of Equine Practitioners. He was chairman of the 2001/2002 ACVS examination committee and was on the ACVS Board of Regents from 2003-2005. He has spoken many times at the American Association of Equine Practitioners annual meeting and is currently chairman of the equine lameness wet lab that is given every year. Dr. Baxter came to CSU as an Assistant Professor in Clinical Sciences in 1990, became an Associate Professor in 1994 and a Full Professor in 2000. He is currently an equine clinician and surgeon at the Veterinary Teaching Hospital Large Animal Chief of Staff and Equine Section Chief as well as Assistant Department Head in the department of Clinical Sciences overseeing the veterinary residency and graduate program. He has been actively involved in research since coming to CSU and has authored or co-authored nearly 100 scientific publications, review articles and book chapters. He is certified in Medical Acupuncture for Veterinarians.

*Honors include:* Outstanding Research Publication in "Veterinary Surgery," 1989. Senior author of manuscript that received "Outstanding publication in Equine Veterinary Journal for 1992."



**Nicole Ehrhart, D.V.M., M.S.,** Diplomate ACVS, Professor, Department of Clinical Sciences

*Research Interests:* Guided Bone Regeneration, Allograft Healing, Distraction Osteogenesis, Limb Preservation, Bone Substitutes.

Dr. Ehrhart is one of 20 fellowship-trained veterinary surgical oncologists in the world. She is an Associate Professor in surgical oncology at the highly acclaimed Animal Cancer Center and has been a member of the CSU faculty since 2002. She is the director of the Musculoskeletal Oncology Lab and has been actively involved in limb preservation research and sarcoma research for the last twelve years. She has been an invited speaker at various venues for MD researchers in translation medicine, both nationally and internationally. In addition to her research, she has held several prestigious positions in the American College of Veterinary Surgeons (Scientific Program Chair, Residents Forum Chair, Examination Committee) and Veterinary Orthopedic Society (Scientific Program Chair). She has authored numerous publications on limb preservation and translational cancer research. She is currently the co-director of the Musculoskeletal Oncology section of the University-wide Cancer Supercluster.

*Honors include:* Bloomberg International Sports Medicine Lecturer, 2003; International Musculoskeletal Transplant Foundation Speaker, 2007; President, Veterinary Society of surgical Oncology, 2008-2010.

## Faculty

College of Veterinary Medicine and Biomedical Sciences

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**David D. Frisbie, D.V.M., M.S., Ph.D.,** Diplomate ACVS, Associate Professor, Department of Clinical Sciences

*Research Interests:* Gene therapy, intra-articular therapeutics, new methods of cartilage repair.

Dr. Frisbie began his professional career after obtaining both a Bachelor's Degree in Biochemistry and a Doctor of Veterinary Medicine (D.V.M.) from the University of Wisconsin. He then went to New York, where he completed a Surgical Internship at Cornell University and began his research in joint disease. After completing his internship, Dr. Frisbie came to Colorado State University, where he continued his joint research, completed a Surgical Residency in Large Animal Surgery, and obtained a Master's Degree in Joint Pathobiology. After completion of his residency, Dr. Frisbie began his work on a novel way to treat joint disease using gene therapy, which was the focus of his Ph.D. During work on his Ph.D., Dr. Frisbie became Board certified in Large Animal Surgery and is a Diplomate of the American College of Veterinary Surgeons. He joined the faculty as an Assistant Professor in 1999 and was promoted to Associate Professor (with tenure) in 2007.

His current joint disease research is in two basic fields: 1) the evaluation of intra-articular therapeutics and their effects on joint disease (well known therapeutics he has evaluated include Legend, Adequan, Vetalog and Depo-Medrol, Orthokine (IRAP), stem-cells); 2) new methods of cartilage repair. These methods include cutting-edge technology aimed at arthroscopic repair of cartilage in the athletic horse. Dr. Frisbie is also exploring methods to augment fracture healing using gene transfer.

*Honors include:* Pfizer Animal Health Award for Research Excellence, 2001; American Association Equine Practitioners Presidential Award, 2011.



**Laurie Goodrich, D.V.M., M.S., Ph.D.,** Associate Professor, Department of Clinical Sciences

*Research Interests:* Gene therapy, stem cell therapy.

Dr. Laurie Goodrich joined the faculty at CSU College of Veterinary Medicine in April of 2005 as an assistant professor in Equine Surgery and Lameness. Prior to joining the faculty, she obtained her D.V.M. from the University of Illinois, and completed an internship in Large Animal Surgery and Medicine at Virginia-Maryland Regional College of Veterinary Medicine. Following her internship, Dr. Goodrich joined the faculty at Virginia for one year as an equine ambulatory clinician before going on to complete her residency in Equine Surgery at the Equine Medical Center in Leesburg, Virginia. She also obtained a Master of Science in Pharmacology during her residency. Dr. Goodrich subsequently joined the large animal surgery faculty at Cornell University's College of Veterinary Medicine and became Board Certified in Large Animal Surgery in 1999. At Cornell, she rotated as Chief-of-Service for the Orthopedic, Soft Tissue, and Emergency Surgery Services. In 2000, she began a Ph.D. in Cartilage Repair and Gene Therapy. Her research included the transplantation of genetically modified chondrocytes (cells of cartilage) into the defects of cartilage to improve cartilage healing. She completed her Ph.D. in the fall of 2004. Dr. Goodrich's clinical interests are broad and include joint disease, lameness, arthroscopy, laparoscopy, upper airway disease, and wound healing, neoplasia, and pain management. Dr. Goodrich's research interests are primarily focused on cartilage healing and cartilage repair currently using growth factor gene therapy modalities. Side interests include bone healing and pain management research.

*Honors include:* Orthopaedic Research Society, New Investigator Research Award, Semi-Finalist, 2006; Recipient five-year NIH KO8 Training Grant, 2008-2013; Clinician of the Year Award for Teaching Excellence, 2011; Elastikon Equine Research Award, 2011.



## Faculty

### College of Veterinary Medicine and Biomedical Sciences

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**Kevin K. Haussler, D.V.M., D.C., Ph.D.,** Assistant Professor, Department of Clinical Sciences

*Research Interests:* Etiopathogenesis and objective assessment of musculoskeletal pain, spinal dysfunction, and sacroiliac joint disorders. Spinal kinematics and conservative management of spinal-related disorders. Clinical research in the areas of veterinary chiropractic, acupuncture, physiotherapy modalities, and musculoskeletal rehabilitation.

Dr. Haussler obtained a Bachelor's of Science in Agriculture from the University of Nebraska - Lincoln in 1984. He graduated in 1988 from The Ohio State University, College of Veterinary Medicine, followed by a small animal internship at the Sacramento Animal Medical Group in 1989. Dr. Haussler was a relief veterinarian for multiple small animal practices, emergency clinics, and humane societies from 1989 to 1994, when he became interested in pursuing further specialized training in the diagnosis and management of pain and musculoskeletal disorders in animals. He enrolled in Palmer College of Chiropractic - West, a human chiropractic program, to learn how to apply human chiropractic techniques and principles to the treatment of animals with musculoskeletal-related disorders. Dr. Haussler started veterinary chiropractic practice with equine and small animal patients in 1992. After graduating with a Doctor of Chiropractic (D.C.) degree from Palmer College of Chiropractic - West in 1993, Dr. Haussler obtained a Ph.D. degree in Comparative Pathology from the University of California - Davis, School of Veterinary Medicine in 1997. The focus of his Ph.D. research was the evaluation of the anatomy, pathology and biomechanics of the lower back and pelvis of Thoroughbred racehorses. He then went on to complete a post-doctorate investigating *in-vivo* equine spinal kinematics in 1999 at the Department of Anatomy, College of Veterinary Medicine at Cornell University. As a Lecturer at Cornell University until 2005, he was responsible for teaching equine anatomy, biomechanical research, and initiation of a clinical Integrative Medicine Service at the Cornell University Hospital for Animals in both the large and small animal clinics that provided chiropractic, acupuncture, and physical therapy services. Dr. Haussler's research studies included evaluation of *in vivo* equine spinal kinematics, paraspinal muscle morphometry and histochemistry, and the initiation of equine chiropractic research assessing pain and spinal flexibility.

Currently, Dr. Haussler is an Assistant Professor at Colorado State University at the Equine Orthopaedic Research Center with continued research interests in objective assessment of musculoskeletal pain and spinal dysfunction.

*Honors include:* James M. Wilson Award for Equine Research, School of Veterinary Medicine, University of California, Davis, 1997.



**Thomas R. (Tod) Hansen, B.S., M.S., Ph.D.,** Professor and Director, Animal Reproduction and Biotechnology Laboratory

Collaborating on equine genomic research.



**Ashley Hill, D.V.M., MPVM, Ph.D.,** Assistant Professor, Department of Clinical Sciences

*Research Interests:* Epidemiology of equine athletic injuries, simulation modeling. Research topics have included the effect of mild/moderate injury on the subsequent development of catastrophic injury; the effects of exercise and horseshoe type on development of catastrophic injuries; and simulation modeling of the incidence of metacarpal condylar fractures in California.

Dr. Hill obtained a Bachelor's of Arts in English literature at Haverford College. She graduated in 1998 from the University of California, Davis School of Veterinary Medicine, then completed a Master's in Preventive Veterinary Medicine (MPVM) at UC Davis in 1999, and a Ph.D. in Epidemiology in 2003. Theses for both degrees focused on the epidemiology of forelimb injuries in Thoroughbred racehorses. Dr. Hill came to CSU as an Assistant Professor in the Department of Clinical Sciences in 2006. She is interested in the relationship between exercise, rest, pre-existing injury, and the development of severe or catastrophic injuries. She is also interested in return to function following severe injuries or surgery.

*Honors include:* Mark Gearhart Award for Best Graduate Student Manuscript, Association of Veterinary Epidemiology and Preventative Medicine, 2003.

## Faculty

### College of Veterinary Medicine and Biomedical Sciences



**Christopher E. Kawcak**, D.V.M., Ph.D., Diplomate ACVS, Professor, Iron Rose College Chair in Musculoskeletal Research, Department of Clinical Sciences

*Research Interests:* Subchondral bone histomorphometry, biomechanical modeling of joint loading, and imaging of early subchondral disease in pathogenesis of joint disease.

Dr. Kawcak joined our faculty in 1998 as an Assistant Professor after completing his Ph.D. He is now an Associate Professor in the Iron Rose Ranch Chair in the ORC, and is expanding his duties to include clinical work in the VTH and veterinary student teaching. His collaborations with the Biomedical Engineering Program at CSU, the Mechanical Engineering Program at the University of Texas, the Department of Chemical and Materials Engineering, The University of Auckland, and other laboratories worldwide have allowed for more sophisticated assessment of joint disease and healing. Dr. Kawcak is currently involved with research projects evaluating a new type of horseshoe, the effects of exercise on the incidence of musculoskeletal injury, and the development of computerized models of joints. Specifically, he is collaborating with Dr. Reiser and Puttlitz to develop a functional model of the fetlock joint in horses. He has over 100 publications and has been an invited speaker in the U.S. and Europe and is involved with the American Association of Equine Practitioners and the American College of Veterinary Surgeons. He currently sits on the Research Committee for the Grayson Jockey Club Research Foundation.

*Honors include:* Ken Atkinson Scholar in the College of Veterinary Medicine and Biomedical Sciences, 1995-98; Pfizer Award for Research Excellence, 2003; Elastikon Equine Research Award, Johnson & Johnson Consumer Products Company and Grayson-Jockey Club Research Foundation, 2007.



**John Kisiday**, Ph.D., Assistant Professor, Department of Clinical Sciences

*Research Interests:* Mechanobiology of cartilage and repair tissue, tissue engineering.

Dr. John Kisiday was hired as an Assistant Professor in Clinical Sciences in a research and teaching appointment at the ORC in January 2005 after doing his Ph.D. at MIT in Bioengineering and a collaborative post-doctorate of fellowship with CSU and MIT. His doctorate work primarily focused on mechanobiology, the study of the impact of physical deformation on cells, and the use of a novel peptide-based material (discovered at MIT in the early 1990s), as a three-dimensional scaffold for cartilage tissue repair. Dr. Kisiday's post-doctorate work explored chondrogenesis of equine stem cells for potential applications to equine and human therapies. The research Dr. Kisiday will focus on at the ORC will involve cartilage tissue engineering therapies and mechanobiology in order to build the bridge between basic laboratory studies and beneficial animal models.

*Honors include:* Young investigator Award, Engineering Tissues Workshop, Hilton Head, 2003; NIH Biotechnology Pre-doctoral Training Grant, 2001-2003; MIT President Pre-doctoral Fellowship, 1999.



**Richard D. Park, D.V.M., Ph.D., Diplomate ACVR, Professor, Department of Radiological Health Sciences**

*Research Interests:* Imaging in orthopaedic disease, including radiology, ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI).

Dr. Park is internationally renowned in the field of imaging (previously called radiology). He has been actively involved in the Orthopaedic program, acquiring expertise in CT and CT osteoabsorptiometry (used for quantitative assessment of bone density), as well as the introduction of magnetic resonance imaging (MRI) for imaging in orthopaedic research.



**Natasha Werpy, D.V.M., Diplomate ACVR, Assistant Professor, Department of Clinical Sciences**

*Research Interests:* Imaging in orthopaedic disease, including radiology, ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI).

Dr. Werpy earned her D.V.M. from CSU in 1999, followed by an internship at the San Luis Rey Equine Hospital in California which she completed in 2000. In 2003, she completed a residency directed by Dr. Norman Rantanen in collaboration with CSU, which focused on equine imaging. Dr. Werpy joined the CSU faculty in 2004, overseeing research imaging and directing MRI examination of clinical patients at the Orthopaedic Research Center. Her current research centers on MRI, ultrasound and histology correlation in order to develop imaging protocols for clinical patients.



## Faculty

### College of Engineering

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**Susan P. James, Ph.D.**, Associate Professor, Department of Mechanical Engineering

*Research Interests:* Biomaterials, wear of orthopaedic implants, tissue engineering of cartilage.

Dr. James joined the faculty at CSU in 1994 after receiving her Ph.D. in polymer science and technology from Massachusetts Institute of Technology in September 1993 and working for a year as an engineer at the Failure Analysis Associate in California. She initiated the Biomedical Engineering Program at CSU and served as the program's director from 1999 to 2003, and is currently the Director of BEP. CSU and the College of Engineering recently invested in and institutionalized BEP, which serves multiple colleges on campus. Dr. James is also the Associate Department Head of Mechanical Engineering. Her current research is focused on novel hyaluronan/polyethylene composites for use in joint replacements, cartilage repair and other biomedical applications. She teaches courses in biomaterials, biomedical engineering and materials science at both the undergraduate and graduate level.

*Honors include:* Outstanding Faculty Member, American Society of Mechanical Engineers, Engineering Faculty Award of Excellence at CSU, 1997; Semifinalist for Wallace H. Coulter Award for Medical Innovation and Entrepreneurship, Georgia Tech, Atlanta, Georgia, 2002; Women and Minorities in Engineering Appreciation Award at CSU, 2005; Jack E. Cermak Advising Award at CSU, 2006; George T. Abell Outstanding Faculty Teaching and Service Award at CSU, 2006; Nominated for CSU Best Teacher Award, 2006.



**Christian Puttlitz, M.S., Ph.D.,** Associate Professor, Department of Mechanical Engineering and School of Biomedical Engineering

*Research Interests:* Orthopaedic biomechanics, tissue and biomaterials interactions.

Dr. Puttlitz joined the CSU faculty in 2005 after spending 4 years as an Assistant Professor in the Department of Orthopaedic Surgery at the University of California, San Francisco. After receiving his Ph.D. in Biomedical Engineering at the University of Iowa in 1999, Dr. Puttlitz performed a 2 year Postdoctoral Fellowship in San Francisco. Dr. Puttlitz's research interests are mainly focused on using experimental and computation techniques to investigate orthopaedic conditions and their treatments. Examples of his current research include using the finite element method to study how loading changes in the spine following intervertebral disc replacement. Dr. Puttlitz teaches an undergraduate course in bioengineering and a graduate biomechanics class.

*Honors include:* Mark S. Bloomberg Memorial Award for Outstanding Research, Veterinary Orthopaedic Society, March 2008; Elastikon Equine Research Award, Grayson-Jockey Club Research Foundation, May 2007; Best Basic Science Award, Inman-Abbott Society, San Francisco, May 2005; Finalist, Basic Science Award at the Cervical Spine Research Society, Boston, December 2004; Finalist, Basic Science Award at the Cervical Spine Research Society, Scottsdale, December 2003; Best Poster Award at the International Society for the Study of the Lumbar Spine, Edinburgh, June 2001; Inducted into Sigma Xi, National Research Honorary Society, January 2001; Nordby-Smith Best Paper Award on Minimally Invasive Surgery at the North American Spine Society Meeting, New Orleans, October 2000; Finalist, Doctoral Student Paper Competition, American Society of Mechanical Engineers, November 1999; Inducted into Tau Beta Pi, National Engineering Honor Society, Fall 1995; Inducted into Academic All-American Society, Spring 1993; Inducted into Alpha Sigma Mu, National Materials Science and Engineering Honor Society, Spring 1992.



**Kenneth Reardon,** Professor, Department of Chemical Engineering, College of Engineering, Colorado State University

*Research Interests:* Collaborating on proteomic studies.

## Faculty

### College of Applied Human Sciences

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**Raoul F. Reiser, II, Ph.D.**, Associate Professor, Department of Health & Exercise Science

*Research Interests:* Musculoskeletal biomechanics, fabrication and implementation of custom equipment/instrumentation.

Dr. Reiser completed his B.S. in Mechanical Engineering at Cornell University, his M.A. in Kinesiology with a specialization in Biomechanics at the University of Texas at Austin and his Ph.D. in Mechanical Engineering at Colorado State University. The emphasis of his dissertation was the biomechanics of recumbent cycling and the power output capabilities, pedal force measuring and analysis system, and inverse-dynamics analysis of recumbent versus standard cycling. After working as an Assistant Professor at the University of Wyoming in the Division of Kinesiology and Health, Dr. Reiser began work as an Assistant Professor at CSU in the Department of Health and Exercise Science in August of 2002.

*Honors include:* Elected Fellow, American College of Sports Medicine, 2007; Colorado State University College of Applied Human Sciences Tenure Track Faculty Scholarly Excellence Award, 2007; CSU College of Engineering's Outstanding Research Assistant, 2000; GAANN Three-Year Fellowship, 1997; CSU Graduate Fellowship, 1997; NSCA Challenge Scholarship, 1996.

## Faculty

### College of Agricultural Sciences

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**Jason Bruemmer, Ph.D.**, Associate Professor, Department of Animal Science

*Research Interests:* Maternal recognition, follicular cell differentiation, sperm physiology.

Dr. Jason Bruemmer, Assistant Professor, was born and raised in El Paso, Texas. He received his B.S. in Animal Science and his M.S. in Physiology of Reproduction from Texas A&M University, and his Ph.D. in Reproductive Physiology from New Mexico State University.

While at Texas A&M, Dr. Bruemmer served as a lecturer and manager of the horse farm for more than three years. He bred 60 to 75 mares a year, in addition to teaching reproduction, reproductive short courses, all levels of equine science, and conducting research in nutrition and exercise physiology. During his stay at NMSU, Dr. Bruemmer again taught many equine classes and conducted research in a variety of species including horses, cattle, goats, and sheep. Further studies were conducted at the University of Arizona Medical School.

Dr. Bruemmer joined Colorado State University in 1996. He teaches Equine Management, Equine Production and Industry, and other courses, and continue to conduct research in reproductive physiology with an emphasis in follicular dynamics of the mare, the area in which he did his dissertation work at New Mexico State University.



**Jerry Black, D.V.M.,** Director of Undergraduate Programs, Equine Sciences

Dr. Black completed his D.V.M. degree in 1971 from CSU. He co-founded Pioneer Equine Hospital in Oakdale, Calif., for 34 years from which he retired in 2010 and then accepted the position of Director of Undergraduate Programs in 2010.



**Hariharan K. Iyer, B.S., M.S., Ph.D.,** Professor, Department of Statistics and Center for Bioinformatics

*Honors include:* Fellow of the American Statistical Association, the College of Natural Sciences Graduate Teaching Award, 1993; Fellow Cooperative Institute for Research in the Atmosphere (CIRA), 2004-present.



**Ann Hess, Ph.D.,** Assistant Professor, Department of Statistics and Center for Bioinformatics

Dr. Hess completed her M.S. and Ph.D. in Statistics at CSU. Her research interests are mainly focused on bioinformatics and experimental design. She has been involved in a number of microarray studies as well as other bioinformatics projects.



# Affiliate Faculty

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**Elwyn Firth**, B.V.Sc., Ph.D., Diplomate ACVS, Professor and Director, Massey Equine Research, Massey University, Palmerston North, New Zealand

Dr. Firth is an internationally renowned equine orthopaedic researcher. He has worked closely with Dr. McIlwraith for many years, and, more recently, has become closely involved in a collaborative effort with Drs. McIlwraith and Kawcak, as well as other researchers at Massey University, the University of London, and Utrecht in the Global Equine Research Alliance.

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**Clifford Michael Les**, D.V.M., M.S., Ph.D., Senior Staff Investigator, Bone and Joint Center Henry Ford Health System

Dr. Les is a Senior Staff Investigator at the Bone and Joint Center, Henry Ford Health System in Detroit, Michigan. He is also a member of the Michigan Bone Center at the University of Michigan's School of Medicine and an adjunct Assistant Professor in the Department of Anatomy and Cell Biology at the Wayne State University School of Medicine. Dr. Les received his D.V.M. at the University of California, Davis; his M.S. in Veterinary Biosciences at the University of Illinois, Urbana-Champaign; and his Ph.D. in Comparative Pathology at the University of California, Davis. His dissertation work was on material heterogeneity in the equine metacarpus and biomechanical consequences.

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**Alan J. Nixon**, B.V.Sc., Ph.D., Diplomate ACVS, Professor of Orthopaedic Surgery, Director of the Comparative Orthopaedic Laboratory, Cornell University

Dr. Nixon is a Professor of Orthopaedic Surgery and Director of the Comparative Orthopaedic Laboratory at Cornell University, Ithaca, New York. His research focus is in chondrocyte metabolism and cartilage repair methods using chondrocyte or pluripotent stem cell transplantation. Dr. Nixon's research group has focused on the cloning of growth factor molecules for use in gene therapy protocols, inserting the growth factor gene into cartilage cells at the time of transplantation of synovial cells by direct joint injection. The laboratory group also studies the molecular changes associated with osteochondritis dissecans (OCD) in horses and man, and investigates treatment methods for tendonitis in athletes.

Dr. Nixon's current interests include the use of combination gene therapy using stimulatory growth factors, and, in collaboration with the Orthopaedic Research Center at Colorado State University, the combined use of interleukin receptor antagonist gene therapy to diminish degradation in arthritic joints.

## Affiliate Faculty

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**William G. Rodkey**, D.V.M., M.S., Scientific Director for Regen Biologics and Chief Scientific Officer Steadman Philippon Research Foundation, Vail, Colo.

Dr. Rodkey was formerly Director of Orthopaedic Research at the Letterman Institute in San Francisco. He is currently Scientific Director for Regen Biologics and the Steadman Philippon Research Foundation. Dr. Rodkey is one of three veterinarians with a long-term reputation in human orthopaedic research and collaborated with the CSU Orthopaedic Research Center on articular cartilage resurfacing research.

*Honors include:* Excellence in Research in Basic Science Award (American Orthopaedic Society for Sports Medicine); H. Edward Cabaud Memorial Award for Ligament Research (American Orthopaedic Society for Sports Medicine); Co-recipient of Albert Trillat Award for Excellence in Knee Research (International Society of the Knee); U.S. Army Research and Development Achievement Award (Secretary of the Army); H. Edward Cabaud Memorial Award for Knee Research (2nd) (American Orthopaedic Society for Sports Medicine).

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**Jude Samulski**, Ph.D., Professor, Department of Pharmacology, University of North Carolina, Chapel Hill, N.C.

Dr. Jude Samulski is an important collaborator to our group investigating gene therapy at the ORC. He is a Professor in the Department of Pharmacology and the Director of the Gene Therapy Center at the University of North Carolina at Chapel Hill. Dr. Samulski earned his B.S. at Clemson University, a Ph.D. at the University of Florida in Molecular Biology. He did two post docs at SUNY in New York and Princeton University, respectively. He then was on faculty at University of Pittsburgh from 1986-1992 and recruited to UNC as Associate Professor in Pharmacology and Director of the Gene Therapy Center.

*Honors include:* Outstanding Young Men of America Award and the President's Distinguished Research Award; American Society of Gene Therapy Outstanding Achievement Award, 2009.

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**Kevin Shelburne**, M.S., Ph.D., Associate Research Professor, University of Denver, Department of Mechanical and Materials Engineering; Affiliate Faculty Colorado State University, Department of Biomedical Engineering and Veterinary Medicine

Kevin Shelburne received his bachelor's and master's degrees in Mechanical Engineering from Texas A&M University in 1985 and 1988, respectively. He then worked as a Systems Engineer at McDonnell Douglas Space Systems Company, Houston, Texas, where he designed and tested assembly and servicing tasks and robotics systems for the International Space Station. Kevin completed his Ph.D. in Mechanical Engineering at the University of Texas at Austin in May 1997. The focus of his dissertation was the computer modeling and analysis of the normal and reconstructed knee joint. Following his dissertation, Kevin worked for Lockheed Martin Space Systems in the design of new satellite launch vehicles.

In 2000, he joined the Biomechanics Research Laboratory at the Steadman Philippon Research Institute. Kevin is the author of numerous articles regarding the modeling and simulation of knee mechanics and is a current member of the American Society of Biomechanics and the American Society of Mechanical Engineers. Dr. Shelburne joined the University of Denver in 2010.

*Honors include:* Journal of Biomechanics Award from the World Congress of Biomechanics, 2002.

## Collaborators

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**Alan Boyde**, B.D.S., L.D.S., Ph.D., Professor, Department of Anatomy and Developmental Biology, University College London

Dr. Boyde is the author of many papers, chapters, and abstracts on the development, structure, and mineralization of bone, age changes in skeletal tissue, and osteoporosis. He has developed enabling technologies for the microscopic investigation of mineralized tissues and cell biology.

*Honors include:* Wellcome Trust Biomedical Imaging Awards for Excellence, 1998 and 2002; President of the Anatomical Society of Great Britain and Ireland, 2002-2004; Elected Honorary Member of Bone and Tooth Society, 2002.

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**Neil David Broom**, Ph.D., Associate Professor, Department of Chemical and Materials Engineering, University of Auckland

Dr. Broom's doctoral studies were concerned with mechanical and ultrastructural analysis of the high velocity deformation of metal single crystals. He was personally responsible for establishing the first transmission electron microscopy facility in New Zealand permitting quantitative crystallographic analysis of crystal dislocation structures. His postdoctoral research at University of Cambridge was concerned with fundamental structural (TEM) and mechanical studies of intermetallic single crystal fibers relevant to the development of high strength lightweight metal fiber-reinforced metal composites of interest to the UK aircraft industry. Since 1975, Dr. Broom has been funded continuously by the New Zealand Medical Research Council and Health Research Council to conduct biomechanical/biomaterials research in heart valve biomechanics, joint tissue biomechanics/biomaterials, and intervertebral disc biomechanics.

*Honors Include:* University of Auckland Distinguished Teaching Medal, 1998; Engineering Faculty Award for Excellence in Undergraduate Teaching, 1999-2002.

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**Stephanie Bryant**, Ph.D., Assistant Professor, Department of Chemical and Biological Engineering, University of Colorado

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**Bruce Caterson**, Ph.D., Professor Connective Tissue Biology Laboratories, Cardiff School of Biosciences, Associate Director of Musculoskeletal Research, School of Medicine, Cardiff University, U.K.

Dr. Caterson is a Professor in the Cardiff School of Biosciences and is currently Associate Director of Musculoskeletal Research in the School of Medicine. He was previously head of Connective Tissue Biology at Cardiff and prior to that was the Norfleet-Raney Professor of Research in Orthopaedics and Professor of Biochemistry and Biophysics at the University of North Carolina, Chapel Hill School of Medicine. He is world renowned in articular cartilage biochemistry and pioneered the use of monoclonal and polyclonal antibodies as biomarkers of joint disease. He has received the Kappa Delta Elizabeth Winston Lanier Award for Outstanding Orthopaedic Research from the American Academy of Orthopaedic Surgeons and Orthopaedic Research Society in 1998 and currently has large programme grant from the Arthritis Research Campaign on mechanisms of matrix proteoglycan catabolism in articular cartilage as well as EPSRC Platform Grant on bioresponsive polymer therapeutics: synthesis and characterization of novel nanomedicines.

## Collaborators

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**Chris Evans**, Ph.D., Professor, Brigham and Women's Hospital, Center for Molecular Orthopaedics, Harvard University, Boston, Mass.

Dr. Evans is world-renowned in the area of human joint disease research, particularly in the use of gene therapy to treat arthritis. He was an outside member on the Ph.D. Committee of Dr. Dave Frisbie when he worked on his gene therapy with interleukin-1 receptor antagonist to treat equine traumatic arthritis and osteoarthritis. He continues to collaborate with the scientists at the Orthopaedic Research Center at CSU.

*Honors include:* Kappa Delta Award, AAOS; the Cabaud Award, American Society for Sports Medicine; the Henry Kunkle Award, American College of Rheumatology; Osteoarthritis Research Award, OARSI; and the Synos Award for Orthopaedic Research (with Paul Robbins), Synos Foundation.

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**Steven C. Ghivizzani**, Ph.D., Associate Professor, Research Division; Departments of Orthopaedics and Rehabilitation and Molecular Genetics & Microbiology, Gene Therapy Laboratory, University of Florida, Gainesville, Fla.

Dr. Ghivizzani is an Associate Professor in the Gene Therapy Laboratory at the University of Florida. He has collaborated with the Orthopaedic Research Center on several projects. Currently, he is working with the CSU researchers on adeno-associate virus and lenti virus delivery of interleukin-1 receptor antagonist.

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**Alan J. Grodzinsky**, Sc.D., Professor, Director of the MIT Center for Biomedical Engineering, Department of Mechanical Engineering and Biological Engineering Division, MIT

Dr. Grodzinsky is a Professor in the departments of Electrical, Mechanical, and Biological Engineering at the Massachusetts Institute of Technology. He is also the Director of the MIT Center for Biomedical Engineering. Dr. Grodzinsky research focuses on the mechanobiology of articular cartilage, including the response of native tissue to physiological and injurious loading as well as the mechanobiology of neo-tissue development for applications to cartilage resurfacing.

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**Charles Ho**, M.D., Ph.D., Director Imaging Research, Scientific Advisory Board Steadman Philippon Research Institute

Dr. Ho is experienced and active in musculoskeletal and sports medicine imaging and research, particularly in musculoskeletal Magnetic Resonance Imaging. He is a member of the Radiological Society of North America, the American Roentgen Ray Society, the American Academy of Orthopaedic Surgeons, the American Orthopaedic Society for Sports Medicine, and the ACL Study Group, among other professional organizations. He has published numerous papers and book chapters in the radiologic and orthopedic literature, and presented numerous papers internationally in radiologic and orthopedic conference proceedings. Dr. Ho is Director of Imaging Research and a member of the Scientific Advisory Board of the Steadman Philippon Research Institute in Vail, Colo. He has served as Radiologic Consultant for the San Francisco 49ers, the San Francisco Giants, Cleveland Indians, Denver Broncos, Colorado Rockies, the U.S. Ski Team, and the U.S. Decathlon Team.

# Collaborators

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**Chris Little**, B.Sc., B.V.M.S., M.Sc., Ph.D., Diplomate ACVS, Associate Professor and Director, Raymond Purves Bone & Joint Research Laboratories, University of Sydney Department of Orthopaedics & Traumatic Surgery, Royal North Shore Hospital

Dr. Little received his veterinary training at Murdoch University in Western Australia, where he also undertook an internship in equine medicine and surgery (1978-1984). He then completed a residency in large animal surgery and an M.Sc. studying arthritis in horses at the University of Minnesota. Chris was appointed to the faculty at the Ontario Veterinary College, University of Guelph and during this time passed his certifying examinations to become a Diplomate of the American College of Veterinary Surgeons (1990). He then moved to back to Australia and was awarded a Ph.D. degree from the Faculty of Medicine at the University of Sydney in 1996. Following a 5 year postdoctoral position at Cardiff University School of Biosciences in the UK, he was granted a two year Arthritis Foundation of Australia Ulysses Research Fellowship at the University of Melbourne. In 2004 he was appointed as Director of the Raymond Purves Bone & Joint Research Laboratories at the Royal North Shore Hospital, University of Sydney. Chris's research interests centre on the biochemical and molecular mechanisms of cartilage and more recently tendon breakdown in disease. In particular he has studied changes in aggrecan and small proteoglycan biosynthesis and degradation and the proteolytic pathways responsible in cartilage breakdown in arthritis and during tendon degeneration. Chris has been extensively involved in the development and use of neoepitope antibody methodologies, novel animal models, and most recently genetically modified mice, to study disease pathways. He has received over \$3 million in basic and industrial research grants and has authored/co-authored 53 papers and six book chapters.

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**Marcus G. Pandey**, Ph.D., Professor, Chair of Mechanical and Biomedical Engineering, Department of Mechanical and Manufacturing Engineering, University of Melbourne, Melbourne, Australia

Dr. Pandey is a Professor at the University of Melbourne and a leader in the study of musculoskeletal biomechanics. He is interested in applying the principles of mechanics and control theory to describe and explain the relationships between structure and function of the human body. By combining data obtained from biomechanical experiments with detailed computer models of the neuromusculoskeletal system, he is able to determine muscle, ligament, and joint loading during movement. Dr. Pandey is currently collaborating with CSU Orthopaedic researchers to develop a computer model of the entire equine forelimb to aid in the early detection of joint disease in horses.

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**Michael “Mick” Peterson**, Ph.D., Associate Professor, University of Maine

Dr. Peterson is Libra Foundation Professor of Mechanical Engineering at the University of Maine. Prior to coming to the University of Maine, he was a faculty member at Colorado State University and was a Post-Doctoral Researcher at Northwestern University. He has also worked in industry at General Motors and General Dynamics Corp. His Ph.D. is in Theoretical and Applied Mechanics from Northwestern University in Illinois, and he also holds a B.S. in Mechanical Engineering from General Motors Institute (now Kettering University) and an M.S. in Theoretical and Applied Mechanics from Northwestern University. He has also done additional graduate work in Mechanics, Materials, and Mathematics from Yale University, Cornell University, and the University of Connecticut. His primary expertise is in the dynamic responsive materials and waves in solids.



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**A. Robin Poole**, Ph.D., Professor Emeritus, Director of Joint Diseases Laboratory, McGill University,  
Montreal, Quebec

Dr. Poole is a pioneer in the use of markers in the early diagnosis of arthritis before other imaging techniques can reveal change. He is a world-renowned arthritis researcher, having previously led arguably the most prominent laboratory in the world in this area of research. He was the mentor of Dr. Billingham, and Dr. McIlwraith spent time with him on sabbatical leave. He is the co-author of two publications from the CSU Orthopaedic Laboratory. He is now retired but continues to be active and most recently was a keynote speaker at our 2009 Havemeyer Symposium on Biomarkers.

*Honors include:* Kappa Delta Award of the American Academy of Orthopaedic Surgeons, the Howard and Martha Holley Research Prize in Rheumatology, Carol Nachman International Prize for Rheumatology.

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**Christopher B. Riley**, B.Sc.(Physics), B.V.Sc. (Hons), M.Sc., Ph.D., Diplomate ACVS Associate Professor and  
Service Chief of Large Animal Surgery Department of Health Management, Atlantic Veterinary College,  
University of Prince Edward Island, Charlottetown PE Canada

Following military service in the Royal Australian Air Force, Dr. Riley received degrees in physics and veterinary medicine from the University of Melbourne, Australia. After time spent in an internship and private practice in Australia, he completed a surgical residency at the University of Saskatchewan in Canada. Concurrently he completed M.Sc. and Ph.D. degrees in the fields of tendon *in-vitro* biology and biochemistry. Dr. Riley then worked at briefly at Iowa State University and in private practice during which time he became Board certified as a Diplomate in the American College of Veterinary Surgeons. He joined the faculty at the Atlantic Veterinary College, Canada in 1999 where he is currently an Associate Professor and Service Chief of Large Animal Surgery. Following the granting of tenure, Dr. Riley has focused his research on the development of biomedical tests for animal diseases using the emerging technologies of infrared spectroscopy and bioinformatics. He established the first laboratory of its kind in Canada, developed to investigate the veterinary potential biomedical infrared spectroscopy. Dr. Riley has a special interest in orthopedic disease, but is also interested exploring the full potential of infrared technology as it applies to veterinary and comparative medicine. Dr. Riley has partnered with the workers from the Orthopaedic Research Center at Colorado State University, and the Institute for Biodiagnostics, National Research Council of Canada, to develop the first infrared test for equine traumatic arthritis in the world. He looks further to continued collaboration and advances in this new field of research.

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**Paul D. Robbins**, Ph.D., Professor of Molecular Genetics and Biochemistry and Orthopaedic Surgery, University  
of Pittsburgh School of Medicine, Director of the Vector Core Facility and Basic Research for the  
Molecular Medicine Institute

Dr. Robbins is currently a Professor of Molecular Genetics and Biochemistry and Orthopaedic Surgery at the University of Pittsburgh School Of Medicine. He is also Director of the Vector Core Facility and Director of Basic Research for the Molecular Medicine Institute. He received his Ph.D. from the University of California at Berkeley and worked as a post-doctoral fellow at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. He is an Associate Editor for *Cancer Research* and *Gene Therapy* as well as on the Editorial Boards for *Cancer Gene Therapy*, *The Journal of Gene Medicine*, *Arthritis Research*, and *Genes & Immunity*. Dr.

**Collaborators**

Robbins has co-authored over 180 peer-reviewed manuscripts, 110 book chapters and reviews, and has edited two books on gene therapy. He is a member of the PathB study section, the Telethon Scientific Review Committee and the Scientific Review Board of National Gene Vector Laboratory.

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**Robert Lie-Yuan Sah**, M.D., Sc.D., Professor and Vice-Chair of Bioengineering Affiliate in Orthopaedics, UCSD

Dr. Sah received his Sc.D. in Biomedical Engineering from the Massachusetts Institute of Technology and his M.D. from Harvard Medical School. He did postdoctoral work at Massachusetts General Hospital in Orthopaedic Bioengineering. He is currently a reviewer for Arthritis Foundation, NIH, NSF and Orthopaedic Research & Education Foundation, and the 2004 Chair of Gordon Research Conference on Musculoskeletal Biology and Bioengineering.

*Honors include:* “Mechanical Blueprint for Cartilage” cited as one of the Great Advances in Scientific Discovery in Disease and Injury Treatment, The Science Coalition, 1998; Accelerated academic advancements, UCSD, 1999 and 2001; American Academy of Orthopaedic Surgeons Kappa Delta Young Investigator Award, 2001; American Academy of Orthopaedic Surgeons Best Poster Award, 2003.

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**Kevin Shelburne**, M.S., Ph.D., Assistant Director of the Biomechanics Research Laboratory, Steadman Philippon Sports Medicine Foundation, Vail, Colo.; Faculty Colorado State University, Department of Biomedical Engineering and Veterinary Medicine; Associate Research Professor at the University of Denver

Kevin Shelburne received his bachelor’s and master’s degrees in Mechanical Engineering from Texas A&M University in 1985 and 1988, respectively. He then worked as a Systems Engineer at McDonnell Douglas Space Systems Company, Houston, Texas, where he designed and tested assembly and servicing tasks and robotics systems for the International Space Station. Kevin completed his Ph.D. in Mechanical Engineering at the University of Texas at Austin in May 1997. The focus of his dissertation was the computer modeling and analysis of the normal and reconstructed knee joint. Following his dissertation, Kevin worked for Lockheed Martin Space Systems in the design of new satellite launch vehicles.

In 2000, he joined the Biomechanics Research Laboratory at the Steadman Philippon Research Institute. Kevin is the author of numerous articles regarding the modeling and simulation of knee mechanics and is a current member of the American Society of Biomechanics and the American Society of Mechanical Engineers.

*Honors include:* Journal of Biomechanics Award from the World Congress of Biomechanics, 2002.

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**Roger K.W. Smith**, M.A. VetMB Ph.D. DEO DipECVS MRCVS, Professor of Equine Orthopaedics, Royal Veterinary College, London, United Kingdom

Roger Smith qualified as a veterinary surgeon from Cambridge University in 1987 and, after two years in practice, returned to academia to undertake further clinical training as a Resident in Equine Studies at the Royal Veterinary College. Following his residency, he undertook a three-year research project culminating in the award of a Ph.D. for his studies on the extracellular matrix of equine tendon. He remained at the Royal Veterinary College, first as a Lecturer in Equine Surgery, then as Senior Lecturer in Equine Surgery before his appointment to a Professorship in December 2003. He holds the Diploma of Equine Orthopaedics from the Royal College of Veterinary Surgeons, and is both a Diplomate of the European College of Veterinary Surgeons and a Royal College of Veterinary Surgeons Specialist in Equine Surgery. He currently divides his time equally between running a specialist orthopaedic service within the Royal Veterinary College and continuing to direct research into equine tendon disease. His main area of research is understanding the mechanisms of tendon ageing but also has projects investigating the epidemiology of tendon disease in the horse, the development of a serological assay for tendonitis, and stem cell therapy for tendons in conjunction with a commercial company, VetCell Bioscience Ltd.

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**J. Richard Steadman**, M.D., Head of the Steadman Clinic and Steadman Philippon Research Institute, Vail, Colo.

Dr. Steadman graduated from the University of Texas Southwestern Medical School in Dallas. Following internship, two years in the army, and an orthopaedics residency at Charity Hospital in New Orleans, La., Dr. Steadman moved to Lake Tahoe, Calif., where he practiced orthopaedics with increasing emphasis on the treatment of knee disorders. While living there, he was named Chief Physician for the United States Ski Team. During his time at Lake Tahoe, Dr. Steadman developed special surgical techniques which allowed several ski team members to return to competition and win Olympic medals and championships. At Lake Tahoe, Dr. Steadman started a non-profit sports medicine foundation in order to conduct research in knee surgery and rehabilitation projects. That organization exists today as the Steadman Hawkins Sports Medicine Foundation in Vail, Colo. In 1990, Dr. Steadman moved to Vail, Colo., and was joined in practice there by Dr. Richard Hawkins, a specialist in shoulder disorders. By this time, Dr. Steadman had limited his practice to the surgical and conservative treatment of knee disorders. Today, Dr. Steadman is regarded as a world-renowned human orthopaedic surgeon. He is a prominent knee surgeon and the inventor of two significant new techniques in orthopaedics. His Foundation has supported several research projects at CSU. Dr. Steadman serves as a consultant regarding clinical relevance of our research work, and the CSU orthopaedic research lab has done controlled studies investigating his techniques used in human orthopaedic surgery.

## Collaborators

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**Brigitte von Rechenberg**, Dr. med. vet., Diplomate ECVS, University of Zurich

*Honors include:* SSRS Award 1996-1997 for the abstract, "Spontaneous production of nitric oxide and prostaglandin E<sub>2</sub> in media of cartilage explants."

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**René van Weeren**, D.V.M., Ph.D., Diplomate European College of Veterinary Surgeons and Specialist in Equine Surgery, Royal Dutch Veterinary Association; Associate Professor, Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Paul René van Weeren (1957) graduated in 1983 "*cum laude*" from the Utrecht University Veterinary Faculty (The Netherlands). He obtained his Ph.D. degree in 1989 and became a Diplomate of the European College of Veterinary Surgeons in 1994. Currently he is the coordinator of scientific research of the Department of Equine Sciences of the Faculty of Veterinary Medicine of Utrecht University and a member of the Management Board of the Department. His special interest is in equine orthopaedics. He has been a supervisor of 14 Ph.D. students, who have obtained their degree in the past years and currently supervises five Ph.D. students, who will be graduating within the next few years. He is a member of the board of reviewers of the *American Journal of Veterinary Research* and a member of the advisory board of *Equine Veterinary Journal*. He has been external examiner for Ph.D. students abroad at various occasions in the UK, France, Austria, Sweden, and Finland. He is author or co-author of more than 150 peer-reviewed scientific publications or book chapters.

## 2010-2011 Post Doctoral Fellows

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**Kirk McGilvray, Ph.D.**

Kirk is currently working as a Post-doc at the OBRL. He is a Colorado native and received his B.S., M.S., and Ph.D. from CSU. His research efforts included soft tissue biomechanics and computational simulations, focusing on heart valve replacements, spine instrumentation, and cardiovascular mechanics. Kirk's overarching goals are to improve surgical techniques and cardiovascular health through solid research efforts in the field of biomedical engineering.



**Katie Seabaugh, D.V.M.**

Katie received her D.V.M. degree from Washington State University. Following graduation she performed an internship at Pioneer Equine Hospital, a private equine referral practice in Oakdale, Calif. After completing a residency in equine surgery and lameness at CSU, she is currently a post-doctoral fellow.



**Snehal Shetye, M.S., Ph.D.**

Snehal obtained his B.E. in Mechanical Engineering from the University of Pune in India. He moved to Fort Collins for higher studies and obtained his M.S. in Computer-Assisted Engineering under Dr. David Alciatore in the Mechanical Engineering department. Snehal completed his Ph.D. in Biomechanical Engineering under Dr. Christian Puttlitz. His Ph.D. project involved the development of a finite element model of the canine antebrachium. This model will be instrumental in developing novel designs for limb-sparing endoprostheses for the treatment of canine distal radius osteosarcoma.

## 2010-2011 Equine Sports Medicine Residencies

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**Erin Contino, D.V.M.**

Dr. Erin Contino joined the ORC as an Equine Sports Medicine resident in July 2011. Erin is originally from Concord, Calif., but moved to Fort Collins to attend CSU, graduating in 1999 with a Bachelor's Degree in equine sciences. In 2009, she completed a Master's Degree in equine radiology with Drs. McIlwraith and Park researching abnormal radiographic findings in yearling and two-year-old cutting horses. After graduating from CSU with her D.V.M. degree, she did an internship at Pioneer Equine Hospital in California and is now returning for the Equine Sports Medicine residency.



**Dora Ferris, D.V.M.**

Dr. Ferris started an Equine Sports Medicine residency at the ORC in July 2010. She initially joined the ORC in July 2008 as the staff veterinarian responsible for the clinical management of research horses, overseeing treadmill training of the horses, assisting with clinical cases, and aiding research associates. She received her D.V.M. from Washington State University's College of Veterinary Medicine in 2007 and she completed an internship focusing on equine lameness and surgery at Oakridge Equine Hospital in Edmond, Okla., prior to coming to CSU.



## 2010-2011 Ph.D. Graduate Students

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**Caroline Adrian, M.S.**

Caroline (Carrie) received her Ph.D. in 2010 in canine biomechanics at Colorado State University. Her research interests include the application of physical therapy on animals, more specifically, compensatory gait analysis, biomechanics, and neuromotor control of normal and pathological canine gait.

She received her B.S. in Biology in 1994 from Allegheny College in Meadville, Pa., and gained animal experience working in veterinary hospitals since 1990. She received her M.S. in Physical Therapy degree from North Georgia College in 1999. Carrie has participated in a number of continuing education seminars on animal rehabilitation, both as a participant and lecturer since 1998. She has lectured nationally and internationally on the topic of animal physical therapy. She is a contributor to the book, *Canine Rehabilitation & Physical Therapy, Veterinary Clinics of North America* and the upcoming edition of the *Clinical Textbook for Veterinary Technicians*. She presently serves as Vice President for the Animal Special Interest Group within the American Physical Therapy Association. Carrie is the Director of Physical Therapy Services for VCA Hospitals and manages the Physical Therapy and Sports Medicine Department at VCA Alameda East Veterinary Hospital in Denver. Her department serves as one of the few nationally approved clinical practicum sites for the first formal animal rehabilitation training program offered in the country. Carrie also teaches canine anatomy and pathology at the Boulder College of Massage Therapy.



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

Kaydence 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. Kaydence joined the ORC in May 2009 as a Ph.D. student under Dr. Frisbie and Dr. 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.



**Ben Gadomski, B.S.**

Ben received his B.S. in mechanical engineering from Tri-State University in 2009. Since then he has been a Ph.D. candidate under the guidance of Dr. Christian Puttlitz. Ben's past research areas include spinal implant design as well as lumbar spine finite element modeling. His current research focus is in the area of bone fracture healing in microgravity environments using a large animal model.

## 2010-2011 Ph.D. Graduate Students



**Daniel Hemphill, B.S.**

Daniel Hemphill graduated with a B.S. in chemical engineering in 2008 from CSU and started his Ph.D. in Bioengineering. He worked with Dr. Laurie Goodrich doing gene therapy research after completing lab rotations through the school of biomedical engineering.



**Devin Leahy, B.S.**

Devin graduated with a B.S. in mechanical engineering from The Ohio State University in 2004. He is working toward a Ph.D. under Dr. Christian Puttlitz. Devin has industry experience in the areas of composite materials, ergonomics, aerospace, and motorsports, and is currently researching ligamentous trauma in the cervical spine.



**Valerie Moorman, D.V.M.**

Valerie Moorman 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. at the Orthopaedic Research Center. She has an interest in equine sports medicine and surgery, as well as lameness and imaging. In her free time, Valerie enjoys sailing, hiking, and riding hunter-jumpers.



**Trinette Ross, M.S.**

Trinette Ross is an instructor and advisor for the undergraduate Equine Sciences Program and is also the Equine Teaching and Research Center Event Coordinator at CSU. She received her undergraduate degree from Montana State University and then completed an M.S. at Texas A&M University. Trinette is now a Ph.D. student at the ORC (Advisor, Dr. McIlwraith) evaluating the effect of omega 3 fatty acids in equine osteoarthritis model.

## 2010-2011 Ph.D. Graduate Students

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**Kevin Troyer, M.S.**

Kevin received his B.S. and M.S. degrees from Colorado State University. He is currently a Ph.D. candidate studying soft tissue mechanics at the Orthopaedic Bioengineering Research Laboratory under Christian Puttlitz. His dissertation work seeks to characterize and model the complex time-dependent mechanical behavior of musculoskeletal soft tissues, such as ligament and tendon.

## 2010-2011 M.S. Graduate Students

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**Myra Barrett, D.V.M.**

Myra's undergraduate degree was awarded by Stanford University. She went on to receive her D.V.M. from Colorado State University in 2006. After a year internship at Oakridge Equine Hospital, a busy referral practice in Oklahoma, Myra returned to Colorado to pursue a specialty in equine orthopaedic imaging. She received an M.S. in 2010 at the CSU Orthopaedic Research Center as well as a resident in an equine-focused non-traditional diagnostic imaging residency. Myra's master's research is focused on the clinical significance of various radiographic lesions in cutting horses. In 2011, Dr. Barrett became an Assistant Professor CVMBS Environmental & Radiological Health Services Department.



**Hannah Hudson, B.S.**

Hannah joined the James Lab as a sophomore during her undergraduate studies in Mechanical Engineering and is currently pursuing her M.S. under the supervision of Dr. Susan James. Currently, Hannah's work is investigating the feasibility of penetrating DOPS coatings into 3D titanium lattice structures and expects to graduate May 2012.

## 2010-2011 M.S. Graduate Students

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**Lacy Kamm, D.V.M.**

Lacy, originally from Toledo, Ohio, graduated from the University of Michigan with a B.S. in cellular and molecular biology and a minor in Spanish language. She then went to vet school at CSU. Afterwards, she completed an internship at Rood and Riddle Equine Hospital in 2008 and spent a year as a graduate student at Cornell University where she studied cytokine expression and inhibition. Lacy returned to CSU as a resident at the Veterinary Teaching Hospital in 2009 and joined the ORC as a Master's student under the direction of Drs. Laurie Goodrich and Dave Frisbie. Lacy, under the direction of Dr. Goodrich, is working on a project where she will explain the anatomy of the pastern joint in order to perform arthroscopy on the joint. Lacy is also working on a project with Dr. David Frisbie comparing protein biomarkers in osteoarthritic joints with microarray and PCR results. The goal of this study is to determine if gene expression in peripheral white blood cells can diagnose osteoarthritis in the horse.

## Research Scientist

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**Kirk C. McGilvray, Ph.D.**

Dr. McGilvray received his Ph.D. in mechanical engineering from Colorado State University in 2009 and is a research scientist within the Orthopaedic Bioengineering Research Lab. His research foci include monitoring and improving fracture healing, bio-micro-electro-mechanical systems, and cardio-vascular biomechanics. Dr. McGilvray leverages comparative animal models, ex vivo biomechanical techniques, and computer modeling methods to assess both basic science and industry driven questions in the field of medical bioengineering.

## Research Associates

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**Lynsey-Ann Bosch, B.S.**

Lynsey graduated from Michigan State University with a Bachelor's Degree in Veterinary Technology, and worked there as a technician throughout her education and for one year after graduation. At MSU, Lynsey helped with equine emergencies, daily treatments, and out-patient appointments. Lynsey moved with her husband to Colorado and worked at an equine private practice for one year, and taught at a veterinary technician training college for two years. Lynsey came to the lab in 2005 as an administrative assistant, and to implement an archiving computer program which will digitally document the research studies and associated data, and will make the wealth of information produced at the ORC easily searchable.

## Research Associates

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**Cecily Broomfield, M.S.**

Cecily received a B.S. in microbiology from California Polytechnic State University in 2000, and an M.S. in agriculture from Colorado State University in 2006. She is currently working as the research coordinator for the OBRL.



**Susan James, B.A.**

Susan earned her B.A. in Biology from CU and worked as a Biologist at the National Institutes of Health in Bethesda, Md. before returning to her native Colorado. Susan has worked for CSU in the histology field for the past two years. Previously she worked as a research associate at the CSU Arthropod-borne Infectious Diseases Laboratory where she assisted with research on ticks as vectors of West Nile Virus and Lyme disease. She also assisted with cancer treatment research at CSU's Vet Teaching Hospital. Susan joined the EORC team in June 2007 as a Research Associate and Histology Technician.



**Christina Lee, Ph.D.**

Christina Lee received her B.S. in animal science at UC Davis in December 2002, during which time she worked in Dr. Sue Stover's lab for Dr. Hill collecting data to investigate correlations between equine suspensory apparatus injury with suspensory apparatus failure and metacarpal condylar fractures. She then entered graduate school at UC Davis in 2003 in the Molecular, Cellular, and Integrative Physiology graduate group working in Dr. Clare Yellowley's laboratory. For her dissertation studies, Dr. Lee investigated the effects of oxygen tension on the expression of proteins associated with bone remodeling and hypoxic regulation of gene expression in osteoblastic cells. In 2007, Dr. Lee came to Colorado State University to work at the Orthopaedic Research Center as a Post-Doctoral Fellow under the mentorship of Dr. David Frisbie and Dr. John Kisiday to develop an *in vitro* model to investigate the cellular and molecular responses of chondrocytes to cartilage injury. In early 2011, she transitioned from her postdoctoral position into her current role as Laboratory Manager and Research Coordinator for the Orthopaedic Research Center.



**Nate Jensrud, B.S.**

Nate Jensrud joined the EORC as a Research Associate in March of 2010. He earned his B.S. in Forest Resources with an emphasis in Biotechnology from the University of Georgia in Athens, Ga. Jensrud managed a Plant Pathology laboratory at UGA for several years, studying the effects of *Phytophthora ramorum*, Sudden Oak Death, before moving to Colorado in 2008. He spent several seasons working for the federal government with the U.S. Forest Service and U.S. Geological Survey before coming to the EORC.



## Research Associates



**Bob Zink, B.S.**

Bob received his B.S. in biological sciences in 1969 from California Polytechnic State University. He has been at Colorado State University as a histotechnologist since 1990 and with the OBRL since 2007. His specialty is immunohistochemistry.



**Nikki Phillips, B.S.**

Nikki received her B.S. in Cell and Molecular Biology in May 1997 from Tulane University. She has been at Colorado State University since 2001 working in the Department of Pathology for a year before working for both Clinical Sciences and Biomedical Sciences. Nikki joined the ORC in January 2008 as a research associate to assist in the ORL.



**David A. Prawel, Ph.D., Associate Director, James Biomaterials Group, School of Biomedical Engineering, Department of Mechanical Engineering**

*Research Interests:* Biomaterials, biomimetic orthopaedic implant coatings, drug-delivery, tissue engineering of bone analogs.

Dr. Prawel is the Associate Director of the James Biomaterials Group, which is part of the Orthopaedic Bioengineering Research Laboratory and also a Senior Research Scientist in the School of Biomedical Engineering, Department of Mechanical Engineering. His current research is focused on developing “smart” coatings for orthopaedic implants, which are capable of enhancing bone growth while delivering drugs such as antibiotics directly to implant sites.

David earned his Ph.D. in the School of Biomedical Engineering at Colorado State University in 2010. He earned a B.S. in biology (1978) and an M.S. in natural sciences (1980), both at the State University of New York at Buffalo. He earned a second M.S. in comparative physiology at Rutgers University in 1982 while working towards a Ph.D., which was overshadowed by the then-fledgling computer industry. David also earned a certificate in Technology Mergers and Acquisitions from the University of Michigan Business School in 1992.



## **Director Gait Analysis Center/ ORC Staff Veterinarian**

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**Melissa King, D.V.M.**

Melissa graduated from Colorado State Veterinary School in 1997. After graduating, she did a one year internship at Rood and Riddle Equine Hospital in Lexington, Ky. Upon completion of her internship, Melissa returned to northern Colorado to begin her career as an equine ambulatory clinician focusing on equine lameness. After practicing for 10 years, Melissa sold her ambulatory practice to pursue a Ph.D. in equine lameness and rehabilitation, which she recently completed. Dr. King has moved into a faculty position as well as the Director of the Gait Analysis Center and the ORC Staff Veterinarian. Melissa's research interests are orthopaedic rehabilitation and the effects of underwater treadmill exercise on the biomechanics of the equine limb.

## **Research Coordinator**

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**Jennifer Suddreth, B.S.**

Jennifer Suddreth, originally from Altamont, Utah, graduated from Colorado State University in 2009 with a bachelor's degree in equine science and agricultural business. Suddreth started at the ORC on Feed Crew, and then came back after graduation working as an animal care technician. Jen joined the ORC full time as Barn Manager and Volunteer Coordinator in June 2010.

## **Administrative Staff**

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**Paula Vanderlinden, Program Coordinator**

Paula joined the ORC in March 2007 as Program Coordinator and as Dr. McIlwraith's personal assistant. Paula manages the Annual Stallion Auction, publishing of the annual newsletter and bi-annual lab report. Prior to working at CSU, Paula worked in the pharmaceutical industry.



**Candice Hastings, Accounting Manager**

Candice is the Accounting Manager for the Department of Clinical Sciences and in May 2011 began managing the accounting activity for the ORC.

## Orthopaedic Research Program

### Areas of Expertise of Personnel

#### Core Faculty

College of Veterinary Medicine and  
Biomedical Sciences

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C. Wayne McIlwraith, B.V.Sc., Ph.D.,  
D.Sc., FRCVS, Diplomate ACVS,  
University Distinguished Professor  
Clinical Orthopaedics  
Joint Pathobiology  
Gene Therapy  
Medical and Surgical Treatment  
Rehabilitation  
Cartilage Healing

David D. Frisbie, D.V.M., Ph.D.,  
Diplomate ACVS  
Cartilage Healing  
Biochemistry  
Molecular Biology  
Gene Therapy  
Clinical Orthopaedics

Laurie Goodrich, D.V.M./Ph.D.,  
Diplomate ACVS  
Clinical Orthopedics  
Gene Therapy  
Vector Development  
Cartilage Healing

Kevin Haussler, D.V.M., D.C., Ph.D.  
Complementary (Integrative  
Medicine)  
Rehabilitation  
Spinal and Sacroiliac Disorders  
Anatomy  
Biomechanics

Christopher E. Kawcak, D.V.M., Ph.D.,  
Diplomate ACVS  
Pathogenesis of Subchondral Bone  
Disease and Traumatic Joint  
Injury  
Histomorphometry  
Biomechanics  
Clinical Orthopaedics

John Kisiday M.S., Ph.D.  
Mechanobiology  
Cartilage Healing  
Biomechanical Characterization

Natasha Werpy D.V.M.  
Orthopaedic Imaging including  
Radiology, Computerized  
Tomography, MRI, and  
Ultrasonography

#### Collaborating Faculty

College of Veterinary Medicine and  
Biomedical Sciences

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Gary M. Baxter, VMD, M.S., Diplomate  
ACVS  
Clinical Orthopaedics  
Medical and Surgical Treatment  
Vascular Physiology

Nicole Ehrhart, D.V.M., M.S.,  
Diplomate ACVS  
Orthopaedic Oncology  
Gene Delivery and Tissue  
Engineering

Thomas R. (Tod) Hansen, B.S., M.S.,  
Ph.D.  
Gene Chip Technology

Ashley Hill, D.V.M./Ph.D.  
Epidemiology  
Experimental Design

Robert Norrdin, D.V.M., Ph.D.,  
Diplomate ACVP  
Orthopaedic Pathology  
Bone Histomorphometry

Richard D. Park, D.V.M., Ph.D.,  
Diplomate ACVR  
Orthopaedic Imaging including  
Radiology, Computerized  
Tomography and MRI

College of Engineering

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Susan P. James, Ph.D.  
Biomechanics

Ketul Popat, Ph.D., Assistant  
Professor, Department of Mechanical  
Engineering, School of Biomedical  
Engineering

Christian Puttlitz, Ph.D.  
Orthopaedic Biomechanics

College of Agricultural Sciences

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Jason Bruemmer, Ph.D.  
Gene Chip Technology

College of Natural Sciences

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Hariharan K. Iyer, B.S., M.S., Ph.D.  
Ann Hess, Ph.D.

College of Applied Human Science

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Raoul F. Reiser II, Ph.D.  
Musculoskeletal Biomechanics  
Custom Equipment/  
Instrumentation

## **Student Hourly Assistants at the ORC 2010-2011**

Alyssa Ball  
Kaylan Campbell  
Annaliese Catlin  
Casey Cooper  
Lauren Farrington  
Sarah Johnson

Stephanie Lowe  
Lauren Luedke  
Whitney McMillan  
Cassie Powers  
Jami Reed  
Lindsay Richardson

Jordan Schimming  
Ashlee Shelly  
Jen Suddreth  
Meghan Tumlinson

## **Volunteers at the ORC 2010-2011**

Carly Brown  
Liz Carazo  
Jack Conner  
Jenn Hartman

Nick Lemmel  
Kalie Petefish  
Maggie Rollert  
Amy Scott

Mason Vickerman  
Haley Wilson



## Graduate Students – Placement Since Inception

Student	Degree	Date Graduated	Current Position
Fahd Al-Sobayil	M.S.	1997	Assistant Professor, King Saud University, Riyadh, Saudi Arabia
Abigail Dimock	M.S.	1997	Currently a Ph.D. student, Equine Nutrition (Orthopaedic Related), Rutgers University
Becky Woodward	M.S.	1998	Graduate Researcher on S-V Dagon Research Vessel, University of British Columbia
Tina Anderson	Ph.D.	1998	Director of Marketing
Louise Southwood Perante	M.S.	1998/2002	Associate Professor, University of Pennsylvania School of Veterinary Medicine
Charles Hubbeling	Ph.D.	1999	Private consulting
Guy Beauregard	Ph.D.	1999	Senior scientist/researcher for private industry
Andrew Green	M.S.	1999	Engineering manager for private industry
Elisha Rentfrow	M.S.	1999	Private consulting
Tara Ruttley	M.S.	2000	Engineer for NASA
Carson Shellenberger	M.S.	2000	Engineer for private industry
Al Kane	Post-Doc	2000	Analytic Epidemiologist, USDA; Affiliate Faculty for Colorado State University's Center of Veterinary Epidemiology and Animal Disease Surveillance Systems
Julie Dechant	M.S.	2000	Assistant Professor, University of California Davis
Troy Trumble	M.S.	2000, 2004	Associate Professor, University of Minnesota
Chengcheng Lui	M.S.	2001	Continuing in school
Jana Read	M.S.	2001	Employed in quality control
Erin Peterson	M.S.	2001	Faculty Member, Department of Animal Science, University of Maryland
Anne DePalma	M.S.	2002	
Joel Millets	M.S.	2002	Employed at Osteotech, Allograft Company
Carolyn Skurla	Ph.D.	2002	Assistant Professor, Baylor University
Louise Southwood Perante	Ph.D.	2002	Faculty Member, University of Pennsylvania School of Veterinary Medicine
Awad Al-Zaben	Ph.D.	2003	Faculty Member, Electronics Engineering Department, Yarmouk University, Irbid, Jordan

## Graduate Students – Placement Since Inception

Student	Degree	Date Graduated	Current Position
Sophie Morisset	Ph.D.	2003	Assistant Professor, Department of Clinical Sciences, Université de Montréal
Thomas Young	M.S.	2003	Currently job searching
Colin Scruten	M.S.	2004	Private practice, Alberta, Canada
Lea Rempel	Ph.D.	2004	Post-Doctoral Fellow, University of Kansas Medical School, Currently, Research Scientist, United States Meat Animal Research Center, Clay Center, Neb.
Chris Sorensen	Ph.D.	2004	Post-Doctoral, National Mass Spectrometry Facility, Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Wash.
Brandon Santoni	Ph.D.	2006	Postdoctoral Research Fellow, ORBL, Colorado State University
Katja Duesterdieck	Ph.D.	2006	Assistant Professor, Oregon State University
M. Shearin	D.V.M./Ph.D.	2006	Assistant Doctoral Fellow, University of Tennessee
Valerie Perino	M.S., Ph.D.	2007	Completed Ph.D., Equine Orthopaedic Research, Colorado State University
Sam Hendrix	M.S.	2008	Equine practice, Utah
Ty Wallis	M.S.	2008	Equine specialty practice
Erin Contino	M.S.	2009	Final year D.V.M. student
Ryan Carpenter	M.S.	2009	Equine practice, Southern California
Jennifer Antonnici	Ph.D.	2010	University of California
Christina Lee	Post-Doc	2010	Lab Manager, Orthopaedic Research Center
Myra Barrett	M.S.	2010	Assistant Professor CVMBS, CSU
Carrie Adrian	Ph.D.	2011	Director of Rehabilitation Services, VCA Animal Hospitals
Katrina Easton	D.V.M./Ph.D.	2011	University of Sydney
Melissa King	Ph.D.	2011	Staff Veterinarian, Orthopaedic Research Center, Clinical Instructor Sports Medicine and Rehabilitation Service, CSU

## **Surgery Residents Supervised (and Outcome)**

<b>Resident</b>	<b>Years of Residency</b>	<b>Date Achieved Board Certification in the American College of Veterinary Surgery</b>
G.W. Trotter	1979-1981	1983
A.J. Nixon	1980-1983	1985
G.S. Martin	1980-1983	1986
R.M. De Bowes	Phase III, 1983-1984	1985
K. Sullins	1981-1984	1986
J.V. Yovich	1983-1986	1987
A.L. Bertone	1983-1986	1988
K.J. Easley	Phase II 1986, Phase III 1986-87	
C. Kobluk	1987-1988	1990
K.T. Gibson	1986-1989	1990
S.B. Gustafson	1986-1989	1990
M.J. Reeves	1986-1989	1990
D. French	1988-1990	1992
J.F. Foland	1989-1991	1994
R.D. Howard	1990-1992	1994
C.R. Ray	1991-1994	1998
C.E. Kawcak	1992-1995	1996
D.D. Frisbie	1993-1996	1999
L. Southwood	1995-1998	2000
T. Trumble	1996-1999	2000
J. Dechant	1997-2000	2001
J. Alldredge	2000-2003	2004
C. Scruton	2001-2004	2004
E. Farstvedt	2002-2005	2005
S. Hendrix	2003-2006	2006
J. Joyce	2005-2007	2007
T. Wallace	2006-2008	2008
R. Carpenter	2007-2009	2010



### History

The Orthopaedic Research Center (ORC) began as a multidisciplinary equine program dedicated to finding methods to treat and prevent equine musculoskeletal disease and injury. Prior to 1984, the program's research was primarily clinical. During this time, many of the techniques for arthroscopic surgery currently used to treat joint problems more effectively and to enable continued athletic function were developed at CSU. We also identified and defined a number of new clinical conditions and documented some of the best methods for diagnosis and treatment. A major goal of the program has always been to find solutions to musculoskeletal problems, especially joint injuries and arthritis. The researchers strive to offer the best possible treatment of clinical cases with continual and critical assessment of the results, which are then used to modify treatments and direct the research toward disease prevention. The program's goals are to use state-of-the-art research techniques to find new methods to rehabilitate damaged joints, to prevent or decrease the occurrence of joint disease and musculoskeletal injuries, find methods of early detection and develop better treatments to prevent permanent damage to injured joints and validate manual therapies and rehabilitation techniques. Significant collaboration with the College of Engineering, School of Bioengineering and the Orthopaedic Bioengineering Research Laboratory (OBRL) as well as the Department of Health and Exercise Sciences has added considerably to our research strengths. In recent years considerable human-based funding (Foundation, NIH and corporate) has been added to ORC and OBRL support.

### Research Activities

The following are the research focuses of the Orthopaedic Research Center. Details of recent and current projects can be found on pages 105-182.

#### 1. *Musculoskeletal Tissue Healing*

Until a few years ago we have principally addressed articular cartilage healing and will continue to do so, but we have enlarged the focus to include tendons, ligaments, and menisci. For instance, treatments of tendonitis including A-cell therapy, extracorporeal shock wave therapy (ESWT), and mesenchymal stem cell therapies have been assessed.

#### 2. *Early Diagnosis of Bone and Joint Disease*

This area includes the development of novel imaging techniques (present and future), body fluid biomarkers, and also molecular monitoring. The uses of these early diagnostic techniques include a) Evaluation of the pathogenesis of musculoskeletal disease, b) Early detection of disease processes, c) Monitoring of therapy, with the long term goal of preventing severe osteoarthritis or failure of joints, tendons, ligaments, and menisci.

#### 3. *Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*

Catastrophic injury is a major problem in the equine athletic industry and we, as well as researchers elsewhere, have demonstrated that the severe fractures and injuries start as microfractures in the subchondral bone. Our ongoing mission is to develop methods of detecting this damage in the clinical patient before it becomes severe, irreversible damage. Exercising horses have been followed with imaging techniques including computed tomography (CT) and MRI, nuclear scintigraphy, defined sentinels of early damage, and fluid biomarkers as a means of identifying horses at risk studied with promising results. Recently, biomechanical and modeling studies have been done to monitor early events in bone disease. Modeling has been used to look at the pathogenesis of condylar fractures and other disease processes as well as mapping of pressure distribution and articular cartilage thickness in equine joints. Other factors that can potentially contribute to traumatic musculoskeletal injury including race track surface and conformation have also been part of this research focus.

## Program Synopsis

### *4. Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse*

Objective evaluation of currently available pharmaceutical agents as well as new potential ones have been a significant focus of our work. These evaluations also include examination of specific biological inhibitors including gene therapy, novel protein therapies, and mesenchymal stem cells therapies. These newer therapies offer the potential of inhibiting the disease process sufficiently early so that the need for palliative drugs currently used is decreased.

### *5. Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease*

This is a newer focus that includes objective assessment of integrative therapies including physical manipulation and acupuncture for management of musculoskeletal disease and pain as well as rehabilitative techniques of swimming under water treadmilling and hyperbaric therapy. This area also includes study of the pathogenesis of musculoskeletal problems biomechanically and using gait analysis (kinetics, kinematics) and electromyography (EMG), as well as novel methods of pain detection.

In recent years, the Orthopaedic Research Center has acquired the personnel and technical abilities to do more sophisticated orthopaedic research and to address critical questions at a more basic level. Development of this expertise has allowed us to use the horse as a model to resolve problems in human arthritis where conditions are comparable to those in horses. This has led to collaborations with human health researchers, foundations, and industry.

## Impact

As a preeminent equine orthopaedic research program, both nationally and internationally, the Orthopaedic Research Center provides critical new findings of significant clinical impact and has been able to attract talented students who wish to pursue

careers in orthopaedic research. Students choose this program because of its excellent reputation and because of the opportunities they have to be involved in research during their undergraduate and pre-veterinary programs. Many pre-veterinary students have served as volunteers in the equine orthopaedic research program over the past 10 years; this allows students to develop a high level of research expertise during this undergraduate experience. This involvement encourages students to pursue advanced degrees and ultimately research careers rather than traditional private veterinary practice. Our program also impacts undergraduate and pre-veterinary education by applying findings from research studies to clinical veterinary medicine.

The breadth of dissemination of information from the Orthopaedic Research Center is extensive, with information distributed to graduate and undergraduate students in eight Departments within five Colleges at Colorado State University. Many faculty members from these five Colleges who are participants in the Orthopaedic Research Program are internationally recognized; they are therefore able to share research findings worldwide to academia, the equine industry, the scientific community, and private biomedical industry. The Orthopaedic Research Center's extensive collaboration with the Steadman Philippon Sports Medicine Foundation and biotechnology companies has significantly impacted the treatment of humans with orthopaedic injuries and osteoarthritis. Human medicine, as well as veterinary medicine, has been positively affected by the dissemination of the Orthopaedic Research Center's findings.

## Program Trends

Over the last 10 years, funding for our orthopaedic research and specialized personnel availability has increased dramatically. Until 1994, orthopaedic research was being performed by faculty members within the Department of Clinical Sciences. Since that time, the Orthopaedic Research Center has acquired seven full-time faculty senior scientists and also has two Bioengineering Faculty in our Center. To

## Program Synopsis

support the work of the Faculty Researchers, we now have eight research associates. We have had 10 Ph.D. students and seven M.S. students in the program the past two years. Current funding is around \$4 million annually. Thanks to generous private donors, the construction of a new Orthopaedic Research Center facility and the remodeling of the existing laboratory have been completed. In addition, a state-of-the-art equine MRI facility has been in operation for five years, and this has also been funded by private donations. We have also received three \$3 million University Endowed Chairs from Barbara Cox Anthony, Iron Rose Ranch, and Abigail K. Kawanakoa. We have also acquired a \$1.5 million Chair in Musculoskeletal Imaging from the estate of Kenneth and Virginia Atkinson. We continue to pursue endowed funding to make all of our positions permanent. In addition, the Orthopaedic Bioengineering Laboratory has had two full-time faculty senior scientists, five Ph.D. students and 12 M.S. students in the past two years.

### Program Goals

#### Goals Accomplished 2010-2011

**1. Construction of Equine Gait Analysis Building.** This building has been completed and been critical in the completion of two major Ph.D. projects: 1) The assessment of underwater treadmill in an experimental model of osteoarthritis by Dr. Melissa King in which kinetic and kinematic evaluations enabled us to show that underwater treadmill not only decreased the amount of osteoarthritis developing but also improved proprioception and postural balance in these patients and 2) The evaluation of electromyographic and kinematic changes following cruciate ligament injury and rupture in the dog by Dr. Carrie Adrian. Dr. King remains at the Orthopaedic Research Center as a clinical instructor and in addition to being the supervisor of the Gait Analysis Center, will also be the ORC staff veterinarian and is playing a pivotal role in the new Sports Medicine Program.

**2. Achieve Extramural Research Funding to Continue Quality Orthopaedic Research.** The NIH Program grant in collaboration with MIT (Dr. Frisbie PI of sub-contract) has been completed. The NIH KO8 training grant of Dr. Laurie Goodrich completed its second and third years with excellent progress in developing an adenoassociated viral gene vector. In addition, Dr. Goodrich (PI Sub-contract) and Dr. McIlwraith collaborated in an NIH RO1 grant with Dr. Connie Chu at the University of Pittsburgh in which we did a 12-month study evaluating the value of bone marrow derived mesenchymal stem cells (BMSC) in diluted fibrin to repair articular cartilage. This project also involves Dr. Bob Sah and we have added a number of critical biomechanical and imaging parameters to evaluate outcomes and results will be forthcoming. Last but not least, McIlwraith and Frisbie are Co-PIs on a sub-contract with Dr. Steve Trippel of Indiana University on another NIH RO1 grant evaluating gene therapy and cartilage repair. It is expected that the 12-month equine study that is part of this grant will commence at the beginning of 2012.

**3. Development of an Equine Ambulatory Sports Medicine Service.** An equine ambulatory sports medicine service has been developed over the period of this report by Dr. Chris Kawcak which is an extension of the work that Dr. Kawcak was doing with research associate Jodi Callison in providing service to some of our major donors. There is increasing demand from equine clients for this service offering state of the art expertise in equine musculoskeletal problems in athletic horses. Dr. Melissa King has been retained to work with Dr. Kawcak and Jodi as well as our equine sports medicine residents. This service commenced in 2011 and has exceeded our expectations in demand.

**4. Establishment of Equine Sports Medicine and Rehabilitation Residencies.** A new American veterinary specialty, The American College of Veterinary Sports Medicine and Rehabilitation has been developed and was accredited by the American Veterinary Medical Association in May 2010. There are 27 Charter Diplomates established

## Program Synopsis

by a nomination and Delphi election system. Four of our faculty, Drs. McIlwraith, Haussler, Kawcak, and Frisbie, were made Charter Diplomates of the new College. We have established an equine sports medicine and rehabilitation residency program to train future specialists. Our first resident, Dr. Dora Ferris commenced in July 2010 followed by our second resident, Dr. Erin Contino starting in July 2011. At the moment, we have the only equine sports medicine residency program in the U.S. and the College is using our program as a template.

*5. Unrestricted Funding from Donors and Foundations: The period 2010-2011 has been one of continuing to function without any loss of faculty and staff in these recessionary times.* In 2008-2009, the corpuses of our four endowed chairs decreased markedly. Fortunately, thanks to the generosity of Herbert Allen, Jim Kennedy (The Cox Family Foundation), Gail Holmes, and Abigail Kawanakoa, we have been able to make up for necessary operating expenses and continue to fund new cutting edge projects. In the 2010-2011 period we have made up some of the deficit with increase in corpus by 6.25% in FY10 and 16.75% in FY11. As mentioned before, we have been able to maintain all our positions thanks to support with operating expense deficits.

*6. Promotion of Orthopaedic Research Center Faculty in 2011.* Both Drs. John Kisiday and Laurie Goodrich were promoted to Associate Professor and gained tenure and Dr. Chris Kawcak was promoted to Full Professor. In 2010, Dr. Christian Puttlitz, the Director of OBRL, was promoted to Associate Professor with tenure and in 2011, was made a Monfort Chair. This is a two year position with supplementary funding and is only given to two faculty members in the whole university.

### Current Goals

1. Continue to achieve adequate funding from Federal Grant Agencies, industry, and private funding.

2. Identify funding for construction of a building to house offices for faculty and graduate students for both the Orthopaedic Research Center and the Orthopaedic Research Bioengineering Laboratory.

3. Create endowed funding for two staff positions, one post-doctoral fellow and scholarships for graduate students.

4. Provide quality education to Undergraduate PVM and Graduate Students.

5. Continue to do State-of-the-Art Research within the Orthopaedic Research Center's Research Focuses.

### Research Goals

#### Research Goals Achieved 2010-2011

##### 1. Focus 1 Musculoskeletal Tissue Healing

We continued to explore the use of mesenchymal stem cells (MSCs) for repair of articular cartilage and other tissues in the joint and how the cells can be optimally manipulated. Current practices for the preparation of bone marrow mesenchymal stem cells (BMSCs) for equine musculoskeletal injuries involve culture-expansion to obtain millions of cells for single treatment. While tissue culture plastic is the standard surface on which BMSCs are culture-expanded, we reported that coating tissue coated plastic with fibrinogen precipitated from blood plasma increases BMSC proliferation without effecting differentiation potential. Fibrinogen harvested from autologous blood is an inexpensive means of creating a growth surface that is superior to tissue culture plastic.

The comparison of the sternum and ilium as a source of BMSCs was evaluated. It was found that both sternum and ilium offer a rich supply of BMSCs that have similar growth rates. It was also shown that the highest concentration of BMSCs is in the first 5 mls of bone marrow aspirate. In a second related study it was found that the cells acquired from equine sternum and ilium were also similar in their differentiation capacity *in vitro*. In a third study, we showed that



## Program Synopsis

BMSCs from the ilium were superior in chondrogenic potential (ability to grow new cartilage) compared to BMSCs from the sternum.

The basic science of subchondral bone microfracture in articular cartilage repair in the horse (and how this information can be extrapolated to human cartilage repair) was reviewed by Drs. Frisbie and McIlwraith and published in the journal *Cartilage*. In addition, Drs. McIlwraith and Frisbie did a collaborative review with Drs. Fortier and Alan Nixon from Cornell University on the use of equine cartilage repair models to evaluate the best technique for horses and humans. This involved excellent discussion as to the comparison of results between the two research groups and what as the best way forward in the use of the equine model that has become widely accepted as a human pre-clinical model. This review has also been recently published in the journal *Cartilage*.

Another pivotal study that was completed in 2011 was a 12-month study evaluating the effect of bone marrow derived mesenchymal stem cells (BMSCs) administered intra-articularly four weeks after creation of full thickness subchondral microfractured defects on the medial femoral condyle. There was superior firmness of the repair tissue in the BMSC treated groups as well as enhancement of aggrecan content (a critical component that is typically very deficient in repair studies done thus far). This study was published in *Arthroscopy* in 2011.

Another study headed up by Dr. Frisbie evaluated the effects of clinically relevant autologous conditioned blood products (IRAPII™, ACP™, and various other PRP products) on the anabolic properties equine digital flexor tenocytes and suspensory ligament fibroblasts to examine these products on tendon healing. This was recently presented at the AAEP meeting and is being submitted for publication.

### 2. Focus 2 Early Diagnosis of Bone and Joint Disease

Work has continued in the development of a wireless gait analysis system for horses to enable us to measure three dimensional forces on the hoof at fast exercise. This project is the Ph.D. project of Dr. Valerie

Moorman, supervised by Drs. Chris Kawcak and Raoul Reiser. The sensor developed by EquySys has been evaluated in comparison to the Gold Standard of force plate analysis and although promising, some inadequacies found. This work is in press in the *American Journal of Veterinary Research*. Work is continuing on a modified system of an inertial measurement unit (IMU) in collaboration with Dr. Mick Peterson at the University of Maine (as well as a strain gauge system in collaboration with Dr. Peterson and Dr. Jeff Thomason).

Dr. Lacy Kamm, a large animal surgery resident (working with Drs. Goodrich and McIlwraith), has also done a detailed descriptive study of the equine proximal interphalangeal joint (pastern joint) (PIP) using magnetic resonance imaging, contrast arthrography, and arthroscopic examination which has defined completely our ability to diagnose and treat problems in the dorsal and palmar pouches of the PIP joint. It was found arthroscopic approaches allow adequate access to the proximal interphalangeal joint for diagnosis and anatomy surrounding the joint is described. There are limitations with a surgical approach to certain areas.

The study on radiographic changes in yearling cutting horses has progressed from a detailed evaluation of the incidence of lesions of looking at the significance of those changes with regard to soundness/lameness and cutting performance. The initial study was performed by Dr. Erin Contino (and is in press in *Equine Veterinary Journal*) and the latter study by Dr. Barrett (see research reports) is in submission at *Equine Veterinary Journal*.

Dr. Werpy has also done studies on a novel technique for ultrasound examination of the suspensory ligament, the pioneering comparative study of MRI changes compared to histologic changes of the navicular region in the horse. Working with Dr. Frisbie and Lauren Farrington, a pre-veterinary student, she also developed better techniques for radiographic-guided needle placement into the collateral ligaments of the distal interphalangeal joint. These were presented at AAEP and are included in the abstracts.

## Program Synopsis

### 3. Focus 3 Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease

Dr. Kawcak, working with student Chelsea Zimmerman, showed in previous work that horses that suffered from third metacarpal condylar fractures had a significant difference in the shape of their third metacarpal bones compared to those horses that did not fracture. In order to determine how abnormal shape influences the stresses across the joint, Dr. Katrina Easton, a recent Ph.D. graduate from the ORC, recently completed a finite element model of the fetlock joint and showed that the abnormal shape pattern found in horses that fractured can lead to a significant reduction in loading at the site of fracture, leading to the conclusion that those bones may not be properly conditioned for racing due to this shape.

Two additional studies have been recently completed as part of the Global Equine Research Alliance (GERA) in New Zealand. Drs. Kawcak and McIlwraith with Dr. Elwyn Firth at Massey University investigated the effects of early exercise on the metacarpophalangeal (front fetlock) joints assessing bone density, distribution using computed tomography (CT) as well as assessing articular cartilage metabolism and histologic assessment of synovial membrane, articular cartilage, and subchondral bone. Horses that were exercised since near birth had fewer gross lesions in the joints, greater bone fraction in the dorsal lateral aspect of the distal metacarpal condyle and higher bone formation rate compared to non-exercised horses. This paper has been published in *American Journal of Veterinary Research*, 2010.

A second study continuing GERA work was done by Drs. Firth and Neil Broom at Auckland University in New Zealand with Drs. Kawcak and McIlwraith looking at severity of articular lesions in the mid carpal joints between exercised and non-exercised horses as well as quantifying swelling behavior of the articular cartilage extracellular matrix. The investigators found a wide range of defects in the joint surfaces but there were no significant differences in the number or severity of lesions between the two

groups. There were also no significant differences in material properties of the articular cartilage between both groups with regard to swelling behavior and the conclusion was that early conditioning had no negative effects of the articular cartilage on the mid carpal joints. Previous work had shown beneficial effect on the chondrocytes in the articular cartilage of the metacarpophalangeal joints.

Dr. Christina Lee, working with Drs. Frisbie and McIlwraith has been developing a reproducible model of cartilage injury that can be done in a dish in the laboratory. Mechanical load is applied to rapidly compressed cartilage explants to 60% of the total thickness and success was demonstrated at inducing histologic changes in cartilage that mimic injury induced OA that we see clinically. Using co-culture models of cartilage with synoviocytes under load we hope to be able to screen molecular based therapies including gene therapy to alter the progression of OA in response to injury and minimize the use of testing in live horses. This research has been accepted for publication and is hoped that it will be a useful *in vitro* model (avoiding any sacrificing of live animals) in evaluating putative therapies for OA.

### 4. Focus 4 Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse

An *in vitro* study evaluating two different platelet rich plasma (PRP) formulations on anabolic and catabolic activities in equine cartilage and meniscal implants was led by Drs. Kisiday, working with Drs. McIlwraith and Frisbie in collaboration with Drs. Rodkey and Steadman of the Steadman Philippon Research Institute. Various proclamations have been made regarding the various formulations and the importance of platelet numbers and cells. Single spin technique with relatively low platelet counts was shown to be associated with higher protein and proteoglycan synthesis than a traditional double spin PRP technique. In addition, gene expression of the proteoglycan catabolic enzyme (ADAMTS-4 expression) was lowest for single spin PRP and suggest that single spin PRP preparation may be the



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most advantage for intra-articular applications and double spin systems (despite higher platelet counts) should be considered with caution.

In another study, mesenchymal stem cell proliferation in expression of growth factors in response to shockwave treatment was evaluated *in vitro*. Although using proliferation cultures of stem cells, the first application of shockwave treatment decreased growth relative to unshocked MSCs but subsequent treatments did not affect MSC proliferation. This result suggests that MSCs are capable of acclimating to shockwave therapy. The conclusion was that no immediate benefit of shockwave therapy was observed when stem cells were administered but it seems that multiple shockwave treatments can be administered to defects without harming the repair response subsequent to MSC injection.

In addition, a second study on shockwave therapy in our *in vivo* equine OA model showed positive results in the subchondral bone (and no harmful side effects) in addition to the symptom modifying effects in the articular cartilage as previously reported.

Considerable work has continued with the adenoassociated viral vector that Dr. Goodrich has been working with Dr. Jude Samulski. The study done by Nikki Phillips (research associate in the lab) in collaboration with Drs. Goodrich, Samulski and McIlwraith showed that age does not appear to significantly influence the efficacy of gene transduction using a self-complimentary adeno-associated viral vectors (scAAV) in synoviocytes and chondrocytes in horses. Because the adult population may be more prone to receiving gene therapy then neonatal or young animals this is valuable information.

Another study headed up by Dr. Goodrich showed that the scAAVIL-1ra vector injected into metacarpophalangeal and middle carpal joints of horses produced IL-1ra protein for 23 and 183 days, respectively. There was no evidence of intra-articular toxicity which has been a concern with previous regimens and the horses remained sound

for the duration of the testing period. This work is continuing with higher numbers in order to evaluate the ideal dose to be used in osteoarthritis. The length of expression is particularly exciting.

Another study on scAAV transduction efficiencies in joint tissue monolayer and explant cultures and the effects of synovial fluid neutralization was done by Dr. Goodrich's Ph.D. student, Daniel Hemphill, working with Drs. Goodrich, McIlwraith and Samulski. We've shown that gene transduction of chondrocyte and synovial monolayers significantly different than cartilage synovial explants and this study highlights the necessity to test gene therapeutic tissue *in situ*. Neutralizing antibodies were found to exist in both the synovial fluid and the serum which could reduce efficient gene transduction. It is important to determine which serotypes have antibodies that exist in the horse. A pivotal *in vitro* study was done looking at gene and protein expression with autologous conditioned plasma (ACP™) and comparing the newer IRAPII™ to the earlier product IRAPI™. This study has recently been published in the Equine Veterinary Journal and showed superior IL-1ra protein production and more particularly, significantly improved IL-1ra/IL-1 ratios with the IRAPII™ system compared to the IRAPI™. In addition, the concentrations of a harmful cytokine, TNF-α were higher in the IRAPII™ system.

### 5. Focus 5 Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

The last two years have seen the completion of the pivotal underwater treadmilling study in horses. Dr. Melissa King completed her Ph.D. working with Drs. Kawcak, Haussler, Reiser, and McIlwraith and showed marked therapeutic effects of underwater treadmilling in decreasing symptom and disease effects in equine experimental osteoarthritis in the knee. In addition, the underwater treadmilled horses showed considerable improvement in proprioception parameters. This is the first scientific evaluation of underwater treadmilling in horses and reinforces the positive clinical results obtained. A randomized controlled study in clinical cases after arthroscopic

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surgery in a study at Pegasus Training Center in Seattle by Drs. McIlwraith and Haussler working with Drs. Jim Bryant and Mark Dedomenico is ongoing.

Another project under Focus 5 was the use of an equine back profiling system for objectively measuring dorsal trunk contours as it relates to saddle fitting. The equine back profiling system (EBPS) was able to reliably capture a wide variety of dorsal trunk contours and provides a significant advancement as we are now able to better communicate findings or changes in dorsal trunk contours related to saddle fit to colleagues, owners, and trainers.

Details of these projects above are in the Research Summaries.

### Future and Current Research at the ORC

The 2010-2011 years have been challenging but exciting times for the Orthopaedic Research Center. The scientists have continued to achieve considerable extramural funding in the last two years, including long-term funding to offset the economic difficulties associated with endowed funding.

The research projects continue to revolve around the programs five main focuses.

#### 1. Focus 1 Joint Tissue Healing

Another study headed up by Dr. Frisbie is evaluating the effects of clinically relevant autologous conditioned blood products (IRAPII™, ACP™, and various other PRP products) on the anabolic properties equine digital flexor tenocytes and suspensory ligament fibroblasts to examine these products on tendon healing.

A 12-month study to evaluate the effect of implantation of the novel chondroprogenitor cells produced from the superficial layers of articular cartilage is under way. Earlier work by Dr. Helen McCarthy, working with Professor Charlie Archer at the University of Cardiff, has shown superiority in

*in vitro* production of cartilage tissue and avoidance of terminal differentiation of the repair tissue into bone. The technique is now being evaluated in a 12-month study with full thickness defects on the medial trochlear ridge of the femur with the novel chondroprogenitor cells implanted in fibrin.

An eight-month equine study evaluating bone marrow derived stem cells in a fibrin/PRP scaffold has been completed. This project has been led by Dr. Laurie Goodrich (with Dr. McIlwraith) as part of a sub-contract for an NIH RO1 grant with Dr. Connie Chu at the University of Pittsburgh. Dr. Bob Sah at UC San Diego, and Dr. Lisa Fortier at Cornell University, are also collaborators.

A long-term study evaluating a new model of tendonitis is being led by Dr. Dave Frisbie working with Drs. Alex Valdez and Wayne McIlwraith. We are using a traumatically induced model previously described by investigators at Utrecht University in Holland. This 12-month study is comparing imaging of the lesions with ultrasound compared to contrast CT and contrast MRI. It is hoped that a better system of evaluating therapies for tendonitis will be the result of this work and the plan is to incorporate preliminary finding into a critical study on a novel stem cell technique. In conjunction with this project, Dr. Melissa King is collecting kinematic and kinetic data from this group of horses to investigate the changes in forelimb biomechanics associated with acute tendon injury and repair. This portion of the study is expected to be a noninvasive method for determining the biomechanical extent of the repair process and may be used in future studies to evaluate the efficacy of various treatment options for tendon injury.

#### 2. Focus 2 Early Diagnosis of Bone and Joint Disease

Dr. Kawcak is leading two studies at this time that focus on the use of computed tomography for diagnosis of stifle and suspensory ligament injuries. The goal is to develop a rapid scanning technique that can be used for these problems.

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We continue to pursue validation of synovial fluid and serum biomarkers for musculoskeletal disease in horses. Analysis of the biomarkers from the three-year GERA study has been completed with the help of Dr. John Cockrem at Massey University. These biomarkers are currently being analyzed but will show changes from birth to the end of the three-year-old year of racing Thoroughbreds.

The study where gene chip microarray was used to find novel markers in our experimental OA model has finally been analyzed and a manuscript submitted. This work has been done by Dr. Lacy Kamm with Drs. Frisbie and McIlwraith.

Also we are embarking on exploration of other biomarkers in collaboration with Dr. Peter Clegg at the University of Liverpool as well as examining potential tendon biomarkers with Dr. Dick Heinegård of the University of Lund in Sweden.

A long-term goal of this work is identifying biomarkers that may predict exercise-induced tendinopathy.

### *3. Focus 3 Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*

Work continues with finite element modeling to understand the pathogenesis of traumatically induced osteochondral disease and fractures in the equine athlete. This work is headed by Dr. Kawcak.

### *4. Focus 4 Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse*

Work continues with the evaluation of intra-articular PRP, IRAP<sup>™</sup> as well as mesenchymal stem cells (MSCs) as intra-articular therapies for equine traumatic arthritis and OA.

Dr. Laurie Goodrich is continuing work on gene therapy vectors, specifically in an adenoassociated virus (AAV) to deliver important genes to cells of joint tissues such as cartilage, synovium and mesenchymal stem cells. The collaboration with Dr. Jude Samulski

at The Gene Therapy Center at UNC has already resulted in a paper published in the journal Human Gene Therapy Journal and describes the best serotypes of these vectors in transfection to synovial membrane and cartilage after intra-articular injection as identified by green fluorescent protein expression. Currently *in vivo* dose titration studies are being done and this will lead to testing an AAV-IL-1ra in our equine osteoarthritis model. It appears that these vectors will safely and efficiently deliver important gene sequences to the cells of the normal or injured joint and result in long-term protein expression. This work was initially supported by an NIH KO8 grant obtained by Dr. Goodrich (with Dr. Jude Samulski of the University of North Carolina and Dr. McIlwraith as co-mentors) and the development of vector costs for the initial study as well as of *in vivo* dose titration study has been supported by funds donated by Herbert Allen of Allen & Company for cutting edge techniques. These funds will also support the study of AAV-IL-1ra in our equine osteoarthritis model.

In addition to assessment of these new therapies, our pursuit of better biological therapies continues. The use of bone marrow-derived mesenchymal stem cell therapies has been used in a clinical study of soft tissue healing in joints has been submitted for publication.

A 12-month-long term study with the traumatically induced tendonitis model is evaluating intralesional bone marrow-derived MSCs for tendonitis. This 12-month study is also using novel imaging techniques of contrast CT and contrast MRI to evaluate the healing and compare it to tendon strength at the end of twelve months.

Dr. Kisiday is also assessing the influence of dynamic loading on mesenchymal stem cells and their activity.

### *5. Focus 5 Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease*

We are currently applying rehabilitation techniques through the Equine Sports Medicine service. Dr. Melissa King is leading this effort and applying the results from her graduate work to this endeavor. Dr.

## Program Synopsis

King submitted two manuscripts on her Ph.D. work in underwater treadmill exercise in horses.

Carrie Adrian is in the process of finalizing the first manuscript reporting EMG changes after canine cruciate ligament injury and rupture for submission. Dr. Adrian will also be involved in a new collaboration on clinical canine rehabilitation.

The collaborative studies with Pegasus Rehabilitation on carpal chip rehabilitation and SDFT rehabilitation with hyperbaric oxygen therapy, underwater treadmill exercise, swimming, and a progressive exercise program in Thoroughbred racehorses is ongoing. It has been very difficult getting sufficient cases for this randomized controlled study, but over 50 percent of the horses have been treated and it is hoped that this study will be completed in 2012.

With the development of the new clinical Sports Medicine Program as well as offering rehabilitation to

patients, it is hoped to extend our database regarding rehabilitation and this work will be done along with our two Sports Medicine and Rehabilitation residents, Drs. Ferris and Contino.

Dr. Haussler is collaborating on a NASA grant with Dr. Puttlitz investigating *in vivo* bone healing in unweighted space conditions and the role of physical therapy modalities to stimulate bone healing in this environment. Low intensity pulsed ultrasound will be used in pilot studies to assess its effect on bone healing in this animal model during treadmill locomotion.

Dr. Haussler is also collaborating with Dr. Puttlitz investigating a biological microelectromechanical system (Bio-Mems) for *in vivo* application for measuring tendon strain and healing in response to physical therapy in a sheep model. Passive joint range of motion will be used in pilot studies to assess its effect on tendon healing in this animal model.

## Research Techniques Available at the Orthopaedic Research Center

The Orthopaedic Research Center at Colorado State University is a comprehensive research facility predominantly focusing on the prevention and repair of orthopedic disease in humans and animals. In addition to protein biomarker analysis and development, this program is supported by several molecular biology applications such as antibody purification, real time PCR assay development and gene expression analysis, cell and tissue culture techniques, adenoviral construction and cloning, gene chip microarray, biomechanical testing, and histological procedures. As the support structure for biomedical research continues to expand with modern medical discoveries and advances, the Orthopaedic Research Center will continue to provide ground-breaking research for the future.

Below is a brief list of the laboratory applications and services provided by the ORC.

### 1. Biomarker Analysis

*Fully equipped to run any commercially available absorbance or fluorescence biomarker immunoassay in 96 or 384-well plate format, using Molecular Devices SpectraMax 384 plus, microplate absorbance/transmittance reader, as well as a Gemini-XS Fluorometer.*

*Extensive experience with the following biomarker assays:*

#### • Detection of Cartilage Markers:

- **Alcian Blue:** Standardize measurement of 35S labeled proteoglycan complexes.
- **C1,C2:** An assay to standardize the measurement of Types I and II collagen degradation.
- **CPII:** An assay to measure type II collagen carboxy propeptide (C-propeptide).
- **CS-846:** Measurement of Aggrecan Chondroitin Sulfate 846 Epitope.
- **Eq. Col 2  $\frac{3}{4}$  (CEQ):** An assay to quantify equine specific Type II collagen, which has also been proven to work with canine fluid.

- **GAG DMMB:** An assay for standardized measurement of glycosaminoglycans in biological fluids and/or tissues.
- **Prolagen-C:** Measurement of C-Terminal propeptide Type-I collagen.
- **Pyd Assay:** An assay to standardize measurement of pyridinoline crosslinks in serum and urine.
- **Pyrilinks-D:** To standardize measurement of deoxypyridinoline crosslinks in urine.
- **TCA:** Assay to measure 3H content in media or cartilage digested samples.
- **YKL-40:** Assay for measurement of YKL-40, human cartilage glycoprotein 39, in serum.

#### • Detection of Bone Markers:

- **C1,2C:** An assay to standardize measurement of Type I and II collagens (378 assay, MMP1 and MMP13).
- **Metra™ BAP:** Quantification of bone-specific alkaline phosphatase in serum and synovial fluid samples.
- **Metra™ Osteocalcin EIA:** An enzyme immunoassay for the quantification of intact (de novo) osteocalcin.
- **Serum Cross Laps® (CTX):** Assay for the quantification of degradation products of C-terminal telopeptides of Type-I collagen in serum and plasma.

#### • Cytokine Assays:

- **HIL-1ra:** To standardize the measurement of interleukin 1 receptor antagonist concentrations in cell culture supernatant, serum and plasma.
- **IGF:** To standardize the measurement of Insulin-like Growth Factor in Serum, Cell culture and plasma.
- **TGF-α:** An assay to quantify measurement of Transforming Growth Factor-beta in serum, cell culture supernatant, plasma, and urine.
- **TNF-alpha:** An assay to quantify levels of Tumor Necrosis Factor-alpha in serum, plasma, synovial fluid, and cell culture supernatant.
- **IL-10:** An assay to quantify levels of Interleukin-10 in serum, plasma, and cell culture supernatant.



## Research Techniques Available at the Orthopaedic Research Center

- **PDGF-BB:** An assay to quantify levels of Platelet-Derived Growth Factor-BB subunit in serum, plasma, and cell culture supernatant.
- **PGE2:** An assay to quantify levels of Prostaglandin E2 in serum, plasma, synovial fluid, cell culture supernatant, and urine.
- **Pre-assay sample processing including:** papain, hyaluronidase, and collagenase digestion, as well as chromatography extraction of synovial fluid, serum, and tissues.
- **Western, Southern, and Northern Blotting**
- **Many other assays available. Please inquire.**
  - **PDGF-BB:** An assay to quantify levels of Platelet-Derived Growth Factor-BB subunit in serum, plasma, and cell culture supernatant.
  - **PGE2:** An assay to quantify levels of Prostaglandin E2 in serum, plasma, synovial fluid, cell culture supernatant, and urine.
- **Real Time PCR Analysis**
  - **ABI Prism® 7000 Sequence Detection System**
  - **Optimization of PCR Primers**
- **RNA/DNA Extractions/Isolations**
  - **cDNA synthesis from RNA**
  - **RNA from cells, tissue, or whole blood**
  - **Primer and probe design**
  - **Gel extraction and purification**
  - **Purification of plasmid DNA**
  - **PCR amplification**
- **Isolation of Synoviocytes, Chondrocytes, and Tenocytes**
  - **Cell culture expansion of freshly collected cells**
- **Culturing of Mesenchymal Stem Cells (bone-marrow derived or fat-derived)**
  - **Cell culture expansion of bone-marrow derived or adipose-derived cells, including three-dimensional culturing for clinical use**

### 2. Biomechanical Testing

- **Displacement control testing for compressive, tension, and shear material properties**
- **Tissue explants or cell-seeded scaffolds**
- **Light to moderate load cells are suitable for testing small tissue explants or cell-seeded scaffolds**

### 3. Molecular Biology

- **Evaluation of metabolic activity in living tissues**
  - **Radiolabel protocols available**
- **GeneChip® Microarray Analysis**
  - **Complete Affymetrix GeneChip® 3000 scanner, fluidics 450, and hybridization system**

- **Adenoviral Vector construction and cell transfection**
  - **The development of adenoviral vectors for the delivery of genes into cells**

### 4. Histology Services

- **Decalcified tissue histology**
- **Immunohistochemistry**
- **Paraffin and frozen Sectioning and staining of paraffin embedded samples**
- **Histomorphometric analysis**



## **Research Techniques Available at the Orthopaedic Bioengineering Research Laboratory**

The Orthopaedic Bioengineering Research Laboratory (OBRL) is an interdisciplinary research and educational effort bringing together engineers, clinicians, biologists, and scientists all over campus. The goal of the laboratory is to provide an environment for undergraduate and graduate education in Biomedical Engineering while advancing treatment and/or prevention of muscular, neuromuscular or skeletal injury and/or disease. The primary research focuses include:

### **1. Computational Simulation of Orthopaedic Conditions and Treatments**

- a. Finite element analysis
- b. Cadaver and animal experiments to validate and augment the computational models

### **2. Biomaterials Development**

- a. Enhancing wear resistance of polymeric orthopaedic implant bearing materials
- b. Biopolymer derivative synthesis and characterization
- c. Bioactive and osteoinductive bone graft materials

### **3. Engineering and Growth Factor Therapy for Cartilage and Bone Repair**

- a. *In vitro* cell culture assessment
- b. Animal models to evaluate repair
- c. *In vitro* micro-assessment of mechanics of regenerated and normal tissue
- d. Development and assessment of biomaterial carriers

### **4. Retrieval Analysis for Failure Assessment, Design Improvement, and Tissue Interface**

- a. Orthopaedic implants
- b. Allograft bone composites
- c. Synthetic bone graft materials

### **5. Biocompatibility and Biomaterial/Tissue Interface**

- a. Interface biomechanics
- b. Tissue response to biomaterials

### **6. Comparative Orthopaedics and Animal Models**

- a. Animal model development and validation
- b. Comparison of human and other animal disease mechanisms and treatment efficacy

### **7. Biomechanical Analysis**

Equipment available includes: minibionix MTS machine, standard MTS, spine tester, biaxial tester

- a. Range of motion/kinematics
- b. Materials testing for shear strength
- c. Tension and compression analysis

### **8. Hard Tissue Structural Analysis**

- a. MicroComputedTomography ( $\mu$ CT) – High resolution imaging of bone to determine bone volume and morphology
- b. Non-decalcified hard tissue histology
- c. Histomorphometric analysis

## Scientific Publications and Presentations

### Textbook Chapters

2010

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## Scientific Publications and Presentations

- Les C.M., Pechey C.L., Michels E.M., Fang M., MacLeay J.M., Turner A.S., Banaszak-Holl M. Estrogen Depletion is Associated with a Loss in Anatomic Variability in Both Bone Collagen D-spacing, and in Bone Material Pre-stress. In *Proceedings 57th Orthopaedic Research Society*, Long Beach, Calif. 2011:2266.
- McIlwraith C.W. Use of corticosteroids. In, *Proceedings AAEP Annual Convention*, San Antonio, Texas, November 18-22, 2011;57:107-110.
- Mixco A., Reynolds M., Tracy B., Reiser R. Age-related co-contraction effects on balance recovery using the ankle strategy after tether release. In, *Proceedings Med. & Science in Sports & Exercise*. 2011;43:S365.
- Oldinski R.A., Ruckh T.T., Staiger M.P., Popat K.C., James S.P. Dynamic mechanical analysis and biomineralization of hyaluronan–polyethylene copolymers for potential use in osteochondral defect repair, *Acta Biomaterialia*, Volume 7, Issue 3, March 2011, Pages 1184-1191.
- Pechey C.L., Michels E.M., Fang M., MacLeay J.M., Turner A.S., Banaszak-Holl M.H., Les C.M. Histologic and Mineralization Correlates of Estrogen Depletion-related Changes in Ovine Bone Collagen I D-spacing. In, *Proceedings 57th Orthopaedic Research Society*, Long Beach, Calif. 2011:177.
- Phillips J.N., McIlwraith C.W., Samulski R.J., Goodrich L.R. Influences of age on scAAV transduction of chondrocytes and synoviocytes. In, *Proceedings Orthopaedic Research Society Annual Meeting*, Long Beach, Calif., January 13-16, 2011;Poster:2052.
- Reiser R., Hodges S., Tracy B., Browning S., Troup L., Diehl M. Early improvements in Wii Fit Balance Game Scores by older adults. In, *Proceedings Med. & Science in Sports & Exercise*. 2011;43:S361-2.
- Reynolds M., Ehlen K., Reiser R., Browning R. Metabolic rate and cost of transport in obese and non-obese adults during level and gradient walking. In, *Proceedings Med. & Science in Sports & Exercise*. 2011;43:S39.
- Shetye S., Ryan S., Ehrhart N., Puttlitz C.M. Novel endoprosthesis for canine limb sparing of canine distal radius osteosarcoma patients – a modular approach. In, *Proceedings ASME Summer Bioengineering Conference*, Farmington, Pa., June 22-25, 2011.
- Troyer K., Puttlitz C.M. Comparison of A novel nonlinear viscoelastic finite ramp time correction method to a heaviside step assumption. In, *Proceedings ASME Summer Bioengineering Conference*, Farmington, Pa., June 22-25, 2011.
- Werpy N.M., Denoix J.M., McIlwraith C.W., Frisbie D.D. Ultrasound of the suspensory ligament using perpendicular and oblique beam angles for identification of anatomy with comparison with MRI. In, *Proceedings AAEP Annual Convention*, San Antonio, Texas, November 18-22, 2011;57:446-447.
- Wu Z., Slaboch C., Ovaert T., MacLeay J.M., Turner A.S., Niebur G. Ovariectomy Affects the Creep Behavior of Ovine Trabecular Bone Tissue. In, *Proceedings 57th Orthopaedic Research Society*, Long Beach, Calif. 2011.

## Scientific Publications and Presentations

### *Oral Presentations*

2010

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Smith B.S., Yoriya S., Grimes C.A. , and Popat K.C. "Blood Interaction and Protein Adsorption on Engineered Titania Nanotubular Arrays," Society for Biomaterials 2010 Annual Meeting (April 21-24, 2010) Seattle, Wash.

Browning R., Ehlen K., Carter C., Reiser R. Biomechanics and energetics of walking uphill: a better form of exercise for obese adults? American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Golden, Colo., February 26-27.

Prawel D.P., Popat K.C., and James S.P., "Novel Electro-spray Technique for Applying Phospholipid Coatings to Titanium," Society for Biomaterials 2010 Annual Meeting (April 21-24, 2010) Seattle, Wash.

Dolan N., Ross A., Reiser R. Anterior cruciate ligament intervention program: helping the youth? American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Golden, Colo., February 26-27.

Ehlen K., Reiser R., Browning R. Biomechanics and energetics of walking uphill: a better form of exercise for obese adults? ACSM 2010 Annual Meeting. Baltimore, Md., June 4, 2010.

Frisbie D.D. Florida Association of Equine Practitioners Promoting Excellence Symposium, Orlando, Fla., November 4-6, 2010.

Frisbie D.D. Current Therapies & Novel Biologicals for Musculoskeletal Injuries. Boston, Mass., September 2, 2010.

Frisbie D.D. Treating Performance Horses: The horizon. Boehringer Ingelheim Presentation, Dixon, Calif., July 28, 2010.

Frisbie D.D. Treating Performance Horses: The horizon. Boehringer Ingelheim Presentation, Houston, Texas, June 29, 2010.

Frisbie D.D. Current Therapies and Novel Biologics for Musculoskeletal Injuries. Boehringer Ingelheim Presentation, Aiken, S.C., May 4, 2010.

Frisbie D.D. The clinical use of intra-articular stem cells. 1st North American Veterinary Regenerative Medicine conference. UC Davis, Santa Ynez Valley, Calif., March 5-6, 2010.

Frisbie D.D. Defining IRAP, advances and its clinical use. Northwest Equine Practitioner's Association, Bend, Ore., March 12-14, 2010.

Frisbie D.D. Defining stem cells, what is out there, their clinical use in joints and tendons. Northwest Equine Practitioner's Association, Bend, Ore., March 12-14, 2010.

Frisbie D.D. Defining shockwave therapy, what is out there, its clinical use on tendons and joints. Northwest Equine Practitioner's Association, Bend, Ore., March 12-14, 2010.

## Scientific Publications and Presentations

- Frisbie D.D. Defining PRP, what is out there, its uses in tendon/ligaments. Northwest Equine Practitioner's Association, Bend, Ore., March 12-14, 2010.
- Frisbie D.D. Routine medications used in joint disease: NSAID's, Corticosteroids, HA, PSGAG and oral products. Northwest Equine Practitioner's Association, Bend, Ore., March 12-14, 2010.
- Goodrich L.R. Gene therapy for musculoskeletal repair. 1st American Veterinary Regenerative Medicine Conference, Los Olivos, Calif., 2010.
- Goodrich L.R. Gene therapy in regenerative medicine. 1st North American Regenerative Medicine Conference, Los Olivos, Calif., 2010.
- Haussler K.K. 6th International Association of Veterinary Rehabilitation and Physical Therapy Symposium, Auburn University, College of Veterinary Medicine, Auburn, Ala., 2010.
- Haussler K.K. 56th Annual Convention of the American Association of Equine Practitioners, Baltimore, Md., December 2010.
- Haussler K.K. 71st Annual Conference for Veterinarians, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colo., January 2010.
- Haussler K.K. Alternative Therapies in Veterinary Medicine Symposium. Colorado State University, Fort Collins, Colo., April 2010.
- Haussler K.K. Colorado Veterinary Medical Association Annual Convention, Loveland, Colo., September 2010.
- Haussler K.K. The DeeDee Arrisone Holistic and Integrative Wellness Seminar Series. College of Veterinary Medicine, Cornell University, N.Y., October 2010.
- Haussler K.K. Diagnosis & Treatment of Lameness in Horses, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colo., February 2010.
- Haussler K.K. Equine Physical Rehabilitation Certificate Program. College of Veterinary Medicine, University of Tennessee, Knoxville, Tenn., November 2011.
- Haussler K.K. Research and Diagnostic Seminar Series. Department of Veterinary Science, University of Kentucky, Lexington, Ky., May 2010.
- Haussler K.K. Student Chapters of the American Association of Equine Practitioners and the Pain Management Club, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colo., October 2010.
- Kawcak C.E. Surgery of the digital sheath and proximal suspensory ligament. American Board of Veterinary Practitioners 15th Annual Symposium, Denver, Colo., April 18, 2010.
- Kawcak C.E. Joint medications PRP, ESWT, IRAP, Tildren, Rehabilitation. American Board of Veterinary Practitioners 15th Annual Symposium, Denver, Colo., April 18, 2010.

## Scientific Publications and Presentations

- Kawcak C.E. Suspensory ligament Disease. American Board of Veterinary Practitioners 15th Annual Symposium, Denver, Colo., April 18, 2010.
- Kawcak C.E. Advances in diagnosis of equine lameness. Iowa Veterinary Medical Association, February 10, 2010.
- Kawcak C.E. Farm based management of equine orthopaedic diseases. Iowa Veterinary Medical Association, February 10, 2010.
- Kawcak C.E. Treatment for equine orthopaedic diseases Part I: First aid and medical management. Iowa Veterinary Medical Association, February 10, 2010.
- Kawcak C.E. Treatment for equine orthopaedic diseases Part II: Surgical and post-op management. Iowa Veterinary Medical Association, February 10, 2010.
- McIlwraith C.W. Symposium on the Last Ten Years Advances in Equine Medicine – Roissy, France. “Ten years of surgery and medical treatments for articular cartilage and bone in joints,” February 27, 2010.
- McIlwraith C.W. International Convention of Racing Analysts and Veterinarians (ICRAV), Queenstown, New Zealand. Keynote lecture, “Use of fluid biomarkers for early detection of equine bone and joint disease – identifying the horse at risk from catastrophic injury,” March 8-10, 2010.
- McIlwraith C.W. AO North America, Equine Principles of Fracture Management course, Faculty member: 3 lectures and 3 two hour laboratories, Columbus, Ohio., April 22-25, 2010.
- McIlwraith C.W. 17th KER Equine Nutrition Conference. Feeding and veterinary management of the sport horse. “Advances in the diagnosis and treatment of joint disease” and “Prevention and management of joint disease in the sport horse,” April 26-27, 2010.
- McIlwraith C.W. Basic arthroscopic surgery course, Muenster, Germany. Three 1-hour lectures and three 2-hour laboratories, May 28-29, 2010.
- McIlwraith C.W. Basic arthroscopic surgery course, Colorado State University, Fort Collins, Colo. Four hours of lecture and four hours of laboratory, June 10, 2010.
- McIlwraith C.W. Advanced arthroscopic surgery course, Colorado State University, Fort Collins, Colo. Eight hours of lecture and four hours of laboratory, June 11-12, 2010.
- McIlwraith C.W. eCM meeting: Cartilage and disc repair and regeneration, Davos, Switzerland. Keynote speaker, Animal models for cartilage repair, June 28-30, 2010.
- McIlwraith C.W. Pegasus Training and Equine Rehabilitation Center Racetrack Veterinary Seminar, Washington. Symposium with 4 papers, “Evaluation of rehabilitation – randomized controlled study in midcarpal joint chip fragmentation,” “Mesenchymal stem cells – what do they do? Does it matter where we culture them from?” “Platelet-rich plasma – basic science to clinical application,” “Update on new targeted therapies – gene therapy,” July 13, 2010.

## Scientific Publications and Presentations

- McIlwraith C.W. Graduation ceremony Royal Veterinary College of London, Guild Hall, London, United Kingdom. Speech to graduating veterinary class after conferment of DVetMed (honoris causa) degree, July 20, 2010.
- McIlwraith C.W. Steadman Philippon Research Foundation Advisory Board Meeting, Vail, Colo. Participation as member of board and 2 presentations, "Bone marrow derived mesenchymal stem cells (BMSC) to augment microfracture," "Evaluation of PRP effects *in vitro*: preliminary results," July 30-31, 2010.
- McIlwraith C.W. FEI Congress on NSAIDs usage in the Equine Athlete, Lausanne, Switzerland. Two invited lectures, "What are NSAIDs used for in equine practice?" "How effective are NSAIDs at pain control in horses?" Chaired another session, August 16-17, 2010.
- McIlwraith C.W. Third World Veterinary Orthopaedic Congress, Bologna, Italy. Three invited lectures, "Critical review of the clinical use of IRAP," "Indications for and results of intra-articular use of stem cells," "Is there a role for arthroscopy in the management of OA?" September 16-18, 2010.
- McIlwraith C.W. Alltech FEI World Equestrian Games Veterinary Sport Horse Symposium, Lexington, Ky. Invited lecture, "Use of medication for the protection of joints," September 22-23, 2010
- McIlwraith C.W. World Equestrian Games, Promoting Peak Performance in Equine Athletes – Sports Horse Workshop. Invited lecture, "Use of joint medications for the sport horse," September 24, 2010.
- McIlwraith C.W. International Cartilage Research Society (ICRS), Sitges, Barcelona, Spain. Proteomics, genomics and biomarkers introductory lecture and moderator, and invited presentation for instructional course, "How to set up an animal model study for assessing repair of articular cartilage," September 26-29, 2010.
- McIlwraith C.W. International Advanced Course in Arthroscopic Surgery, Muenster, Germany. Six hours of lecture and three hours of laboratory, October 15-16, 2010.
- McIlwraith C.W. ACVS Veterinary Symposium "The Surgical Summit," Seattle, Wash. Presentation of cutting-edge panel on management of stifle lameness in the equine athlete: advances and limitation (with Drs. Lisa Fortier and Dean Richardson), October 21-23, 2010.
- McIlwraith C.W. November 11-12, 2010 – AONA Challenges in Fracture Care across Disciplines, Phoenix, Ariz. Invited lecture, "Applied basic science research: cartilage repair as it relates to articular fracture, injury and joint function."
- McIlwraith C.W. NIHU-13AOSSM Post-Joint Injury Osteoarthritis Conference II, New Orleans, La. "Biomarker predictors of impending injury," December 3, 2010.
- McIlwraith C.W. International Veterinary Supply, Pentaussie Round Table, Hyatt Regency Inner Harbor, Baltimore, Md. Moderator and lead discussant of three-hour round table, December 4, 2010.



## Scientific Publications and Presentations

- McIlwraith C.W. 56th Annual Convention American Association of Equine Practitioners, Baltimore, Md. Three lectures, "Review of the economic impact of osteoarthritis in oral joint-health supplements in horses," "Review of the scientific basis for use of intra-articular corticosteroids in the horse," "Use of bone marrow-derived cultured expanded mesenchymal stem cells to augment healing of chondral defects treated with microfracture," December 4-8, 2010.
- Mixco A., Reynolds M., Tracy B., Reiser R. The relationship between strength and rate of force development with balance recovery in young and older adults. American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Golden, Colo., February 26-27.
- Riedel N., Williams J., Popat K.C. "Mesenchymal Stem Cell Response on Ion Beam Sputter-coated Hydroxyapatite Surfaces," Society for Biomaterials 2010 Annual Meeting (April 21-24, 2010) Seattle, Wash.
- Reiser R. EMG in Exercise Science. Second Annual ERC Interdisciplinary Forum on Measurement Systems for Occupational Health and Safety Research. Colorado State University, April 29, 2010.
- Reynolds M., Troup L., Reiser R. Effect of foot placement on Wii Fit™ measured standing weight-bearing asymmetry. American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Golden, Colo., February 26-27.
- Bechara S.L., Judson A.C., Popat K.C. "Polymeric Nanowire Templates as Scaffolds for Improved Neuronal Cell Functionality," Society for Biomaterials 2010 Annual Meeting (April 21-24, 2010) Seattle, Wash.
- Bechara S.L., Popat K.C. "Polymeric Nanowire Templates as Scaffolds for Improved Neuronal Cell Functionality," LabAutomation 2010 (January 23-27, 2010), Palm Springs, Calif., Susan S. Yonemura, Barbara S. Smith, Marcio Forleo, Susan P. James and Ketul C. Popat, "Hemocompatibility of a Novel Hyaluronan-High Density Polyethylene Composite." 2010 Annual BMES Meeting (October 6-8, 2010) Austin, Texas.
- Ruckh T.T., Smith B.S., Carrol D.A., Popat K.C. "Oleic acid delivered by nanofibers increases bone matrix formation by differentiated marrow stromal cells," Society for Biomaterials 2010 Annual Meeting (April 21-24, 2010) Seattle, Wash.

### *Oral Presentations*

#### **2011**

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- Adams M., Goodrich L.R., Kisiday J.D., McIlwraith C.W. (2011). Equine bone-marrow derived mesenchymal stem cells (BMDMSCs): comparing the sternum and ilium. Proceedings of CSU Research Day, Fort Collins, Colo.
- Abelbeck K., Lipsey T., Reiser R. Athletic performance assessment of SWAT and patrol police officers over a 12-week training program. ACSM 2011 Annual Meeting. Denver, Colo., June 3, 2011.
- Board W., Reynolds M., Ehlen K., Reiser R., Browning R. The effects of obesity on the biomechanics of gradient walking. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.

## Scientific Publications and Presentations

- Brown K., Reiser R. Weight-bearing asymmetries when lifting: symmetric versus staggered stance. American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Fort Collins, Colo., February 25-26.
- Carter K., Trumper R., Smidt C., Rice E., Reiser R. The influence of secondary lateral meniscal injury on recovery following anterior cruciate ligament reconstruction surgery. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.
- Frisbie D.D. Perineural and intrasynovial anesthesia, foot to fetlock. American Association of Equine Practitioners 360 meeting, Fort Collins, Colo., July 31-August 3, 2011.
- Fritz M., Hitchcock L., Trabert M., Jones A., Paxton R., Reiser R., Tracy B. Postural steadiness in young, elderly, elderly fallers, and neuropathy patients. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.
- Hodges S., Patrick R., Reiser R. Fatigue does not increase vertical ground reaction force asymmetries during the barbell back squat. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.
- Hudec M., Reynolds M., Ehlen K., Reiser R., Browning R. Accuracy of metabolic prediction equations for obese adults walking at various speed and grade combinations. ACSM 2011 Annual Meeting. Denver, Colo., June 2.
- McIlwraith C.W. 72nd Annual Conference for Veterinarians, Colorado State University, Fort Collins, Colorado Keynote Speaker "Arthritic and damaged joints – finding solutions for horses and humans," January 8, 2011.
- McIlwraith C.W. Colorado State University Delegation to visit UADY Merida, Mexico. "A one health approach to addressing arthritic and joint diseases of humans and animals," January 17, 2011.
- McIlwraith C.W. Montana State University Equine Conference. Catlin Memorial Lectures "Equine osteoarthritis; cause and diagnosis", "Equine osteoarthritis; treatment and prevention," March 5, 2011.
- McIlwraith C.W. 10th Anniversary Polish Association of Equine Practitioners, Lotz, Poland. "Arthrosis and arthritis – diagnostic procedures and conventional (non-surgical therapy)," "Arthroscopy – still the best method for treatment of joint injuries," 6 hour workshop on basics of arthroscopy in horses, April 8-10, 2011.
- McIlwraith C.W. 21st Annual Australasian Society for Biomaterials and Tissue Engineering Conference. Keynote lecture "Regenerative medicine in articular cartilage repair," April 27-29, 2011.
- McIlwraith C.W. Havemeyer Conference on Regenerative Medicine, Sagauro Lake Ranch, Ariz. "Equine joint models" and 4 days discussion, May 2-5, 2011.
- McIlwraith C.W. Basic Equine Arthroscopic Surgery course, Berlin, Germany. 4 hours lecture and three 4-hour laboratories, May 27-28, 2011.

## Scientific Publications and Presentations

- McIlwraith C.W. British Racing Summer Meeting, Chester, UK. Principle speaker “Talking to the press,” “Exercising young Thoroughbreds. What is the impact of this?” “Intraarticular medication what to use and when to use it,” “New developments in biomarkers and track surfaces in injury susceptible horses,” June 7, 2011.
- McIlwraith C.W. University of Liverpool, Department of Musculoskeletal Biology Annual Science Day. Keynote lecture, “Attempts at cartilage resurfacing: studies in equine models,” June 8, 2011.
- McIlwraith C.W. AAEP 360 Imaging and Lameness from the Fetlock to the Ground, Colorado State University, Fort Collins, Colo. “Arthroscopic normal anatomy of foot to fetlock,” “Arthroscopic disease of foot to fetlock,” July 31-August 3, 2011.
- McIlwraith C.W. Basic Equine Arthroscopic Surgery Course, Colorado State University, Fort Collins, Colo. 4 hours of lecture and 4 hours laboratory, August 4, 2011.
- McIlwraith C.W. Advanced Arthroscopic Surgery Course, Colorado State University, Fort Collins, Colo. 8 hours lecture and 4 hours laboratory, August 5-6, 2011.
- McIlwraith C.W. 30th Anniversary Workshop, Dorothy Russell Havemeyer Foundation. Chair Sports Medicine Program, “Havemeyer Foundation Symposium on Biomarkers and Regenerative Therapies for Joint Injury and Disease,” August 18-20, 2011.
- McIlwraith C.W. Advanced Arthroscopic Surgery of the Stifle course, Cornell University, Ithaca, New York. 5 lectures and 2 laboratories (2-day course), September 16-17, 2011.
- McIlwraith C.W. Racing Medication and Testing Consortium, Board of Directors Meeting, Scottsdale Plaza Resort, Ariz. Invited lecture, “Update on corticosteroid research,” October 6, 2011.
- McIlwraith C.W. Seminar on New Research on PPS Combinations for Osteoarthritis in Horses, Sydney, Australia. Two one hour lectures, “Principles of treatment of synovitis, capsulitis and osteoarthritis including new developments,” “Evaluation of the effectiveness of intramuscular sodium pentosan polysulfated in an osteochondral fragment model of osteoarthritis in horses,” October 28-29, 2011.
- McIlwraith C.W. Japanese Racing Association (JRA), lecture at Miho Training Center, Japan, “Treatment of osteoarthritis with novel therapies and scientific evidence for pentosan polysulfated being an effective systemic therapy for osteoarthritis,” November 9, 2011.
- McIlwraith C.W. Japanese Racing Association (JRA) seminar at Ritto Training Center, Japan, “Treatment of osteoarthritis with novel therapies and scientific evidence for pentosan polysulfated being an effective systemic therapy for osteoarthritis,” November 10, 2011.
- McIlwraith C.W. 57th Annual Convention American Association of Equine Practitioners, San Antonio, Texas. Lecture, “The use of corticosteroids” and participation in panel, Choosing joint therapies for the best results. Two Table Topics (1.5 hrs each), “The FDA and drug compounding” (with Jeff Berke, Lynn Oliver & Scott Stanley) and “The American College of Veterinary Sports Medicine and Rehabilitation: What is it about and how might one qualify?” (with Chris Kawcak), November 19-22, 2011.

## **Scientific Publications and Presentations**

- Mixco A., Reynolds M., Tracy B., Reiser R. Age-related co-contraction effects on balance recovery using the ankle strategy after tether release. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.
- Reiser R., Hodges S., Tracy B., Browning S., Troup L., Diehl M. Early improvements in Wii Fit Balance Game Scores by older adults. ACSM 2011 Annual Meeting. Denver, Colo., June 3, 2011.
- Reynolds M., Ehlen K., Reiser R., Browning R. Metabolic rate and cost of transport in obese and non-obese adults during level and gradient walking. ACSM 2011 Annual Meeting. Denver, Colo., June 2, 2011.
- Taylor M., Reiser, R. Effect of stance width, vision, and trial duration on standing weight-bearing asymmetries. American College of Sports Medicine: Rocky Mountain Chapter Annual Meeting. Fort Collins, Colo., February 25-26, 2011.
- Virgin J., Goodrich L.R., Baxter G.M., Rao S. Incidence of support limb laminitis in horses treated with a half or full limb cast: a retrospective study of 113 horses (2000-2009). Proceedings of CSU Research Day, Fort Collins, Colo. Oral presentation. 2011.

## Funded Research Projects

Title	Investigators	Sponsor	Project Period	Amount
Track Banking and the Asymmetry of Hoof Loading	J.J. Thomason (PI) University of Guelph; C. Wayne McIlwraith (Co-I)-1678	Grayson-Jockey Club Research Foundation	2010-2011	\$36,761.00
Incidence of Nonfatal Injuries in Racing Thoroughbreds	C. Wayne McIlwraith (Primary PI)-1678	Grayson-Jockey Club Research Foundation	2009-2010	\$44,397.00
Performance Parameters for Engineering Track Management	C. Wayne McIlwraith (Primary PI)-1678	Grayson-Jockey Club Research Foundation	2008-2010	\$43,838.00
Evaluation of Intra-Articular Polyglycan Versus Intravenous Polyglycan or Saline (0.9%NaCl) for Osteoarthritis Using an Equine Model	C. Wayne McIlwraith (Primary PI)-1678; Christopher E Kawcak (Co-PI)-1678; David D Frisbie (Co-PI)-1678	ArthroDynamic Technologies, Inc.	2007-2011	\$644,832.00
Effect of Rehabilitation on Carpal Osteoarthritis	Melissa King (PI)-1678; C Wayne McIlwraith (Co-I)-1678	Grayson-Jockey Club Research Foundation	2009-2010	\$19,994.00
Gene Transfer Treatment of Articular Cartilage Damage	David D. Frisbie, C. Wayne McIlwraith (Co-PIs)-1678; Steve Trippel (PI) University of Indiana	NIH R01AR047702-07A1	2011-2014	\$516,060.00
Effects of Clinically Relevant Autologous Conditioned Blood Products (ACBP) on the Anabolic Properties of Equine Digital Flexor Tenocytes and Suspensory Ligament Fibroblasts	David D. Frisbie (Primary PI)-1678; C. Wayne McIlwraith (Co-PI)-1678; Thomas Howard Hraha (Co-PI)-1678	AQHA-American Quarter Horse Assoc.	2009-2010	\$19,560.00
Self-Assembling Peptide for Tissue Engineering	David D. Frisbie (PI of subcontract)-1678; C. Wayne McIlwraith (Co-PI)-1678; with Dr. Alan Grodzinsky (MIT)	HHS-HIH (NIH MIT subaward amendment) (5-R01-EB003805-03) 5710002213)	2007-2010	\$590,205.00
Evaluation of Intra-Articular Polysulfated Glycosaminoglycan (Adequan) Versus Intra-Articular Hyaluronate Sodium (Hyvisc) or Saline (0.9% NaCl) for Osteoarthritis	David D. Frisbie (Primary PI)-1678; C. Wayne McIlwraith (Co-PI)-1678; Christopher E. Kawcak (Co-PI)-1678	Luitpold Pharmaceuticals	2007-2008	\$357,601.00
Evaluation of Intra-Articular Polysulfated Glycosaminoglycan (Adequan) and Amikacin, Versus Intra-Articular Polysulfated Glycosaminoglycan (Adequan) and Triamcinolone Acetonide with Amikacin or Saline (0.9% NaCl) with Amikacin for Osteoarthritis Using an Equine Model	David D. Frisbie (Primary PI)-1678; C. Wayne McIlwraith (Co-PI)-1678; Christopher E. Kawcak (Co-PI)-1678	Luitpold Pharmaceuticals	2009-2010	\$357,602.00
ARRA: Multicenter Cartilage Repair Preclinical Trial in Horses	Laurie R. Goodrich (PI subcontract)-1678; C. Wayne McIlwraith (Co-I)-1678; John D. Kisiday (Co-I)-1678; Connie Chu (PI) University of Pittsburg	NIH ARRA (5327810)	2009-2011	\$459,989.00
ARRA: Gene Therapeutic Approaches to Cartilage Repair	Laurie R. Goodrich (Primary PI)-1678; C. Wayne McIlwraith (Co-mentor); Jude Samulski (Co-mentor) (University of North Carolina)	HHS-NIH-National Institutes of Health	2009-2011	\$108,000.00

## Funded Research Projects

Title	Investigators	Sponsor	Project Period	Amount
AAV-IRAP Gene Therapy to Prevent Osteoarthritis	Laurie R. Goodrich (Primary PI)-1678; David D. Frisbie (Co-PI)-1678	Grayson-Jockey Club Research Foundation	2011-2013	\$134,757.00
Orthokine Protocol to Assess Serum Protein Factors	Frisbie D.D., McIlwraith C.W., Reardon K.F., Carbone B.A.	Arthrex	11/1/2006-10/31/2008	\$49,307.00
Evaluating Reduction in Lameness or Prevention of Pathological Bone Changes Following Application of Dynamix Shoes	Frisbie D.D., Kawcak C.E.	Dynamix	1/1/2001-6/30/2009	\$432,174.00
Evaluation of Intra-Articular Polysulfated Glycosaminoglycan (Adequan) Versus Intra-Articular Hyaluronate Sodium	Frisbie D.D., McIlwraith C.W., Kawcak C.E.	Luitpold Pharmaceuticals	1/8/2007-4/7/2010	\$357,602.00
Evaluation of MRNA in Periphal White Blood Cells Count – Research Plan #1 Under MRA	Frisbie D.D., McIlwraith C.W., Hess A.M.	Pfizer Inc - Animal Health	12/10/2007-12/10/2009	\$197,652.00
Pilot Study to Assess the Short Term Effects of Chondrofix in Equine Model #18,000.01	Frisbie D.D., McIlwraith C.W.	Zimmer, Inc.	12/19/2005-6/30/2009	\$296,061.20
Horse Gait Trials at CSU	Kawcak C.E., Frisbie D.D., Werpy N.M., McIlwraith C.W.	Sharp Foundation	11/1/2008-10/31/2009	\$28,500.00
Evaluation of Nuclear Magnetic Resonance (MBST) Therapy for Osteoarthritis Using an Equine Model	Kawcak C.E., Frisbie D.D., McIlwraith C.W.	MBST Medical Devices, Inc.	6/1/2007-5/31/2008	\$230,270.00
An Experimental Model for Tendon Strain Injury and Mesenchymal Stem Cell Interactions	Kisiday J.D., Frisbie D.D.	CRC Funding	7/1/2008-6/30/2009	\$10,500.00
Colorado Racehorse Postmortem Evaluation Project	Kawcak C.E., Werpy N.M.	CRC Funding	7/1/2008-6/30/2009	\$15,000.00
Mesenchymal Stem Cell Proliferation and Migration Out of Fibrin Scaffolds in Response to Shockwave Treatment	Kisiday J.D., Frisbie D.D., McIlwraith C.W.	Pulse Veterinary Technologies LLC	7/15/2009 – 7/31/2010	\$20,420
Gene and Protein Expression in Autologous Conditioned Plasma: A Comparison of IRAP I and IRAP II	Frisbie D.D., McIlwraith C.W.	Arthrex	2/10/2009 – 8/31/2009	\$9,876
Effects of Clinically Relevant Autologous Conditioned Blood Products (ACBP) on the Anabolic Properties of Equine Digital Flexor Tencytes and Suspensory Ligament Fibroblasts	Frisbie D.D., McIlwraith C.W., Hraha T.	American Quarter Horse Foundation	10/1/2009-9/30/2010	\$19,560
Evaluation of Intrarticular Polysulfated Glycosaminoglycan (Adequan) and Amikacin, Versus Intrarticular Polysulfated Glycosaminoglycan (Adequan) and Triamcinolone Acetonide with Amikacin or Saline (0.9% NaCl) with Amikacin for Osteoarthritis Using an Equine Model	Frisbie D.D., McIlwraith C.W., Kawcak C.E.	Luitpold Pharmaceuticals	4/8/2009-4/7/2010	\$357,601



## Funded Research Projects

Title	Investigators	Sponsor	Project Period	Amount
Evaluation of Polyglycan (At a Single Dose Level and Three Time Dose Level) Versus Saline (0.9% NaCl) After Intraarticular Injection	Kawcak C.E., McIlwraith C.W., Frisbie D.D.	ArthoDynamic Technologies, Inc	6/1/2008 – 5/31/2009	\$75,990
Self Assembling Peptide for Tissue Engineering	Frisbie D.D. (Subcontract PI), Grodzinsky, A.J. (Program PI)	HHS-HIH/MIT (NIH-Program grant) Grant # RO1-003805-01A1	4/1/2007- 3/31/2010	\$479,876
Gene Therapeutic Approaches to Cartilage Repair	Goodrich L.R., McIlwraith C.W.	NIH Mentored Clinical Scientist Research Career Development Award (K08)	6/01/2008- 5/01/2013	\$677,875
A Gene Therapy Approach to Cartilage Healing Utilizing Adenoassociated Viral Vectors in Bone Marrow-Derived Mesenchymal Stem Cells	Goodrich L.R., McIlwraith C.W.	College Research Council, Colorado State University	3/01/2008- 3/01/2009	\$26,000
The Effect of Adenovirus Mediated Co-expression of Combined Bone Morphogenetic Protein-2 and 7 on Osteoblastic Differentiation of Equine and Human Bone Marrow-Derived Mesenchymal Stem Cells	Goodrich L.R., McIlwraith C.W.	College Research Council, Colorado State University	3/01/2007- 3/01/2008	\$30,000
Effect of Rehabilitation on Carpal Osteoarthritis	King M.R., McIlwraith C.W.	Grayson-Jockey Club Research Foundation	4/1/2009- 3/31/2010	\$15,000.00
Investigation of Osteochondral Disease Through Finite Element Modeling	Easton, Katrina	HHS-NIH-National Institutes of Health	4/1/2008- 5/13/2011	\$29,777.00
Collaborative Research: Nanostructured Titania for Orthopedic Biomaterials	Popat K.C.	NSF - National Science Foundation	9/1/2008- 8/31/2011	\$180,000.00
Fibrotic Effects and Regulation of MMP Proteins in Thrombus Resolution	Puttlitz C.M.	N. CA Inst. for Research and Education	5/12/2006- 4/30/2009	\$126,298.00
The Annual Symposium on Computational Methods in Orthopaedic Biomechanics	Puttlitz C.M.	HHS-NIH-Arthritis, Musculoskel, and Skin	2/20/2009- 8/31/2009	\$15,000.00
A Development Proposal for an Instrumented Cervical Intervertebral Disc Space Distractor	Puttlitz C.M.	CSURF-CSU Research Foundation	7/12/2007- 12/31/2008	\$107,285.00
Performance Parameters for Engineering Track Management	McIlwraith C.W., Peterson M.	Grayson-Jockey Club Research Foundation	4/1/2008- 3/31/2009	\$43,838.00
Partial Joint Resurfacing with Biopoly <sup>TM</sup> RS – A Hydrophilic Polymer	James S.P., Puttlitz C.M., Kisiday J.D.	Schwartz Biomedical, LLC	12/1/2006- 12/31/2008	\$400,000.00
Augmentation of a Bone Tendon Reattachment with a PDGF Soaked Collagen Matrix in a Sheep Model	Puttlitz C.M.	Biomimetic Therapeutics, Inc.	11/1/2008- 7/1/2009	\$66,371.00

## Funded Research Projects

Title	Investigators	Sponsor	Project Period	Amount
<i>In Vivo</i> and <i>in Vitro</i> Measurements of Human Cervical Stress Relaxation during ACD	Puttlitz C.M.	Synthes	1/1/2007-3/31/2009	\$39,000
Acute Rotator Cuff with Biosynthesize Cellulose Repair Device: A Histological Study in Sheep: Phase 3	Puttlitz C.M.	Xylos Corporation	8/15/2008-4/30/2009	\$27,615
Evaluation of the Magellan Intervertebral Body Fusion Device in a Sheep Spine Model	Puttlitz C.M.	Magellan Spine Technologies, Inc.	10/20/2008-7/1/2009	\$10,393
Effect of Disc Degeneration, Nucleus Replacement, and Disc Replacement On Facet Force Transmission – A Finite Element ...	Puttlitz C.M.	Synthes	4/3/2007-10/31/2010	\$33,172
STS/GCD Ovine Histology	Puttlitz C.M.	NuVasive, Inc.	7/1/2007-7/1/2008	\$53,071
A Finite Element Investigation of Cervical Intervertebral Disc Replacement Biomechanics	Puttlitz C.M.	Synthes	4/1/2006-3/31/2009	\$102,015
Prevention of Epidural Fibrosis in a Sheep Laminectomy Model Phase II	Puttlitz C.M.	Kuros Biosurgery AG	10/1/2007-2/1/2009	\$18,861
Prevention of Dural Leakage in a Sheep Craniotomy Model	Puttlitz C.M.	Kuros Biosurgery AG	11/15/2007-11/14/2008	\$44,232
Prevention of Epidural Fibrosis in a Sheep Laminectomy Model Phase III	Puttlitz C.M.	Kuros Biosurgery AG	3/1/2008-2/28/2009	\$32,677
Prevention of Epidural Fibrosis in a Sheep Laminectomy Model Phase IV	Puttlitz C.M.	Kuros Biosurgery AG	3/1/2008-2/28/2009	\$10,650
Comparison of Bone Cements in Sheep	Puttlitz C.M.	Medtronic Spine LLC	3/17/2008-8/30/2008	\$8,842
A Biomechanical and Histological Assessment of Tissue Ingrowth for a Dynamic Stabilization Micromotion System (DSF ...	Puttlitz C.M.	IST-Innovative Spinal Technologies, Inc.	10/1/2007-4/30/2009	\$58,503
Triple Damper System Chronic Sheep Study: Biomechanical and Histomorphometric Evaluation of Sheep Lumbar Region	Puttlitz C.M.	Blackstone Medical, Inc.	5/2/2008-11/30/2008	\$134,180
Evaluation of Bone Void Filler Resistance to Bleeding and Irrigation and Correlation with New Bone Formation in ...	Puttlitz C.M.	Kuros Biosurgery AG	5/1/2008-5/1/2009	\$73,961
Evaluation of the Anti-Adhesion Properties of Human Amnion Tissue Patch Following Laminectomy in a Sheep Model	Puttlitz C.M.	AlloSource	6/17/2008-12/1/2008	\$44,898

## Funded Research Projects

Title	Investigators	Sponsor	Project Period	Amount
Evaluation of Polyglycan (At a Single Does Level and Three Times Dose Level) Versus Saline (0.9% NaCl) After ...	Kawcak C.E., Frisbie D.D., McIlwraith C.W.	ArthroDynamic Technologies, Inc.	6/1/2008-5/31/2009	\$75,990
Failed Spinal Fusion Analysis	Puttlitz C.M.	NuVasive, Inc.	6/17/2008-7/31/2008	\$784
Effect of Locally Delivered, Modified PTH in an Anterior Lumbar Interbody Fusion Model in Sheep	Puttlitz C.M.	Kuros Biosurgery AG	3/1/2009-4/1/2010	\$183,615
Long Term Implantation Effects of Flexion Limiting Device in an Ovine Model	Puttlitz C.M.	Simpirica Spine	12/1/2008-12/1/2009	\$130,784
Evaluation of an Allograft Anchor for Pedicle Screw Augmentation in an Ovine Model	Puttlitz C.M.	Synthes	1/15/2009-12/1/2009	\$83,353
Screw Insertion Device Torque Assessment	Puttlitz C.M.	High Plains Technology Group LLC	6/15/2008-1/15/2009	\$744
Acute Rotator Cuff with Biosynthesize Cellulose Repair Device: A Histological Study in Sheep: Phase 4	Puttlitz C.M.	Xylos Corporation	10/1/2008-11/30/2009	\$27,048
The Evaluation of Dermagraft® to Accelerate the Healing of Acute Rotator Cuff Injuries in a Sheep Model – Phase I: ...	Puttlitz C.M.	ABH-Advanced BioHealing	5/15/2009-11/15/2009	\$33,451
Biodistribution Study of Single Dose Intravenous Radium223 in Normal Dogs	Ehrhart N., Ryan S., Steyn P., Dornish M.	Algeta Inc, Norway	6/1/2007-6/30/2009	\$367,000
			<b>TOTAL</b>	<b>\$8,125,573</b>



## Revenue and Expense, FY10 to FY11

FY 11 07/01/10 to 06/30/11		FY 10 07/01/09 to 06/30/10	
REVENUE			
Donations – 64 Accounts			
Hargett, Carla (Raymond James Char. Endowment Fund)	5,000	Employee's Community Fund Heidi Gordon on behalf Denise Steensland	65
Armstrong, Elizabeth	50	Emery, Jaynn	50,000
Powers, James	50	Equusys Incorporated	50,000
Carpenter, Cathy (Dea Family Foundation)	13,000	Ochsner, Michael R.	100
William T. O'Donnell (for Lezlie Rehagen)	500	American Quarter Horse	4,011
Jeffrey S. Matthews – Franklin Street Partners	3,750	Walton Family Foundation (Alice Walton)	10,000
James M. Cox Jr. Foundation (Leigh Ann Launius)	250,000	Mill Creek Veterinary (James Latham)	250
Ms. Gail Holmes, Double Dove Ranch	52,663	Allen, Herbert A. (Allen & Co.)	250,000
McIlwraith	20,000	McKelvie, David K. and Linda	150
Allen & Company, Kim Wieland	250,000	Elsevier Inc	67
Pro Sports Club – on Behalf of Mark Dedomenico	50,000	Pro Sports Club – Dedomenico	50,000
C. George Dewell D.V.M.	2,000	Oak Tree Racing Association	1,667
Mace Siegel	10,000	Community Foundation of Greater Memphis – Lee and Melanie Taylor	3,000
Wildenstein Family, LLC	2,500	McIlwraith, Wayne and Nancy Goodman McIlwraith	20,000
Voth, Roy H.	25	Simonds, William Jo	25
Ms. Cynthia Chesnutt	40	Oak Tree	1,667
Marilynn Dammon – King's Hill Stables	150	Rood and Riddle	20,000
Cauleen Glass – in memory of Denison P. Glass	350	Community Fund Boehing	60
Community Fund Boehing	95	Piper, Cynthia	100
		Kuzyk, Zory (Oak Creek Ranch)	5,000
		Voth, Marlyn S.	25
		Ketch, R.J. (RJ Ketch Equine Inc)	75
		Kawananakoa Foundation (Abigail)	35,100
		Shideler, Robert K., D.V.M.	1,000
Total Donations	660,173		502,361

## Revenue and Expense, FY10 to FY11

FY 11 07/01/10 to 06/30/11		FY 10 07/01/09 to 06/30/10	
Interest on Endowments			
Wayne and Nancy McIlwraith Scholarship	5,602	McIlwraith Scholarship	4,859
Cox Anthony Chair	125,819	Cox Anthony Chair	118,418
Iron Rose Ranch Chair	111,457	Iron Rose Ranch Chair	104,901
Atkinson Chair	48,048	Atkinson Chair	45,222
Kawananakoa Chair	97,510	Kawananakoa Chair	91,774
Total Interest	388,437		365,173
Medical Center Clinical Services			
Digital Xrays	2,178	Digital Xrays	864
Outpatient	19,104	Outpatient	9,631
MRI	47,544	MRI	15,843
Shockwave	26,323	Shockwave	19,250
Surgery	13,946	Surgery	10,168
Client Services Total	109,096		55,756
ORC Ambulatory	77,648		73,365
ORC CORE Lab Revenue – 22 Account	18,723		30,868
Research Projects – 53 Accounts			
AQHA	22,200	Arthro	322,990
HHS-NIH ARRA	189,659	MIT	110,329
Grayson AAV-IRAP	63,566	AQHA	19,560
HHS-NIH Gene	122,364	Pulse	20,420
		PITT ARRA	304,822
		HHS-NIH Gene	122,364
		HHS-NIH ARRA	108,000
		AQHA – Pilot	10,652
		Grayson – King	15,000
Research Accounts Total	397,789		1,034,137
Stallion Auction	31,175		74,264
State Funds – Various 14 and 16 Accounts			
Frisbie Salary Savings	173	CWM Salary Savings	173,350
Goodrich Salary Savings	74,952	Frisbie Salary Savings	5,972
Haussler/Kisiday Start up	33,333	Goodrich Salary Savings	49,036
ICR Return	14,760	Haussler/Kisiday Start up	33,333
Kawcak CRC Grant	15,000	ICR Return	13,477
Kisiday CRC Grant	10,000	Kisiday CRC Grant	14,000
Haussler CRC Grant	16,000	PRSE Grant	12,208
PRSE Grant	20,182		
State Funds Total	184,400		301,375
Total Revenue	1,867,441		2,437,299

## Revenue and Expense, FY10 to FY11

FY 11 07/01/10 to 06/30/11		FY 10 07/01/09 to 06/30/10
<b>EXPENSE</b>		
<b>Salaries</b>		
Faculty Salaries	575,515	756,388
Research Associate Salaries	356,472	361,964
Administrative Salaries	118,822	123,335
Graduate Student Salaries	68,975	119,949
Hourly EORC students	99,848	114,421
<i>Total Salaries</i>	<i>1,219,634</i>	<i>1,476,058</i>
<b>Faculty Travel</b>	36,014	37,662
<b>Materials and Supplies</b>	311,020	505,130
<b>Other Direct</b>	540,556	852,063
<b>Equipment</b>	77,378	–
<i>Expense Subtotal</i>	<i>2,184,600</i>	<i>2,870,912</i>
<b>Facility and Administrative Overhead Costs</b>	181,497	254,258
<i>Total Expense</i>	<i>2,366,097</i>	<i>3,125,170</i>
<b>ACCOUNT BALANCE</b>	<b>(498,657)</b>	<b>(687,871)</b>



## Honors and Awards

**McIlwraith, C.W.** Dr. med. vet. (honoris causa), Royal Veterinary College, University of London, 2010; Life Member, New Zealand Equine Veterinary Association, 2011.

**McIlwraith, C.W.** CSU Graduate School Ceremony, Grand Marshall, 2010.

**Frisbie, D.D.** AAEP President's Award, 2011.

**Goodrich, L.R.** NIH Mentored Clinical Scientist Development Award, 2008-2013.

**Kawcak, C.E.** Clinical and Biomedical Sciences Departmental Nominee – Monfort Scholar Program, 2010.

**Puttlitz, C.M.** Monfort Professor, Colorado State University, 2011.

**Puttlitz, C.M.** Whitecloud Basic Science Award, International Meeting on Advanced Spine Techniques, 2010.

**Puttlitz, C.M.** Editor's Choice: one of the highest impact papers in Anesthesiology in 2009, 2010.

**Reiser, R.F.** Selected for Who's Who in Science and Engineering 2011-2012, 2011.

**Reiser, R.F.** Most Productive Mentor, Rocky Mountain Chapter, American College of Sports Medicine, 2010.



## **Editorial and Scientific Advisory Boards 2010-2011**

### **Baxter, G.M.**

American Journal of Veterinary Research  
The Compendium for Continuing Education, Practicing Veterinarian

### **Frisbie, D.D.**

Journal of Sports Medicine & Doping Studies

### **McIlwraith, C.W.**

Equine Veterinary Journal Advisory Board  
Journal of Equine Veterinary Science  
Equine Veterinary Education Assistant Editor  
Steadman-Hawkins Foundation Scientific Advisory Board  
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New Zealand Equine Trust Board of Trustees  
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Computational Methods in Ortho Biomechanics  
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American Veterinary Medical Association  
American Association of Equine Practitioners  
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Phi Kappa Phi

### **Frisbie, D.D.**

International Cartilage Research Society  
Orthopaedic Research Society  
American College of Veterinary Surgeons  
American Association of Equine Practitioners  
Osteoarthritis Research Society International  
American Veterinary Medical Association  
Veterinary Orthopaedic Society  
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Veterinary AO Society  
International Cartilage Repair Society  
American Society of Gene Therapy  
Orthopaedic Research Society  
American College of Veterinary Surgeons  
Veterinary Orthopedic Society  
California Veterinary Medical Association  
American Veterinary Medical Association

### **Haussler, K.K.**

American Veterinary Medical Association  
American College of Veterinary Sports Medicine  
and Rehabilitation  
American Association Equine Practitioners  
Colorado Veterinary Medical Association  
International Veterinary Academy of Pain  
Management  
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### **James, S.P.**

Society of Women Engineers  
American Society of Mechanical Engineers  
Society for Biomaterials

### **Kawcak, C.E.**

American Veterinary Medical Association  
American Association of Equine Practitioners  
American College of Veterinary Surgeons  
American College of Veterinary Sports Medicine  
and Rehabilitation  
Osteoarthritis Research Society International  
Orthopaedic Research Society  
Veterinary Orthopaedic Society

### **Kisiday, J.D.**

Orthopedic Research Society

### **McIlwraith, C.W.**

Royal College of Veterinary Surgeons (Fellow)  
American College of Veterinary Surgeons  
(Diplomate)  
American Association of Equine Practitioners  
American Veterinary Medical Association  
Phi Zeta Veterinary Honor Society  
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Colorado Veterinary Medical Association  
Orthopaedic Research Society  
Veterinary Orthopaedic Society  
American Association of Veterinary Clinicians  
European College of Veterinary Surgeons  
(Diplomate)  
International Society of Arthroscopy and Knee  
Surgery  
International Cartilage Research Society (ICRS)  
(Fellow)  
American Academy of Orthopaedic Surgeons  
(AAOS) (Associate Member)  
American College of Veterinary Sports Medicine  
and Rehabilitation

## Professional Associations 2010-2011

### Puttlitz, C.M.

Orthopaedic Research Society  
Cervical Spine Research Society  
American Society of Biomechanics  
American Society of Mechanical Engineers  
International Society of Biomechanics  
Spine Arthroplasty Association  
North American Spine Society

### Reiser, R.F.

National Strength and Conditioning Association  
(NSCA)

International Society of Biomechanics in Sports  
(ISBS)

American College of Sports Medicine (ACSM)  
International Sport Engineering Association  
(ISEA)

### Werpy, N.M.

American Veterinary Medical Association  
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American College of Veterinary Radiology  
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### Green Level

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Alamogordo Animal Hospital	David C. Davis	Cindy Guagenti
Dennis and Kerrie Allen	Dearborn Stables	Heidi Gordon on behalf Denise Steensland, Employee's Community Fund
Elizabeth Armstrong	Denali Stud	Hagyard Equine Medical Institute
Ashford Stud	Double JK Ranch	Ed Halpern
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Ms. Cynthia Chesnutt	Melissa Lyons Gardner	Connie English
Cherry Creek Clinic	Loni D. Gattinger	James Irving
Clover Valley Veterinary Hospital	Kate A. Gaughan	J Diamond 3
Columbia Equine Hospital	Barrie and Brenda Gerolamy	Robert A. Jackson
Contract Veterinary Sales	Cauleen Glass – In memory of Denison P. Glass	Jane M. Jennings
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Claire Cox	Martha Goodrich	RJ Ketch, RJ Ketch Equine, Inc.
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LaSalle Veterinary Clinic	Lester Pedicord	John R. Steele and Associates
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***Summary of  
Research Projects  
2010-2011***





## Summaries: Focus 1

### Musculoskeletal Tissue Healing

## Consensus on Equine Tendon Disease: Building on the 2007 Havemeyer Symposium

### Take Home Message

Equine tendon injury accounts for a large number of days out of training/competition and/or retirement. There have been considerable advances in this field over the past 20 years and this consensus document draws conclusions from discussion of four key areas 1) severity grading, 2) genetics of tendon disease, 3) the design of clinical trials and, 4) rehabilitation programs.

### Introduction

This recently published editorial written by Dr. Roger Smith of the Royal Veterinary College, London and Dr. Wayne McIlwraith of CSU ORC presents conclusions following the discussions at a three-day symposium in Iceland sponsored by the Dorothy Havemeyer Foundation in November 2007. It also provides recommendations for future research.

### Terminology

'Tendinitis' is commonly used to describe a nontraumatic overstrain injury to a tendon. Although 'tendinosis' has been suggested in the human field, as many pathologic specimens from human patients show no evidence of traditional histological features of inflammation, it does not rule out components of the inflammatory cascade having some role and so more recently, the nonspecific terms 'tendinopathy' or 'tendon disease' have been adopted.

### Severity Grading and Staging of Tendon Disease

#### Severity

- *Criteria was proposed for severity grading for superficial digital flexor tendon injuries (Table 1)*
- *Staging – An accurate definition of the disease is vital for epidemiological and genetic studies (Table 2)*
- *Clinical definition – heat, pain, swelling reflecting stage (acute vs. chronic)*
- *Ultrasonographic definition – Ultrasonographic criteria of disease were originally correlated with broad histological parameters. However, more recently, more sophisticated computer-assisted*

analysis of the ultrasonographic image shows potential for defining and staging the disease through providing a more accurate determination of the nature of the tissue within the tendon.

- *Magnetic resonance imaging definition – A more accurate and/or sensitive definition of disease may be provided by MRI, although to be practical, this should be performed using standing units.*
- *Histologic definition – Histological analysis could provide the most accurate definition of pathological change, although it is usually only appropriate post mortem because of the trauma created by biopsy techniques.*

### Genetics of Tendon Disease

Genetics have the potential to provide useful markers of susceptible individuals in disease mechanisms which will be beneficial in the future to help prevent injury.

### The Design of Clinical Trials and Objective Outcome Parameters

There are many studies published in both the human and the equine literature on tendon disease that are underpowered. To assist in the design of future studies to compare outcomes between different treatments, a power calculation was performed to give the sizes of the groups required, assuming a P value of 0.05 (two-sided), 90% power and 1:1 treated: non-treated ratio – details in paper.

### Rehabilitation Protocols

There is a need for a standardized rehabilitation protocol. At present, programs are empirical, but the variability of the disease makes scientific testing of these protocols impossible.

### Recommendations for Future Research

The following were considered the most important directions for research into tendon disease in the horse in the immediate future.

- Biomechanics:
  - Evaluation of 'microklutziness' in the horse.



## Summaries: Focus 1

### *Musculoskeletal Tissue Healing*

- Relationship of biomechanics to risk of injury, both in terms of ‘macrobiomechanics’ (i.e. of the limb) and biomechanics at a tissue level.
- Experimental models of tendon disease in the horse:
  - Validation of surgical models in the horse and sheep.
  - Development and validation of *in vitro* models to identify molecular processes of degradation.
- Diagnostic imaging (ultrasonography and MRI) of tendon injuries.
- More sensitive and specific correlation with histology.
- Genetics of tendon disease.
- The influence of genetics on tendon biology and risk of injury.
- Prevention of tendon disease:
  - Development of training regimes to prevent tendon disease in both young and mature horses.
  - Epidemiological studies to identify risk factors.
  - The influence of surface to tendon injury.
- Medication targets in tendons.
- Development of new drugs to reduce the apparent excessive remodeling in tendons.
- ‘Biological’ therapies:
  - Mechanism and evidence-based outcomes for platelet-rich plasma and other growth factor/cytokine-related therapies.
  - Mechanism and evidence-based outcomes for cell-based therapies.
- Clinical trials:
  - Need to be well controlled, with an adequate number of horses per group (see power calculation above).
  - Need for randomized controlled trials, although this may not be possible/practical in the equine industry.
- Rehabilitation programs

### Acknowledgments

The organizers (the authors) would like to acknowledge the considerable financial assistance that was given to hold this meeting primarily from the Dorothy Havemeyer Foundation but also from

Luitpold Pharmaceuticals, and the Orthopaedic Research Center, Colorado State University.

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## Summaries: Focus 1

### Musculoskeletal Tissue Healing

**Table 1.** Proposed criteria for severity grading of superficial digital flexor tendon injuries (consensus includes previously proposed definitions).

Proposed definitions	Havemeyer grade 1 (mild)	Havemeyer grade 2 (moderate)	Havemeyer grade 3 (severe)
BAPTEN protocol	0-15% of tendon 'volume' affected	16-25% of tendon 'volume' affected	>25% of tendon 'volume' affected
Lesion size at MIZ	0<10%	10%-40%	<40%
Maximum tendon CSA	<2 cm <sup>2</sup>	2-5cm <sup>2</sup>	>5 cm <sup>2</sup>

**Table 2.** Summary of the parameters used to define and stage tendon disease

Category	Criteria	Normal	Acute	Chronic
Clinical findings		No heat, pain or swelling	Heat, pain or swelling on palpation	Enlargement only
Ultrasonography	Cross-sectional area	Normal cross-sectional area 80-140 mm <sup>2</sup> for superficial digital flexor tendon at level 2B in Thoroughbreds; <20% difference from contralateral limb	Enlargement of tendon	Enlargement of tendon
	Echogenicity	Even and bright echogenicity	Reduction in echogenicity	Heterogeneous and variable echogenicity
	Longitudinal pattern	Prominent striated pattern (dependent on tendon)	Reduction in longitudinal fibre pattern	'Fibrotic' longitudinal fibre pattern
Magnetic resonance imaging		Homogeneous low signal intensity with normal size/shape	Enlargement of tendon with increased signal intensity	Enlargement of tendon with increased signal intensity; alternatively, enlarged with low signal intensity on all sequences
Histology	Vascular component	Sparse vasculature	Haemorrhage; increased vascularity	Neovascularisation; increased to normal vascularity
	Fascicle organisation	Prominent fascicular pattern with linearly arranged fascicles Crimp pattern to fascicles but reduced in older animals	Fibre disruption, giving rise to a variation in crimp signifying partial rupture	Disorganised fascicular pattern Accumulation of proteoglycan between fascicles Cyst formation; fat necrosis
	Cellular component	Sparse tenocyte population Acellular areas in old tendon	Tenocyte necrosis Inflammation and reparative cellular proliferation and fibroplasias; neutrophil and monocyte infiltration	Increased cellularity with cell rounding Acellular areas

## ***Equine Bone Marrow-Derived Stem Cells: Comparing the Ilium and the Sternum***

### **Take Home Message**

Both the sternum and ilium offer a rich supply of stem cells that have similar growth rates. The highest concentration of stem cells is in the first 5 mls of bone marrow aspirate.

### **Introduction and Hypothesis**

Bone marrow-derived mesenchymal stem cells (BMDMSCs) have been shown to improve healing of cartilage, bone, and soft tissue defects in horses and other species. Many studies have shown the need for sufficient numbers of stem cells to optimize repair. Studies have also shown that the timing of stem cell implantation affects treatment outcome. Currently, stem cell processing techniques strive to produce a large number of pluripotent cells within a short time. However, there are no standards for stem cell processing in the horse, and processing techniques vary widely between laboratories. The two sites of BMDMSC harvest in the horse are the sternum and ilium, and site selection is based primarily on practitioner preference. Also, laboratories differ in their preferred volume of marrow to collect. Differences in stem cell quantity or growth characteristics between harvest sites or harvest volumes could significantly affect the outcome of stem cell therapy. The primary goal of this study performed by McKenzie Adams with Drs. Goodrich, Rao, Olea-Popelka, Kisiday and McIlwraith with Nikki Phillips at the ORC was to determine any differences in cell concentration or growth rates between the equine sternum and ilium. A secondary goal was to determine the affect of aspiration volume on stem cell quantity and rate of growth. We hypothesized that there would be a higher concentration of cells in the sternum compared to the ilium, and that the first 5-ml fraction of marrow from either site would yield the greatest concentration of cells compared to subsequent fractions. Furthermore, we hypothesized that growth rates of cells from each site would not differ.

### **Materials/Methods**

Seven horses from the Equine Orthopaedic Research Center were sampled prior to euthanasia for another study. Horses ranged in age 2-5 years old. Two sequential 5-ml marrow samples were taken from the sternum and ilium of each horse. Nucleated cell counts were obtained for all samples pre and post marrow processing. Cells were expanded in culture for three passages and cell counts were obtained at each passage. Nucleated cell count concentrations between sites pre and post marrow processing were evaluated using a Signed Rank Test for non-parametric paired t-test. Differences in growth rates for cells in all comparisons were evaluated using a multivariable linear regression analysis. The data on number of nucleated cells (in millions) was checked for assumptions of linear regression analysis. If not met, the data was log transformed for analysis purposes. A p-value of 0.05 was used for determining statistical significance.

### **Results**

There was no significant difference ( $p>0.05$ ) between the nucleated cell counts of the first sternum aspirate and first ilium aspirate, both pre and post marrow processing (Figures 1 and 2). However, the nucleated cell counts of the first 5-mL aspirate were significantly higher than the second 5-mL aspirate for both sites, both pre and post marrow processing ( $p<0.05$ ) (Figures 1 and 2). There was no significant difference between growth rates for any of the groups ( $p>0.05$ ) (Figure 3).

### **Discussions/Conclusions**

These data should give practitioners confidence that cell numbers and growth rate characteristics do not vary between sternal and ilial sites and that the first 5 ml aspirate yields the highest concentration of stem cells for both sites. Depending on clinician preference both sites offer a rich supply of BMDMSCs that have similar growth rate characteristics. Further studies will reveal the importance of marrow volume in regard to cell counts and growth rate characteristics.

**Summaries: Focus 1**  
*Musculoskeletal Tissue Healing*

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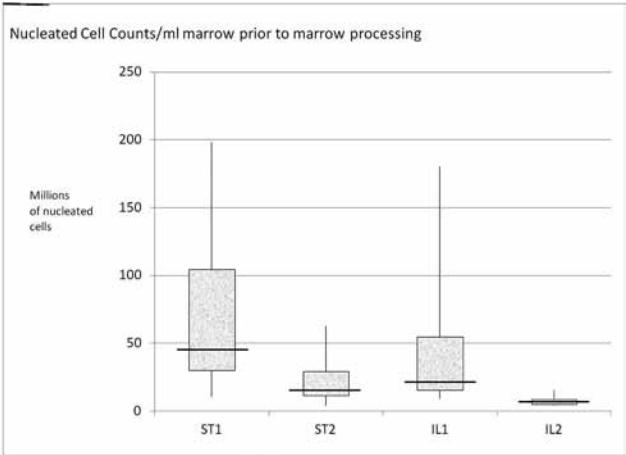
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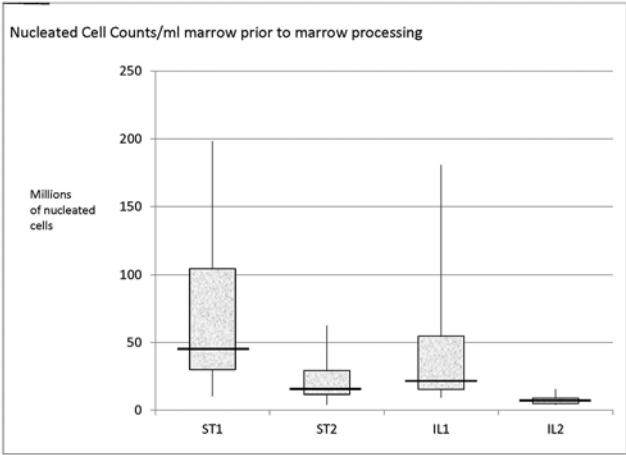
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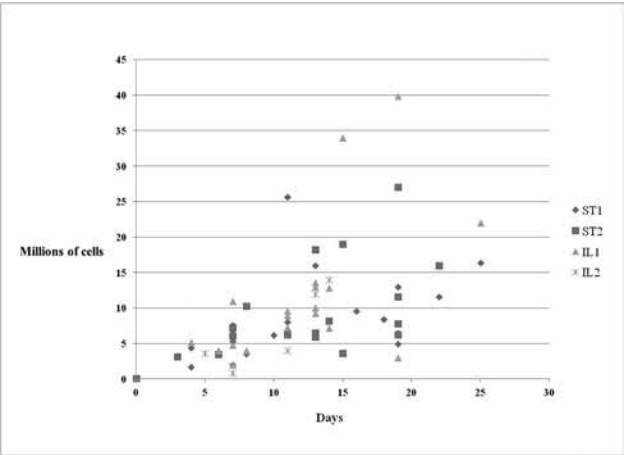
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**Figure 1.** Pre-marrow-processing numbers of nucleated cells. Vertical bars extend for the entire range of values, and boxes encompass all values between the first and third quartile. Horizontal bars represent median values.



**Figure 2.** Post-marrow-processing median numbers of nucleated cells. Vertical bars extend for the entire range of values, and boxes encompass all values between the first and third quartile. Horizontal bars represent median values.



**Figure 3.** Growth of all stem cells in culture over time. Day 0 represents first passage of cells out of colony at 800 cells/cm<sup>2</sup> (60,000 cells/T-75 flask). ST= sternum, IL= ilium 1 and 2 represent the first or second sample from each site.

## **Equine Mesenchymal Stem Cells in Vitro Differentiation Capacity: Differences Between Sternum and Ilium**

### **Take Home Message**

Cells acquired from both equine sternum and ilium are similar in their differentiation capacity *in vitro*. Clinicians can draw marrow from either site without concern that one is superior to another.

### **Introduction**

Equine bone-marrow mesenchymal stem cells (BMDMSCs) have been a primary focus for tissue regeneration, primarily in relation to joint injuries. The two sites of marrow aspiration in the horse are the sternum and wing of the ilium. Each site has been shown to offer a rich supply of BMDMSCs that have similar growth rate characteristics *in vitro*. This study was done by Karla Penman and Jennifer Phillips with Drs. Goodrich, Kisiday, and McIlwraith of the ORC. The next step in guiding practitioners in their aspirate choice involves investigating the capacity of BMDMSCs to differentiate into chondrocytes, osteocytes and adipocytes *in vitro*. We hypothesized that BMDMSCs acquired from the sternum and wing of the ilium in horses will have a similar capacity to undergo chondrogenic, osteogenic, and adipogenic differentiation regardless of their aspiration location.

### **Materials and Methods**

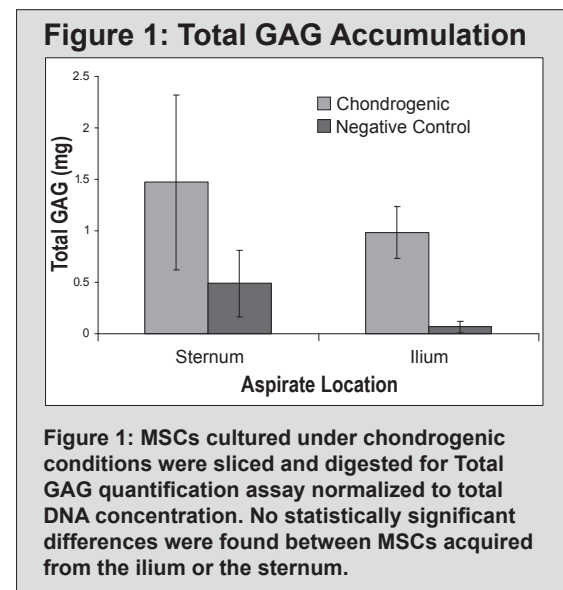
#### *Bone Marrow Aspiration and Cell Culture*

Five horses, between 3-5 years of age, had sternum and ilium aspirates performed using sterile technique. Aspirates were then spun down and plasma collected. Cells were cultured with alpha-MEM supplemented with FGF and grown to 80 percent confluency. Cells were passaged three times, were then cryopreserved, and stored for future use in liquid nitrogen. Cells from the third passage were chosen since these are the cells most commonly used clinically.

#### *Tri-Lineage Differentiation*

Cells were removed from cryopreservation and recovered at passage 3. Cells were then plated in 12 well plates at a density of 50,000 cells per cm<sup>2</sup> and treated with osteogenic and adipogenic media. After

14 days under osteogenic and adipogenic conditions cells stained. Osteogenic cells were lifted for quantification assays. Chondrogenic differentiation was conducted in 2% agarose gel with a concentration of 10 million cells per mL of agarose, surrounded with chondrogenic media. After 14 days the gels were sliced for stains and digested for total GAG accumulation.



#### *Statistical Analysis*

Osteogenic and Chondrogenic data results were analyzed using Statistical Analysis Software and a paired t-test was performed on each.

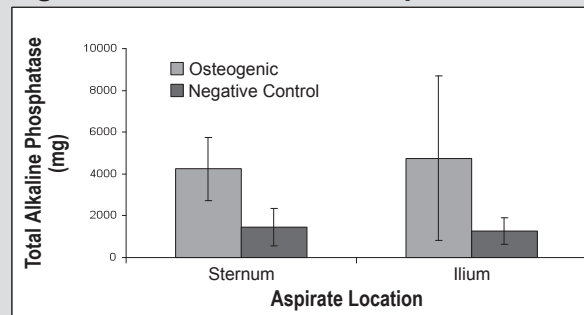
### **Results**

Differentiation assays (adipogenic, osteogenic and chondrogenic) yielded BMDMSCs with morphologic changes consistent with the desired assays. These results confirmed that BMDMSCs from both sternum and ilium were capable of tri-lineage differentiation. Chondrogenic and osteogenic propensity were not found to be significantly different between the two aspirate locations ( $P < 0.05$ ).

## Summaries: Focus 1

### Musculoskeletal Tissue Healing

**Figure 2: Total Alkaline Phosphatase**



**Figure 2: MSCs cultured under osteogenic conditions were lysed for total alkaline phosphatase quantification assay. No statistically significant differences were found between MSCs acquired from the ilium or the sternum.**

### Discussion

The results of this study indicate that there is no difference between the aspiration location on the cells' differentiation capacity *in vitro*. This supports previous work that demonstrated that there is no significant difference between the nucleated cell counts between aspiration locations. This confirms the multipotency of BMDMSCs regardless of their aspirate location. This is consistent with previous EORC studies evaluating *in vitro* differentiation capacity of BMDMSCs. This data illustrates that clinicians can draw from either sternum or ilium when attempts at regenerative therapy are pursued without concern that one site would be superior to the other.

### Future Studies

Evaluating which aspirate location is preferable necessitates testing cells from each location for 'stem cell' criteria. The standards for evaluation include monolayer expansion and tri-lineage differentiation, which have been explored in this study; however, the next step in this investigation is to examine cell surface markers on cells cultured from both aspirate location. Research is currently underway to evaluate cell surface markers to indicate cell quality of BMDMSCs from both sternum and ilium.

### Acknowledgments

Funding provided by PVM student grant.

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## **Equine Models of Articular Cartilage Repair**

### **Take Home Message**

This article is a review of equine chondral defect models that have recently been recognized as having specific advantages for translation into human articular cartilage regeneration co-authored by Drs. McIlwraith and Frisbie at CSU and Drs. Lisa Fortier and Alan Nixon of Cornell University. The defect models in the femoropatellar and femorotibial joints of the horse provide the closest approximation to humans in terms of articular cartilage and subchondral bone thickness. It is possible to selectively remove calcified cartilage, create defects, and do manipulations arthroscopically. Large sized defects allow for more tissue for evaluation, and there is the ability to have controlled exercise and test the ability of the repair tissue to cope with athletic exercise. Rehabilitation regimens can be followed as well.

### **Introduction**

Articular cartilage injuries of the knee are common, and a number of different methods have been developed recently in an attempt to improve this repair. In one review of 31,516 knee arthroscopies, 53,569 hyaline cartilage lesions were documented in 19,827 patients<sup>1</sup>. From a clinical point of view there are two distinct goals of cartilage repair: 1) restoration of joint function, which includes pain relief, and 2) prevention of or at least delay of the onset of osteoarthritis.<sup>2</sup> Methods of assessing putative repair techniques have not been developed *in vitro* and therefore screening of potential procedures for human clinical use is done by pre-clinical studies using animal models of articular cartilage defects.

Articular cartilage lesions encountered within the human joint typically arise as a consequence of trauma (usually a sports injury) or during the course of diseases such as osteoarthritis (OA) and osteochondritis dissecans (OCD), and it is common for them to not encroach significantly beyond the cartilage-bone interface into the subchondral bone compartments<sup>4</sup>. Repair strategy should focus on reestablishing the articular cartilage compartment rather than the bony one, but it is recognized that

augmentation of bone is sometimes necessary.

This article reviews equine chondral defect models that have been recently recognized to have specific advantages for translation into human articular cartilage resurfacing<sup>4,5</sup>. The complete article on this review of equine models of articular cartilage repair has recently been published in the journal *Cartilage*<sup>5</sup>.

### **Recent Equine Models of Cartilage Repair**

Currently, the joints of horses with anatomically equivalence to the human knee and ankle are used for cartilage repair studies. The use of the femoropatellar and femorotibial joints (collectively known as the stifle) which are the anatomic equivalent of the human knee. Equine models for cartilage repair have been performed using the medial femoral condyle (MFC), the lateral trochlear ridge (LTR) of the femur, and the medial trochlear ridge (MTR) of the femur. Compared to other animal models, articular cartilage thickness in the stifle of the horse most closely approximates that of the human knee<sup>6</sup>. Histological measurements of the thickness of noncalcified and calcified cartilage, as well as the subchondral bone plate, were made in three locations on the femoral trochlea and two locations on the MFCs of the species used in pre-clinical studies of articular cartilage and compared to those of the human knee. Species included human, horse, goat, dog, sheep, and rabbit. Average articular cartilage thickness over five locations was 2.2 to 2.5 mm for human, 0.3 mm for rabbit, 0.4 to 0.5 mm for sheep, 0.6 to 1.3 mm for dog, 0.7 to 1.5 mm for goat, and 1.5 to 2.0 mm for horse.

Critical size defects have been tested on both the MFC and the trochlea. An MFC model was initially developed to evaluate the effect of subchondral bone microfracture on articular cartilage repair<sup>6</sup>. It has since been used to look at early events in cartilage repair after subchondral bone microfracture<sup>7</sup> as well as the effective removal or retention of calcified cartilage<sup>8</sup> and the value of augmented gene therapy in the repair of full-thickness defect treated with microfracture<sup>9</sup>. Results have been published as indicated by the references.

## Summaries: Focus 1

### *Musculoskeletal Tissue Healing*

Use of the LTR in the horse as a model of articular cartilage repair was developed by Alan Nixon. This model was first reported as a 12-mm-diameter defect in 1994<sup>10</sup> and a modified 15-mm defect creation reported in 1995<sup>11</sup>. The LTR model has been used to evaluate technologies that require suturing of a membrane<sup>12</sup>. The LTR has been recently used to evaluate osteochondral repair with a biphasic construct<sup>13</sup> and a device developed by Dr. Fortier with Kensey Nash.

In initial work at Colorado State University, the MTR of the femur was used to create five, 4-mm defects for gathering of pilot data. Presently creation of two, 15-mm defects on the MTR of the femur is the standard for test cartilage transplantation techniques to ensure that critical defect size is achieved<sup>14,15</sup>. In this model with two, 15-mm defects on the MTR has been used to evaluate both an autologous chondrocyte implantation (ACI) technique<sup>14</sup> and an autologous fragment transplantation system (CAIS)<sup>15</sup>.

#### **Postoperative Care and Exercise**

All of the procedures are performed arthroscopically so postoperative care is straightforward. At CSU, an exercise regimen of two minutes trot, two minutes gallop and two minutes trot on a high-speed treadmill was implemented at four months and continues out to 12 months. This technique is considered useful to test for durability of tissue. In other studies at Cornell, free exercise is used as a less expensive alternative to high-speed treadmill exercise and typically those studies have gone out two tight months rather than 12 months.

#### **Advantages of Equine Femoropatellar and Femorotibial Models**

Based on the studies presented in this review article and summarized here, the horse provides the closest approximation to humans in terms of articular cartilage and subchondral bone thickness, it is possible to selectively leave the entire calcified cartilage or on the other hand, completely remove it with certainty. The MFC model illustrates that it is possible to emulate MFC lesions in humans. One potential disadvantage of the MFC model is that if the subchondral bone plate is violated, a subchondral

bone cyst can potentially develop which might confound the result, but this has not been a problem in the recorded studies. Both the MTR and LTR locations can be used to generate one or two critically sized cartilage or osteochondral defects.

Other advantages of the equine model include and ability to use the arthroscope to create lesions and to perform second-look arthroscopies. The advantages over other species also include more repair tissue for analysis, the ability to monitor patients clinically, as well as with diagnostic imaging, thereby allowing practical assessment of clinical response to repair techniques. Horses also get similar orthopaedic clinical diseases as humans so clinical evaluation in naturally occurring disease can be additive to the preclinical research studies and both horses and humans can benefit from these studies. It is also the opinion of the authors that the ability to have controlled exercise with horses is an advantage both in the early rehabilitation stage and later to test the ability of the repair tissue to cope with athletic exercise.

#### **Acknowledgments**

This project was funded by the Steadman Philippon Research Institute.

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## Summaries: Focus 1

### Musculoskeletal Tissue Healing

#### Evaluation of Intra-Articular Mesenchymal Stem Cells to Augment Healing of Microfractured Chondral Defects

##### Take Home Message

Studies confirmed that the intra-articular injection of  $20 \times 10^6$  bone marrow-derived mesenchymal stem cells (BMSCs) four weeks after creation of full thickness defects on the central weight-bearing area of the medial femoral condyle in the equine stifle caused enhanced cartilage repair quality with increased aggrecan content and increased firmness of the repair tissue.

##### Introduction

Microfracture technique was developed to enhance chondral resurfacing by taking advantage of the body's own healing capability and is the most frequently used technique for first-line treatment of symptomatic lesions in the articular cartilage in the knee.<sup>1,2</sup> Study microfracture enlarge-, full-thickness articular cartilage defects in a controlled manner, an equine model was developed to mimic lesions often observed in human patients.<sup>2-7</sup> Full-thickness chondral defects are made arthroscopically in the weight-bearing portion of the medial femoral condyle. This model obviates a noted flaw in many studies where the full-thickness defect penetrated deep into the subchondral bone.<sup>8</sup>

Early work using labeled mesenchymal stem cells has shown that they have an affinity for damaged joint tissue with the ability to localize and participate in repair of damaged joint structures including the cruciate ligaments and menisci, as well as cartilage lesions (if administered in sufficient quantities).<sup>7</sup> Most studies have focused on meniscal repair by either direct injection or intra-articular injection of MSCs into the joint stimulated meniscal regeneration in a caprine model of osteoarthritis<sup>9</sup> and this led to the question of potential value in cartilage repair. A recent equine study showed early benefits of MSCs in a fibrin matrix, but no significant difference was noted when MSCs plus fibrin compared with fibrin alone at eight months.<sup>10</sup>

The purpose of this study was to test the potential of bone marrow-derived mesenchymal stem

cells (BMSCs) to enhance the healing response of full-thickness cartilage defects that had been microfractured. We hypothesized that the intra-articular injections of BMSCs would augment healing with microfracture compared with microfracture alone. This study has just been published in *Arthroscopy: The Journal of Arthroscopic and Related Surgery* in 2011<sup>11</sup> and was done by Drs. McIlwraith, Frisbie, Kisiday, Werpy, and Kawcak at the ORC in collaboration with Drs. Bill Rodkey and Richard Steadman of the Steadman Philippon Research Institute.

##### Methods

Ten skeletally mature horses free of musculoskeletal abnormalities were entered into this 12-month study. We performed a power calculation based on a previous study with microfractured defects, an  $n=10$  provided a power of 81% based on the previous study. Defects measuring  $1\text{ cm}^2$  were arthroscopically created on the medial femoral condyle of both medial femorotibial joints in the stifle (analogous to the human knee) as previously described.<sup>3,4</sup> Defects were debrided through the calcified cartilage layer down to the level of subchondral bone plate and both defects were subjective to standard subchondral

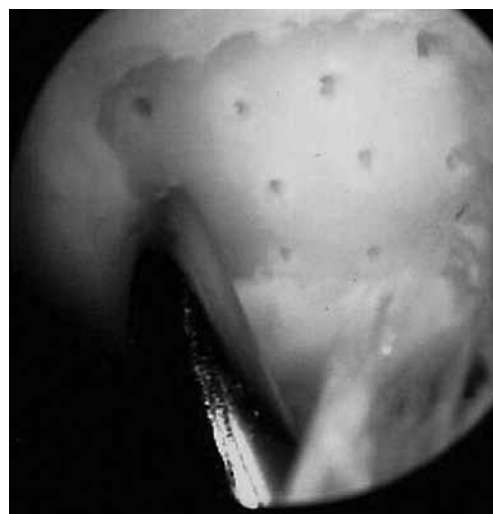
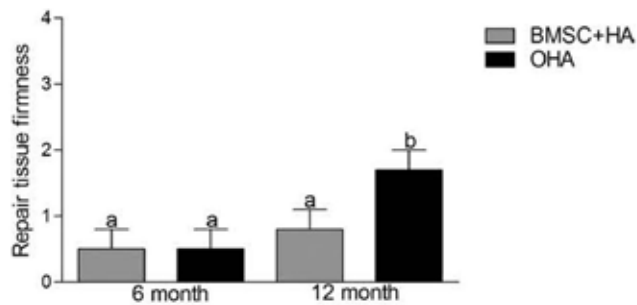


Figure 1.



**Figure 2.** Plot of repair tissue firmness at 6-month second-look arthroscopy and 12-month necropsy evaluation by treatment group. Different letters indicated a significant difference between groups ( $P < .05$ ).

bone microfracture (Figure 1). One month after defect creation, each horse had one joint randomly treated by intra-articular injection of BMSCs with 22 mg of HA (hyvisc) (hyaluronate sodium)  $3 \times 10^6$  Da; Anika Therapeutics, Woburn, M.A. (BMSC plus HA) whereas the opposite joint received 22 mg of HA alone (OHA). A routine post-operative rehabilitation protocol was followed and the horses were subjected to a strenuous treadmill exercise protocol.

Other details of methods are described in the referred manuscript that has just been published.<sup>11</sup>

## Results

At the termination of the study BMSC plus HA compared with OHA joints had significantly firmer repair tissue, whereas no difference existed at the six-month second-look arthroscopy (Figure 2). No deterioration in firmness was noted in the BMSC plus HA-treated joints from 6-12 months but the OHA-treated repair tissue became significantly softer compared with the surrounding cartilage in the same comparison. There was no significant difference with treatment on lameness (very mild) (in both cases), the degree of synovial effusion (again minor) and the response to joint flexion.

Evaluation of the MRI imaging yielded no significant treatment differences between groups and there were no differences in histologic analysis of articular cartilage. However, immunohistochemical evaluation of repair tissue and surrounding tissue significantly

( $P=0.02$ ) greater aggrecan staining in the repair tissue of BMSC plus HA-treated joints ( $75.2\% \pm 8\%$ ) compared with OHA-treated joints ( $57\% \pm 8\%$ ).

## Discussion

Microfracture has been previously shown to enhance the amount of repair tissue in full-thickness articular cartilage defects of the medial femoral condyle in the horse and Type II collagen content is significantly increased.<sup>3,4</sup> It is also been shown that removal of calcified cartilage with retention of the subchondral bone plate in microfractured defects increased the overall amount of repair tissue as assessed by arthroscopic (four months) and gross evaluation (12 months); furthermore, there was improved attachment of the repair tissue to the underlying subchondral bone and the adjacent normal cartilage.<sup>5</sup> Our study showed improvement in aggrecan content in microfractured defects that received intra-articular MSCs, a critical part of achieving cartilage repair. Previous work in evaluating defects treated with microfracture at 12 months showed good content in staining for Type II collagen but inadequate content in staining for aggrecan.<sup>3,5</sup> This finding is consistent with the findings of enhanced firmness of repair tissue as aggrecan is the critical component to provided compressive stiffness in articular cartilage.

## Acknowledgments

This study was funded by the Steadman Philippon Research Institute, Vail, Colo.

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## Summaries: Focus 1

### *Musculoskeletal Tissue Healing*

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## **Expansion of Adult Equine Bone Marrow Mesenchymal Stem Cells on Fibrinogen-Rich Protein Surfaces Derived From Blood Plasma**

### **Take Home Message**

Current practices for the preparation of bone marrow mesenchymal stem cells (MSCs) for equine musculoskeletal injuries involve culture-expansion to obtain millions of cells for a single treatment. While tissue culture plastic is the standard surface on which MSCs are culture-expanded, we report that coating tissue culture plastic with fibrinogen precipitated from blood plasma increases MSC proliferation without affecting differentiation potential. Fibrinogen harvested from autologous blood is an inexpensive means of creating a growth surface that is superior to tissue culture plastic.

### **Introduction**

The low density of mesenchymal stem cells (MSCs) in bone marrow requires culture expansion to obtain sufficient numbers for many proposed therapies<sup>1</sup>. Extensive research has focused on conditions for rapid and sustained MSC growth on tissue culture plastic; however, few studies have explored whether other growth substrates could better support MSC expansion. As an alternative to plastic, Drs. Kisiday, Lee, McIlwraith, and Frisbie with Ben Hale of CSU evaluated MSC proliferation on surfaces coated with a fibrinogen-rich precipitate (FRP) from blood plasma, a material that can be inexpensively obtained and processed using routine clinical and lab equipment and techniques. Chondrogenic and osteogenic cultures were conducted to determine the effect on expansion conditions on differentiation potential.

### **Materials and Methods**

**Bone marrow harvest and MSC isolation:** 10 ml of bone marrow was harvested from the iliac crest of 2-5 year old horses. The nucleated cells were seeded into low glucose DMEM + 10% fetal bovine serum (FBS) at  $0.25 \times 10^6$  cells/cm<sup>2</sup>. Adherent MSC colonies were trypsinized after 6-10 days. FRP surfaces: Plasma was obtained from venous blood drawn into CPDA. Plasma proteins were precipitated by mixing 880  $\mu$ l of 100% ethanol with 5 ml plasma. Precipitated proteins were concentrated by centrifugation, and then

resuspended in an equal volume of plasma (200  $\mu$ l). FRP solution was diluted 1:100 in sterile deionized water and added to flasks at 100  $\mu$ l/cm<sup>2</sup>. The next day, FRP solution was aspirated, and the flasks were rinsed once with water and dried. For Experiment 1, a subset of FRP surfaces was activated with thrombin (110 NIHU/mL in 40 mM CaCl<sub>2</sub>, diluted 1:20 in water) to convert the bound fibrinogen to fibrin.

**Expansion of MSCs:** Cultures were conducted in medium consisting of  $\alpha$ MEM + 10% FBS that had not been screened for stimulating high rates of MSC growth. In some cultures, the medium was supplemented with 2 ng/ml fibroblast growth factor 2 (FGF2). Expansion cultures were established by seeding at high ( $10 \times 10^3$  cells/cm<sup>2</sup>) or low ( $0.5 \times 10^3$ ) density. **Agarose hydrogel chondrogenesis:** MSCs were encapsulated in 2% (w/v) agarose at  $10 \times 10^6$  cells/ml and cultured in high glucose DMEM, 1% ITS+, 0.1  $\mu$ M dexamethasone, 37.5  $\mu$ g/ml ascorbate-2-phosphate, and 10 ng/ml rhTGF $\beta$ -3 [2]. After 15 days, samples were digested in proteinase K, and the total accumulated sulfated glycosaminoglycan (GAG) content was measured via DMMB dye binding assay. Selected samples were evaluated for type II accumulation via immunohistochemistry. **Osteogenesis:** For Experiment 3, MSCs were seeded in monolayer cultures at  $4 \times 10^4$  cells/cm<sup>2</sup> in high glucose DMEM plus 10% FBS, 5 mM  $\beta$ -glycerol phosphate, 0.01  $\mu$ M dexamethasone, and 37.5  $\mu$ g/ml ascorbate-2-phosphate. After 10 days, cultures were fixed in 2% formalin and stained with 0.5% Alizarin Red to identify mineralization. **Statistical Analysis:** Data was analyzed using a mixed model analysis of variance, with donor animal used as a random effect. Individual comparisons were made using least square means procedure. For each experiment, MSCs were tested from five donor horses.

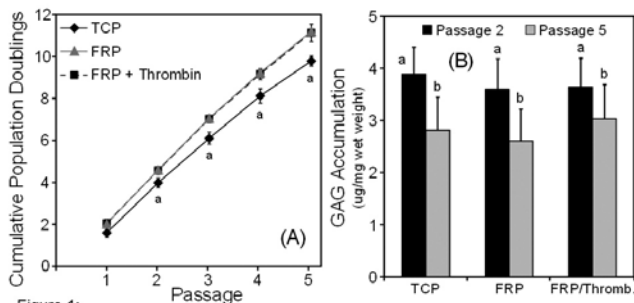
### **Results**

**Experiment 1: Effect of thrombin activation of FRP on MSC proliferation** – MSCs were seeded on FRP, with and without thrombin activation, at high density and cultured for five passages in FGF2 medium.

## Summaries: Focus 1

### Musculoskeletal Tissue Healing

Control MSCs cultured on uncoated tissue culture plastic in FGF2 averaged a population doubling (PD) every 24.5 hours. Cumulative PDs on FRP and FRP/Thrombin were greater than uncoated cultures beyond the first passage (Fig. 1A,  $p<0.05$ ). By Passage 5, growth in FRP and FRP/Thrombin cultures had exceeded uncoated tissue culture plastic by 1.2 and 1.5 PDs, respectively. Cumulative PDs were not significantly different for FRP and FRP/Thrombin surfaces ( $p=0.73-0.93$ ). In chondrogenic cultures, GAG accumulation was not significantly different among surfaces after two and five passages (Fig. 1B,  $p=0.13-0.86$ ). For uncoated and FRP cultures, GAG accumulation at Passage 5 was 72% of Passage 2 ( $p<0.05$ ). For FRP/Thrombin, GAG accumulation at Passage 5 was 82% of Passage 2, although this difference was not significant ( $p=0.08$ ). *Type II collagen* – Type II collagen was detected in all samples (data not shown). At Passage 2 and Passage 5, the intensity of staining was qualitatively similar among culture expansion conditions. The intensity of staining in Passage 5 samples was moderately lower than Passage 2.

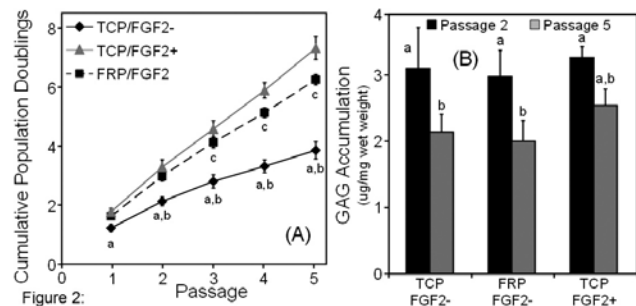


**Figure 1.** A) Cumulative population doublings – 'a' denoted a significant difference between TCP, FRP, FRP/Thrombin surfaces ( $p<0.05$ ). B) GAG accumulation in chondrogenic cultures after 15 days of culture. 'a' and 'b' denote a significant difference between groups ( $p<0.05$ ).

**Experiment 2: Effect of FRP on MSC proliferation in the absence of FGF2** – MSC expansion was evaluated on FRP, without thrombin activation, at high density and in the absence of FGF2. Uncoated control cultures, in the presence and absence of FGF2, were maintained at high density. Cumulative

PDs in uncoated/FGF2- were less than uncoated/FGF2+ at each passage (Fig. 2A,  $p<0.05$ ). In FRP/FGF2- cultures, cumulative PDs were greater than uncoated/FGF2- conditions beyond the first passage ( $p<0.05$ ). Cumulative PDs in FRP/FGF2- was less than uncoated/FGF2+ beyond the second passage ( $p<0.05$ ). By Passage 5, FRP/FGF2- cultures had experienced 3.7 more PDs than uncoated/FGF2- cultures but 1.5 fewer than uncoated/FGF2+.

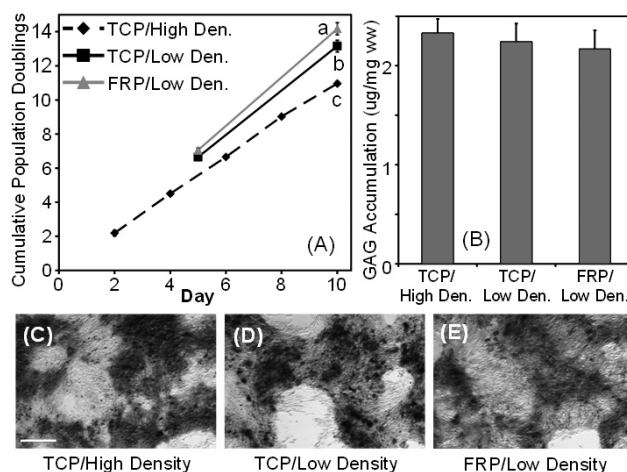
**Chondrogenesis:** After two and five passages, GAG accumulation in MSC-seeded agarose was not significantly different among conditions ( $p=0.46-0.85$ , Fig. 2B). After five passages, GAG accumulation in uncoated/FGF2- and FRP/FGF2- cultures decreased to 69% and 67% of Passage 2 values, respectively ( $p<0.05$ ). In uncoated/FGF2+ samples, Passage 5 GAG accumulation was 77% of Passage 2 values; however, this change was not significant ( $p=0.09$ ).



**Figure 2.** A) Cumulative population doublings – At each passage 'a' denotes a significant difference between TCP/FGF2- and TCP/FGF2+, 'b' denote a significant difference between TCP/FGF2- and FRP/FGF2-, and 'c' denotes a significant difference between FRP/FGF2- and TCP/FGF2+ ( $p<0.05$ ). B) GAG accumulation in chondrogenic cultures after 15 days of culture. 'a' and 'b' denote a significant difference between groups ( $p<0.05$ ).

**Experiment 3: Effect of FRP on MSC proliferation in low density culture** – MSCs in high density TCP cultures experienced at least two population doublings per passage, with an average of 10.9 cumulative population doublings at the end of the 10 day culture period (Fig. 3A). Low density TCP cultures experienced an average of 6.7 and 6.5 population doublings for the first and second passages, respectively. Low density FRP cultures

supported an average of 7.1 population doublings at each passage. At the end of the culture period, the cumulative number of population doublings among all conditions was significantly different ( $p < 0.05$ ). Low density FRP cultures experienced 1.0 and 3.2 additional population doublings relative to low and high density TCP cultures, respectively. *GAG accumulation in chondrogenic cultures* – GAG accumulation in MSC-seeded agarose ranged between 2.2–2.1  $\mu\text{g}/\text{mg}$  wet weight, with no differences among the three conditions ( $p = 0.61$ – $0.82$ , Fig. 3B). *Alizarin red staining in osteogenic cultures* – All cultures accumulated mineralized matrix (Fig. 3C–E). Subjectively, the area and intensity of staining appeared similar among conditions.



**Figure 3.** A) Expansion cultures were maintained over 2 (low density) or 5 (high density) passages in 10 days. 'a', 'b', and 'c' denote significant differences among groups on day 10 ( $p < 0.05$ ). B) Chondrogenic cultures were established after 10 days of expansion - GAG accumulation was not significantly different among groups after 15 days of chondrogenic culture. C) Alizarin red staining of osteogenic cultures established after 10 days of expansion. – Mineralization was detected in all groups after 10 days of osteogenic culture.

## Discussion

The continued advancement of MSC-based regenerative strategies will necessitate clinical expansion techniques that generate a consistent yield of MSCs. Variability among patient populations, such as MSC yield from bone marrow, proliferation rates, and differentiation capacity that can change with age<sup>2-5</sup> may challenge expansion consistency and slow enthusiasm for clinical implementation. Given the ability of FRP surfaces to stimulate MSC proliferation without affecting chondrogenesis or osteogenesis, FRP surfaces represent a simple autologous method for enhancing proliferation in both rapid (FGF+) and slow (FGF-) growing MSCs. Expansion of MSCs in FGF2 at low density appears more promising than high density cultures.

## Acknowledgments

Funded by a Colorado State University College Research Council grant.

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## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease

#### Incidence of Radiographic Changes in Young Cutting Horses

##### Take Home Message

In a large population of young cutting horses there was a high prevalence of radiographic lesions, especially in the tarsi and stifles, on pre-sale survey radiographs. Notably, nearly 90% of the 458 included horses had at least one radiographic finding recorded. Changes in the medial femoral condyle (188/454, 41.4%) and osteophytes in the distal tarsus (201/438, 45.9%) were the most common findings.

##### Background

Radiographic repositories were first established at the Keeneland Thoroughbred yearling sales and due to the success, the National Cutting Horse Association (NCHA) followed suit at the 2005 NCHA Futurity Sales in Fort Worth, Texas. Studies of repository and survey radiographs have reported the prevalence of radiographic changes in various populations, most notably in Thoroughbreds<sup>1,2</sup> and Standardbreds<sup>3-5</sup>. A veterinarian's role is to identify abnormal radiographic changes and correlate them to future performance<sup>1,6</sup> which can be challenging without knowledge, or in the absence, of clinical signs<sup>6</sup>. This problem has been addressed in Thoroughbred and Standardbred populations by studies that correlate radiographic changes with performance<sup>7-10</sup>. There are no such published studies in Quarter Horses. The objective of this study was to quantify the radiographic changes in survey radiographs of yearling and 2-year-old Quarter Horses intended for cutting. This study has recently been published in the *Equine Vet Journal* and was done by Drs. Erin Contino, Richard Park and Wayne McIlwraith.

##### Materials and Methods

Digital radiographs of yearling and 2-year-old horses were obtained from the Western Bloodstock Radiograph Repository at NCHA sales between December 2005 and December 2006. Routine survey radiographs of yearling Quarter Horses foaled between 2003 and 2006 at a private cutting horse farm were also included. One view of each carpus, two views of each fetlock, four views of each tarsus, and two views of each stifle were evaluated. Following

categorization of radiographic lesions, the frequency of each lesion was statistically analyzed by limb, by horse, and by age. The prevalence of radiographic lesions between yearlings and 2-year-olds was compared.

*Grading of the stifle:* In the stifle, the medial femoral condyle (MFC) was classified as convex in contour (grade 0), flattened without evidence of subchondral bone changes (grade 1), a small defect or change in, without extension through, the subchondral bone (grade 2), a shallow, crescent shaped subchondral lucency that is confluent with the joint surface (grade 3) or a well-defined radiolucency in the middle of the MFC that communicates with the joint (grade 4) (Fig. 1). Abnormalities of the trochlear ridges, trochlear groove and patella were also recorded.

*Grading of the carpus, tarsus, and fetlocks:* The carpus was evaluated for the presence of osteophytes, bony fragments, and fractures of the accessory carpal bone. The fetlocks were evaluated for the presence of bony fragments, radiographic evidence of osteochondritis dissecans, palmar/plantar supracondylar lysis, abnormalities of the proximal sesamoid bones, and thickening of the dorsal proximal cortical surface of the middle phalanx (P2). The tarsocrural joint was evaluated for changes of the medial malleolus, distal intermediate ridge of the tibia (DIRT), and the trochlear ridges. The distal tarsal joints were evaluated for radiographic signs consistent with osteoarthritis (OA) including osteophytes, lysis, sclerosis, joint space narrowing, and tarsal bone malformation.

##### Results

The study included 8,857 radiographs of 3,900 joints in 458 horses (278 yearlings and 180 2-year-olds) of which 408 (89.1%) had at least one radiographic change recorded.

*Stifle:* Of the 454 horses with stifle radiographs, 202 (44.5%) had a radiographic change, the majority of which (188, 41.4%) were due to changes of the



MFC. Of these, 98 (52.1%) were categorized as grade 1, 37 (19.7%) as grade 2, 30 (16%) as grade 3 and 23 (12.2%) as grade 4. The right MFC (157, 34.6%) was affected significantly more than the left MFC (128, 28.2%) ( $p < 0.0001$ ). Six (1.3%) horses had an osteophyte on the medial proximal tibia; all were 2-year-olds which was a significant difference when compared to yearlings ( $p = 0.0033$ ). Of the 433 horses in which evaluation of the femoropatellar joint was possible, 14 (3.2%) had radiographic changes that most commonly involved the lateral trochlear ridge.

*Tarsus:* Of 438 horses with tarsal films, 304 (69.4%) had at least one finding recorded, most of which were in the distal tarsus. There were 201 (45.9%) horses with osteophytes, 76 (17.3%) with lysis, 30 (6.8%) with malformation, 29 (6.6%) with sclerosis, and 11 (2.5%) with joint space narrowing in the distal tarsus. In the tarsocrural joint, 54 horses (12.3%) had flattening of the medial trochlear ridge and 59 (13.5%) had changes of the DIRT. Two-year-olds had significantly more changes in the DIRT compared to yearlings ( $p = 0.0107$ ).

*Fetlocks and Carpus:* In the hind fetlocks, 155 of 355 horses (43.7%) had at least one radiographic change recorded. Changes of sagittal ridge were recorded in 69 (19.4%) horses and thickening of the dorsal cortex of P2 affected 49 (13.8%) horses. Osteophytes of the dorsal proximal aspect of P2 were recorded in 43 (12.1%) horses and were significantly more common in 2-year-olds than yearlings ( $p = 0.0004$ ). The front fetlocks were evaluated in 361 horses of which 131 (36.3%) had radiographic changes. In 54 (15%) horses, changes of the sagittal ridge were recorded. Palmar supracondylar lysis affected 47 (13.1%) horses, most of which (36, 76.6%) were categorized as mild. Only 27 of 342 (7.9%) horses had changes in the carpus making it the least affected joint examined.

## Discussion

There was a high prevalence of MFC changes in this population. A recent study of Thoroughbred yearlings found that MFC lesions had a negative impact on the sale of a horse but did not negatively impact performance<sup>11</sup>. Flattening of the MFC affected over

20% of the horses in the study. There have been few studies on the prevalence or clinical relevance of this finding and further research is warranted in Quarter Horses to determine the extent to which changes to the MFC, including flattening, affect future performance.

In the fetlocks there was a relatively high prevalence of palmar supracondylar lysis which has been shown to decrease the likelihood of Thoroughbreds to start a race<sup>7</sup>. Thus, it may be a lesion that proves to negatively affect performance cutting horses. Dorsal cortical thickening of P2 in hind limbs has not been reported in the literature and may be unique to cutting horses. Yearlings were affected as frequently as 2-year-olds suggesting it does not develop in response to training. Osteophytes of P2 were significantly more common in 2-year-olds compared to yearlings suggesting they may form to stabilize the pastern joint in response to the biomechanical stresses of training.

There was a high prevalence of horses with radiographic signs of tarsal OA however, radiographic OA is not a reliable indicator of lameness<sup>6</sup>. It was interesting that there was no significant difference between the age groups since OA advances with age<sup>12</sup>. This indicates that training and discipline are clearly not the only factors contributing to the high prevalence of radiographic tarsal OA in this population of horses. Osteophytes, when seen independent of other changes, may be incidental although in Thoroughbreds, they have been associated with fewer race starts<sup>7</sup>.

This study establishes a baseline for what can be expected in the evaluation of pre-sale radiographs of young Quarter Horses. Work is ongoing to evaluate the clinical significance of these individual changes. This work was performed by Drs. Erin Contino, Richard Park, and Wayne McIlwraith.

## Acknowledgments

This study was funded by the Colorado State University National Cutting Horses Association Stallion Auction Program with additional support from a Colorado State University Research and

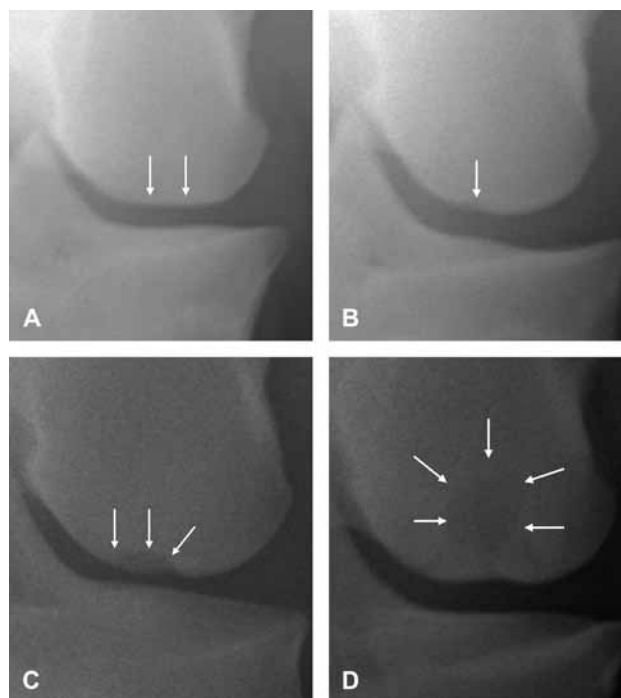
## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease

Scholarly Excellence grant. We wish to thank Jim and Carolyn Ware and Western Bloodstock for facilitating the availability of radiographs.

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**Figure 1.** Caudocranial radiographic projections of the femorotibial joint were used to evaluate the medial femoral condyle (MFC). A: The contour of the MFC is flat (Grade 1). B: There is a small defect in, but not extending through, the subchondral bone of the MFC (Grade 2). C: A shallow dome shaped lucency is seen extending through the subchondral bone in the MFC (Grade 3). D: A well defined subchondral bone cyst that communicates with the articular surface is evident in the MFC (Grade 4). *Reproduced with permission from Contino et al. (2011).*



***A Descriptive Study of the Equine Proximal Interphalangeal Joint Using Magnetic Resonance Imaging, Contrast Arthrography, and Arthroscopy*****Take Home Message**

The dorsal and palmar pouches of the proximal interphalangeal joint are described in depth using MRI, contrast arthrography, and arthroscopy. Arthroscopic approaches allow adequate access to the proximal interphalangeal joint and anatomy surrounding the joint is described.

**Introduction**

The proximal interphalangeal (PIP) joint has the least amount of motion of all the joints of the equine distal limb and is challenging to access arthroscopically. The objective of this study was to assist in the treatment of diseases related to the PIP joint by describing its anatomy and outlining arthroscopic methods as there is very limited description of arthroscopy of the PIP joint in the current literature. This study done by Dr. Lacy Kamm together with Drs. Goodrich, Werpy, and McIlwraith described areas of the PIP joint that are arthroscopically accessible, defines the soft tissue structures that must be avoided during arthroscope and instrument placement, and investigates the differences between the fore and hind PIP joint.

**Materials and Methods**

Cadaver limbs were used to perform anatomic modeling, magnetic resonance imaging (MRI) with MRI-compatible needles, computed tomography (CT) with contrast arthrography, and arthroscopy of the PIP joint. CT contrast arthrography was performed on three fore and three hind limbs. Twenty-four limbs, 12 fore and 12 hind, were used for arthroscopy. Areas that could be viewed arthroscopically were measured, and two arthroscopic approaches to the dorsal joint pouch were compared.

**Results**

Imaging revealed that in order to prevent penetration of the axial palmar/plantar ligaments, abaxial palmar/plantar ligaments, straight sesamoidean ligament, and the branches of the superficial digital flexor tendon, the palmar/plantar pouch instrument and arthroscope portals should be placed dorsal to

the neurovascular bundle and just proximal to the epicondyles of the proximal phalanx.

No significant difference in the joint volume was found between the fore limb and the hind ( $p=0.137$ ), though the mean volume was smaller in the fore limb than in the hind limb ( $8.89\text{cm}^3 \pm 2.1$  [std dev] vs.  $10.76\text{cm}^3 \pm 1.5$ , respectively). The only significant difference in visualization between the fore and the hind limb was the arthroscope portal in the proximal dorsal approach resulted in more visualization abaxially in the fore limb than in the hind ( $p=0.050$ ) when visualizing the cartilage on the same side as the arthroscope portal.

There was no significant difference in the amount of joint viewed when using the more proximal or distal approach to the dorsal joint pouch ( $p=0.586$ ). The average perimeter visualized was  $224.62^\circ$  when the proximal dorsal approach was used. This was only 62.4% of the total perimeter of the joint.

**Conclusion**

The dorsal and palmar/plantar joint pouches allowed for adequate arthroscopic visibility of the axial portions of the articular surface of the proximal and middle phalanx (P1 and P2). The abaxial portions of the articular surface were difficult to view due to the narrowing of the joint pouches abaxially. Palmar/plantar portals were placed dorsal to the neurovascular bundle to prevent injury of tendons and ligaments.

The one statistically significant difference between the fore and hind PIP joints was that the arthroscope could allow visualization more abaxial on the ipsilateral side of dorsal joint pouch in the fore limb compared to the hind limb. The cause for this is unknown as the soft tissues and bones of the PIP joint appeared very similar between the fore and hind on CT and MRI and the joint volumes were not significantly different on contrast arthrography CT. One possibility for the significant difference is that

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the hind pastern is more likely to stay in flexion due to the stay apparatus. Flexion of the joint causes the dorsal aspect to be more restricted and may impede abaxial motion of the arthroscope.

Concerning the dorsal joint, no significant difference in visualization was found when the arthroscope was placed at the level of the PIP joint versus 1.5 cm proximal to the joint ( $p=0.586$ ). The more proximal arthroscopic approach in this study was better to use as it allowed for easy visualization of the medial and lateral aspects of the joint and was not prone to the accidental exiting of the arthroscope or instruments that can happen with the distal approach when the ipsilateral side was visualized. It was found that if the arthroscope portal was placed too far proximally it

can limit “the ability to view the joint space, because the arthroscope is held against the articular surface of the distal end of the proximal phalanx by the joint capsule.” Therefore, it is important to place the arthroscope up to 1.5 cm proximal to the joint surface, but no more proximal than this to allow for maximal manipulation of the arthroscope.

### **Acknowledgments**

Funding for this study was provided by the John H. Venable Grant at the College of Veterinary Medicine and Biomedical Sciences at Colorado State University.

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## ***The Relationship Between Radiographic Changes and Performance Outcomes in Cutting Horses***

### **Take Home Message**

Radiographic repositories are commonly used as a predictor of future performance outcome. This study found that many radiographic changes were not correlated with performance outcomes. Of particular note, no radiographic changes of the medial femoral condyle of the stifle, including grade 4 subchondral cystic lesions, were associated with decreased performance outcome. However, some mild radiographic changes, most specifically mild osteophytosis of the distal tarsal joints, were associated with decreased performance outcomes.

### **Introduction**

Radiographic repositories are becoming increasingly popular in multiple disciplines as a screening tool prior to sale. However, the importance of the radiographic findings must be objectively assessed relative to potential significance. While studies have been done to correlate survey radiographic findings with performance outcomes in Thoroughbreds, no such published study exists in Quarter Horses. This paper serves as part two of a study performed by Dr. Myra Barrett (as part of an M.S.) with Drs. Park and McIlwraith examining the relationship between radiographic changes in survey radiographs relative to objective performance outcomes in Quarter Horse cutting horses.

The goal of this study is to better clarify the potential significance of radiographic changes on repository radiographs relative to performance. This in turn will allow veterinarians and their clients to make more objective, informed decisions prior to purchase about the potential implications of various radiographic changes.

*Methods:* Radiographic changes of 436 Quarter Horses, which were quantified in a previous paper (Contino et al. 2011), were compared to objective performance outcome parameters. The parameters were: 1) likelihood of competing, 2) likelihood of earning money as a three year old, four year old, and as a three and four year old combined, 3) average

amount of money earned as a three year old, four year old, and as a three and four year old combined. Mailed questionnaires and phone calls to owners of horses that did not earn money were used to try to determine why the horse had no recorded earnings.

### **Results**

When the tarsometatarsal (TMT) and distal intertarsal (DIT) joints were examined together (Fig. 1), the presence of mild (grade 2) osteophytes, which affected 19% of the horses, was associated with reduced chance of competing, earning money and mean money earned. Very mild and mild osteophytes of the third and central tarsal bone assessed individually at the level of the TMT and DID also have some significant effects in multiple performance outcome categories. The presence of thickening of the dorsal cortex of hind second phalanx as well as osteophytes at this location was associated with an increased likelihood of earning money. Several other potentially significant findings are reported but affect a relatively small number of the horses included in the study. Radiographic changes of the medial femoral condyle of the stifle were not significantly associated with performance outcome.

### **Conclusions**

Many radiographic changes were not found to be significantly associated with performance outcome. However, some mild changes were associated with decreased performance. In addition, some radiographic changes were correlated with improved performance outcome. The findings of this study can be used to help veterinarians make more objective assessments of survey radiographic findings prior to sale. This research helps lay the groundwork for further investigations of the significance of survey radiographic findings in individual breeds and disciplines.

### **Acknowledgments**

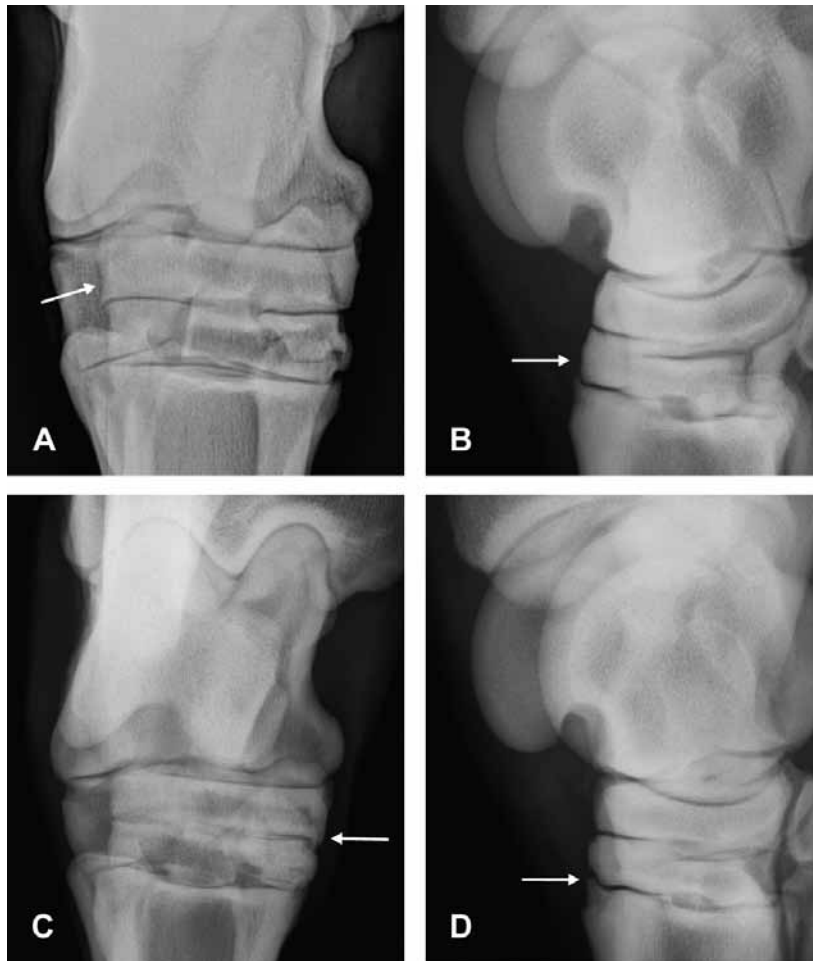
Thank you to Dr. Sangeeta Rao for her statistical analysis and to Taryn Yates for her assistance with data collection.

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**Figure 1.** Osteophytes in the tarsus were recorded by joint and bone involved and graded by size. A very small osteophyte (Grade 1) on the distolateral aspect of the central tarsal bone (A), a small osteophyte (Grade 2) on the proximal dorsolateral aspect of the third metatarsal bone (B), a medium osteophyte (Grade 3) on the distomedial aspect of the central tarsal bone (C) and a large osteophyte (Grade 4) on the proximal dorsolateral aspect of the third metatarsal bone (D) are shown with arrows. *Reproduced with permission from Contino et al. (2011).*



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### ***Suspensory Ligament Ultrasound Using Oblique Incidence for Identification of Ligament Anatomy With Comparison to MRI***

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#### **Take Home Message**

Combining the standard ultrasound examination of the suspensory ligament with oblique incidence technique improves identification of the normal anatomic features of the suspensory ligament (SL).

Injury to the SL is a common condition affecting horses of different ages and disciplines.<sup>1-2</sup> Lameness is often localized to the SL region using local infiltration of anesthetic solution around the ligament or perineural analgesia.<sup>3</sup> Once injury of the SL is suspected based on the clinical examination and response to analgesia, diagnostic imaging of this region is often performed. Radiography can be used to evaluate the proximal palmar aspect (face) of the third metacarpal bone for evidence of sclerosis, lysis, proliferation or avulsion fracture at the attachment of the SL.<sup>4-5</sup> However, examination of the ligament requires a modality that allows visualization of the soft tissue structures. Ultrasound has traditionally been the imaging modality of choice for diagnosis of SL injury. The technique has been described the ultrasound probe being placed at the palmar surface of the limb with the beam oriented perpendicular to the longitudinal axis of the fibers.<sup>6</sup> This technique has several limitations. The traditional technique for ultrasound of the SL creates an image which causes the SL to appear as a rectangular shaped echogenic structure (Fig. 1).

However, the proximal aspect of the SL is a bilobed structure surrounded by connective tissue. The muscle and adipose tissue are most prominent in the central aspect of the each lobe and they are surrounded primarily by ligament fibers. Using the oblique incidence technique the SL is first imaged with the beam perpendicular to the long axis of the ligament creating an echogenic appearance (Fig. 2).<sup>7</sup> The ultrasound beam is then angled obliquely relative to the long axis of the SL (Fig. 2). This causes the

margins of the SL to become evident. The surrounding connective tissue remains bright. The ligament fibers become dark. The adipose tissue remains bright. The muscle becomes dark, but not as dark as the ligament fibers. Preliminary work suggested this technique produced images that provided anatomic information about the fiber versus adipose tissue/muscle distribution similar to MRI images and gross sections. This information was vastly different than what is obtained with the traditional technique (Fig. 2). We hypothesized that the combination of the standard ultrasound examination with additional imaging of the limb while flexed with the probe oriented oblique and perpendicular to the longitudinal axis of the fibers would provide the greatest amount of anatomic information about the SL. Furthermore this ultrasound technique would provide similar information about the distribution of ligament fibers versus adipose tissue/muscle distribution as can be determined with MR imaging.

#### **Methods and Materials**

Ultrasound examination, both with perpendicular and oblique beam orientation as well as MRI was performed on the forelimbs of 10 horses (age 2-5 years). Ultrasound examination was performed beginning at level of the carpometacarpal joint and ending at the level of the SL branches using a GE Logiq E (Carlsbad, Calif.) ultrasound machine with a 10 MHz linear probe. Transverse and longitudinal images were obtained with the ultrasound beam placed perpendicular to the ligament fibers with the limb in a weight-bearing position. Ultrasound and MR images were obtained at 2, 3, 4, 6, and 8 cm distal to the carpometacarpal joint. MR images (3 mm) were obtained post-mortem using proton density, T2-weighted fast spin echo and STIR sequences on a 1.0 Tesla OrthoOne ONI (Wilmington, Mass.).

Following MR imaging, the SL were harvested and sectioned. Gross evaluation of the sections and Masson's trichrome stain was used to identify areas of ligament, muscle, and adipose. The circumferential area of the SL of the SL was measured on all



modalities. Flexed oblique incidence ultrasound and MR images of the right and left SLs were compared at each level and subjective evaluation for asymmetry of lobe size and shape as well as the fat and muscle distribution was performed.

### **Results**

The standard ultrasound examination with probe placed perpendicular to the longitudinal axis of the SL did not allow accurate identification of the ligament margins relative to the surrounding connective tissue. In addition, this examination method did not allow visualization of the entire SL, specifically excluding the medial and lateral extent of the ligament. Examination with the limb in a non-weight bearing position with the carpus mildly flexed and the ultrasound beam perpendicular to the ligament fibers did allow visualization of the entire ligament. This was characterized by consistent identification of the axial margins of the second and fourth metacarpal bones. However, it did not consistently allow identification of the ligament margins and it did not allow differentiation between the ligament fibers versus areas of muscle and adipose tissue. Examination with the limb in a non-weight bearing position with the carpus mildly flexed and the ultrasound beam oblique to the longitudinal axis of the ligament allowed visualization of the ligament margins as well as identification of ligament fibers versus areas of adipose tissue and muscle.

In contrast to the MR images, which allowed areas of adipose tissue versus muscle to be easily distinguished, US images allowed identification of regions of adipose tissue and muscle but differentiation between the two tissue types was not reliably achieved (Fig. 3). Differences in the shape of the ligament margins or lobe size were well detected on the US images (Fig. 4). However, small areas of adipose tissue or muscle tracking through the ligament fibers were not well detected on US images.

In all 10 horses the lobes of the SL were asymmetrical in shape and size at least one level when comparing the right and left limbs (Fig. 4). In eight horses the ligament lobes joined to form an oval shape at approximately 6 cm distal to the carpometacarpal

joint. In one horse this occurred at 5 cm in both forelimbs. This occurred in one horse at 5 cm in the left forelimb and 6 cm in the right forelimb.

The adipose tissue and muscle distribution was asymmetrical to varying degrees in all 10 horses at all levels when comparing the right and left limbs. The degree to which this occurred was variable, some horses only had slight differences when comparing the right and left forelimbs while others had marked differences.

There was a significant difference ( $P < 0.05$ ) between the circumferential area of the different tissue types in the SL averaged over all measurement levels (Fig. 5). The ligament is composed primarily of ligament fibers, followed by muscle tissue and then adipose tissue. The circumferential area of the ligament when measured on oblique incidence ultrasound images at 2 and 3 cm distal to the carpometacarpal joint was significantly different ( $P < 0.05$ ) than when measured at the same levels on the MR images. The remaining levels were not significantly different when comparing oblique incidence ultrasound images and MR images.

There was no statistically significant difference between the cross sectional area of the SL in right and left forelimbs when comparing the measurement performed while the horse is standing with the ultrasound beam perpendicular to the ligament fibers to the flexed oblique incidence technique when averaged over the measurement distances. There was no statistically significant difference between the cross sectional area of the SL or the cross sectional area of fibers, muscle, or adipose tissue in the ligament when comparing the right and left forelimbs.

### **Discussion**

The combination of multiple ultrasound techniques provides the most comprehensive examination of the SL. It will allow visualization of the entire ligament and a more accurate representation of the normal anatomy, both essential requirements for diagnosis of pathologic change. Although MR examination will still be required to diagnose certain types or degrees of injury in the SL and will provide additional information, such as demonstrating the presence of

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fluid in the third metacarpal bone at the ligament insertion, the more comprehensive examination will likely allow diagnosis of abnormalities not previous possible with US. This technique could facilitate diagnosis or monitoring of SL injury in multiple circumstances. It could allow a diagnosis to be obtained without MR examination. This is beneficial for owners who would like to avoid general anesthesia or for those who cannot afford the cost of advanced imaging. In addition it could allow monitoring of lesions over time following MR examination without additional general anesthesia.

Oblique incidence ultrasound provides a reliable method for measuring the circumferential area of the SL creating a detectable peripheral margin. The proximal extent of the ligament measured larger on MR images compared to ultrasound. This difference may originate from fibers extending from the fourth metacarpal bone as well as fibers in the palmar aspect of the medial and lateral interosseous spaces. These fibers are readily identified on MR images but are difficult to visualize on ultrasound images (Fig. 6). A convex probe would allow a beam angle that would make these fibers more echogenic and therefore better defined. However, this could potentially obscure other ligament margins still making it difficult to identify all the ligament fibers and measure the complete circumferential area in one image.

Although the circumferential area between the right and left front SLs is not significantly different, there was detectable difference in either lobe shape or fat and muscle distribution in all horses in the study (Fig. 4). These differences could be confusing when trying to detect injury using comparison to the opposite limb. However, more work showing the scope of normal anatomic variation and comparison to cases with confirmed pathologic change will provide criteria to allow more accurate identification of normal anatomic variation.

## Conclusions

Although this comprehensive ultrasound technique has limitations, it provides additional and essential information not available with the traditional technique. It is currently part of our routine examination when assessing the SL for injury using ultrasound.

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## Acknowledgment

This study was funded by the College Research Council at Colorado State University. Sincere appreciation to Eric Garcia, D.V.M. for his assistance with this project.

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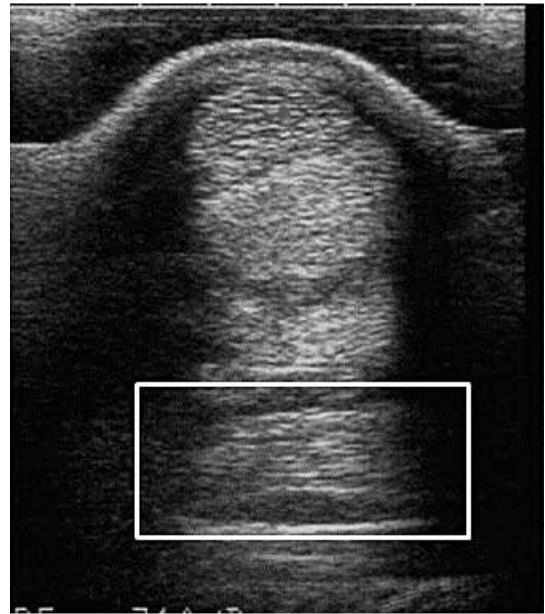
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**Table 1.** Circumferential area of the SL at different levels measured with oblique incidence ultrasound and MRI

Distance (cm)	Modality	
	Oblique Incidence US	MRI
2	1.74* (+0.07)	2.15*(+0.07)
3	1.76*(+0.07)	2.05*(+0.07)
4	1.57(+0.07)	1.68(+0.07)
6	1.52(+0.07)	1.51(+0.07)
6	1.58(+0.07)	1.52(+0.07)

Values with asterisks in the same row are significantly different ( $P < 0.05$ )

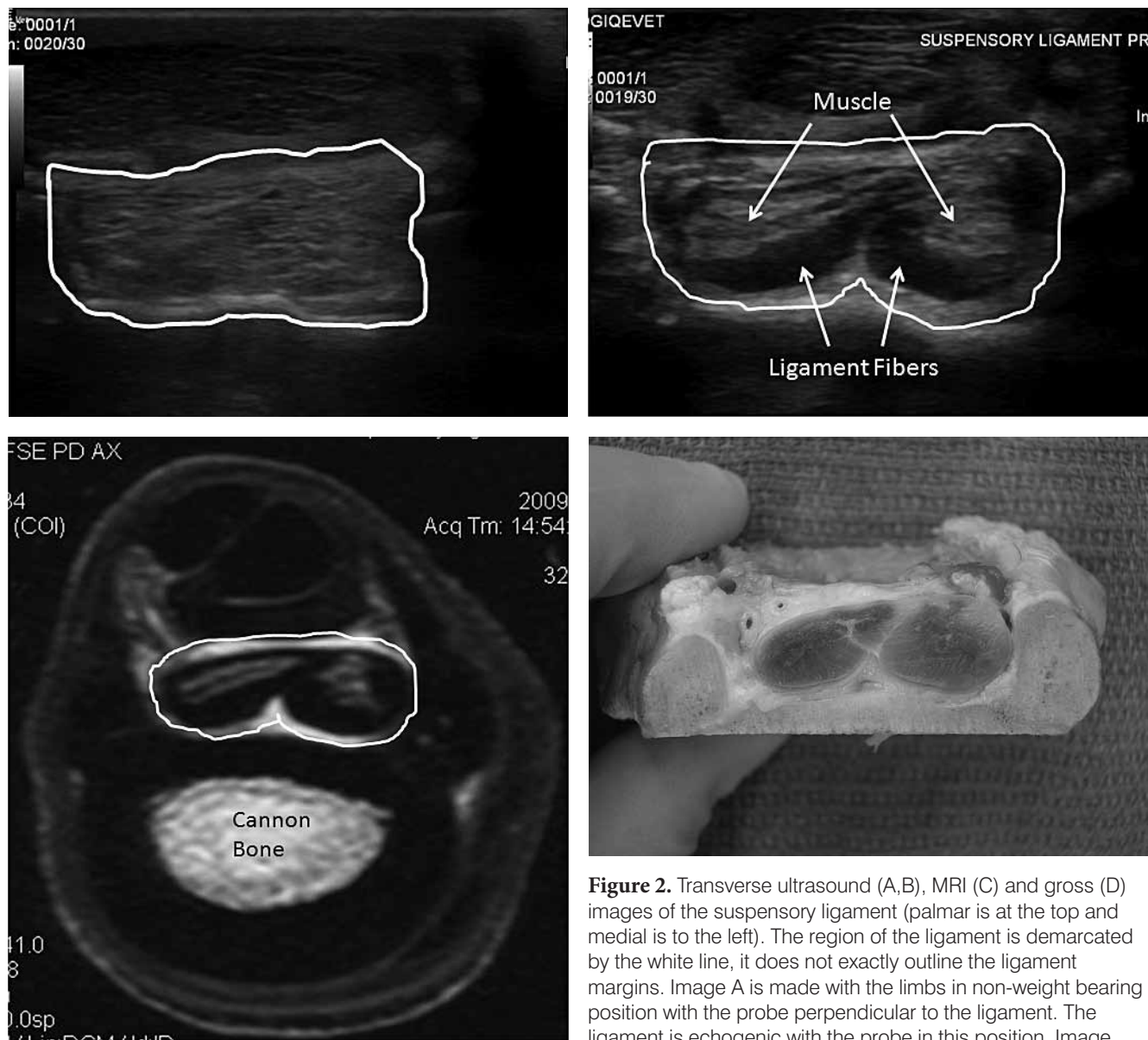
Distance is centimeters (cm) distal to carpometacarpal joint.



**Figure 1.** Transverse ultrasound image made using the current technique with ultrasound probe perpendicular to the ligament fibers in a weight-bearing lime. The suspensory ligament is demarcated by a white rectangle. This ligament appears as a echogenic rectangle shaped structure with hypoechoic areas. The palmar aspect of the third metacarpal bone is the white line just inside the bottom line of the rectangle. This technique causes the suspensory ligament to appear rectangular when in fact it is bilobed with rounded medial and lateral margins at this level.

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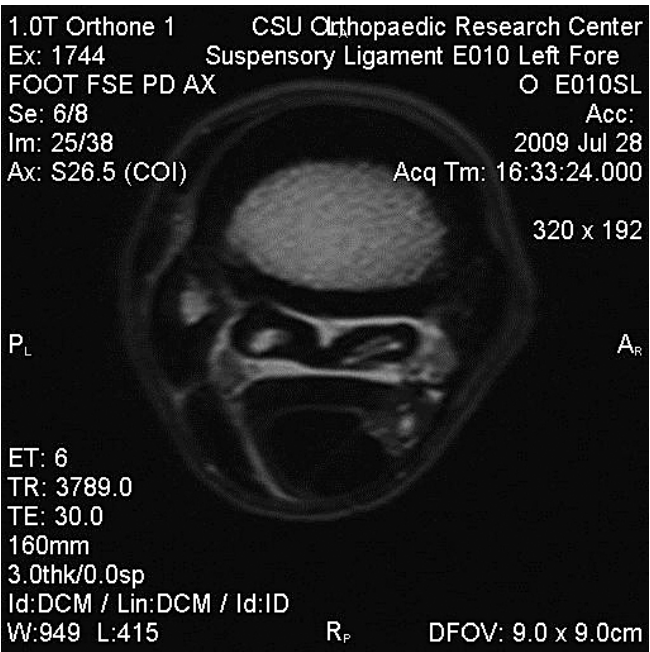


**Figure 2.** Transverse ultrasound (A,B), MRI (C) and gross (D) images of the suspensory ligament (palmar is at the top and medial is to the left). The region of the ligament is demarcated by the white line, it does not exactly outline the ligament margins. Image A is made with the limbs in non-weight bearing position with the probe perpendicular to the ligament. The ligament is echogenic with the probe in this position. Image B is a made at the same level as image A, but the probe is now oriented obliquely in a palmarodistal-dorsoproximal direction to the suspensory ligament. The ligament fibers are now hypoechoic. This horse has primarily muscle in the ligament lobes as opposed to other horses which have a combination of adipose tissue and muscle in the ligament. The muscle is echogenic compared to the ligament fibers with the probe oriented obliquely. The ultrasound image made with an oblique beam angle appears similar to the MR image, with a clear delineation of the ligament margins as well as the ligament fibers in contrast to Figure 1. These two images (B,C) correlate well with the gross image (D) and provide an accurate representation of the anatomy.

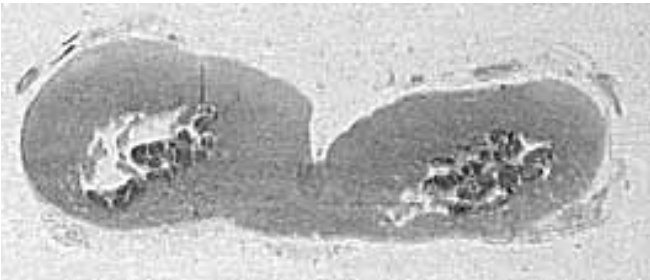


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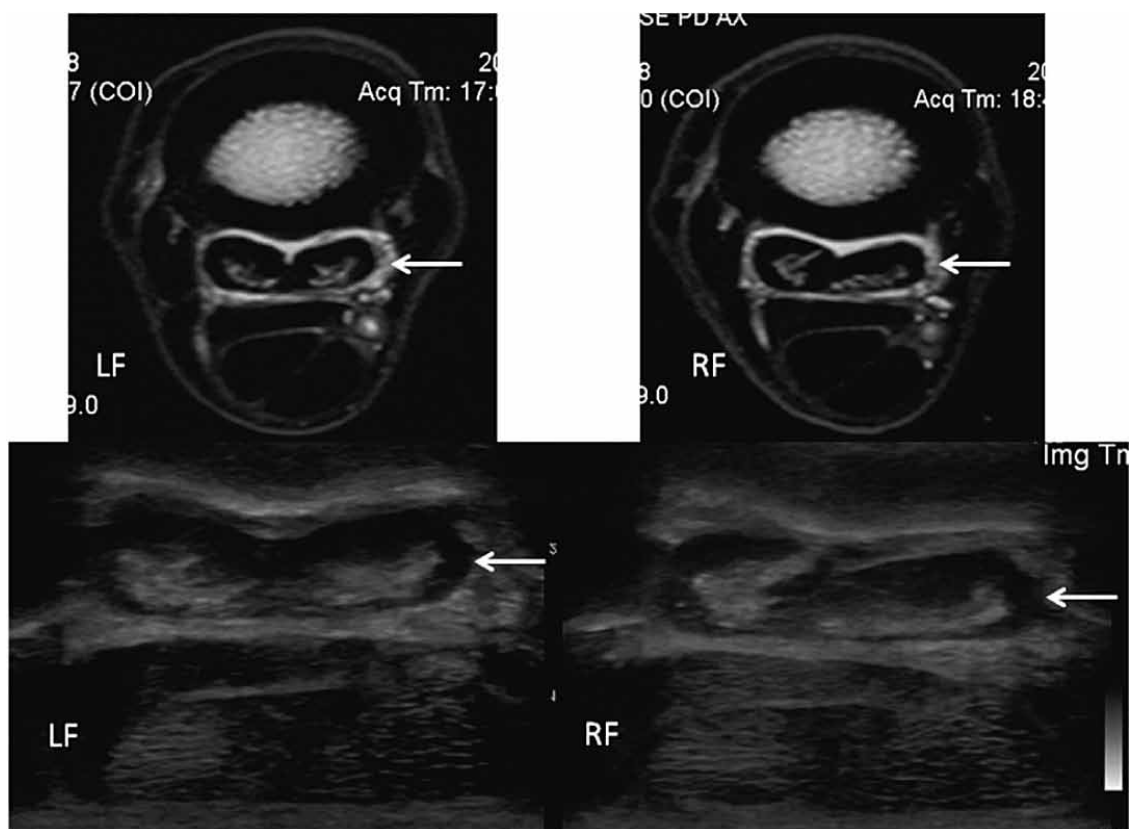


**Figure 3.** Corresponding US, MR and histology images of a limb 4 cm distal to the carpometacarpal joint (Dorsal is at the top and medial is to the right). The entire suspensory ligament is visible on the US image, and the size, shape and margins of the ligament are similar to the appearance on the MR images. The lateral lobe of the ligament (arrow) contains primarily adipose tissue (light gray on MR images) with a small area of muscle (dark gray on MR) within the axial aspect of the lobe. The medial lobe of the ligament (arrowhead) contains a mixture of adipose tissue and muscle. The differences between the fat and muscle distribution in the ligament lobes are clearly delineated on the MR image. However, the demarcation between muscle and adipose tissue is more difficult to identify on the US image. The histology slide (Masson's trichrome) confirms the tissue distribution; ligament fibers are blue, adipose tissue is clear and muscle is red.



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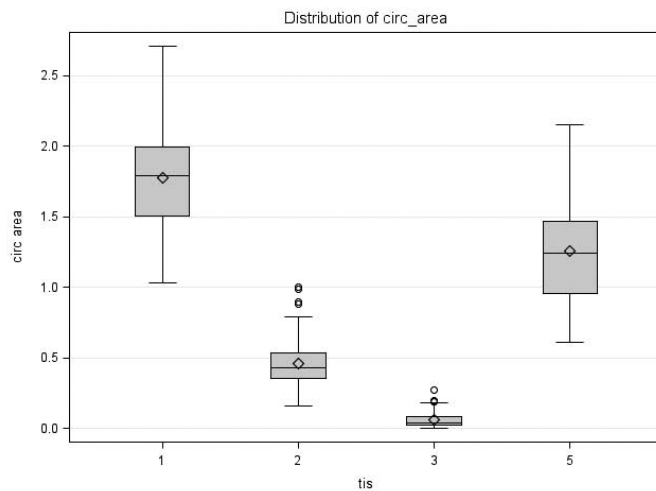


**Figure 4.** Paired US and MR images 4 cm distal to the carpometacarpal joint (Dorsal is at the top and medial is to the right). The suspensory ligament lobes of the left fore are symmetrical in size and shape. In contrast, the suspensory ligament lobes in the right fore are asymmetrical with a thinner longer medial lobe and a thicker shorter lateral lobe. The adipose tissue and muscle distribution is different when comparing the two limbs. The asymmetry of the right and left suspensory ligaments is not the result of pathologic change. It is a consequence of normal anatomic variation.

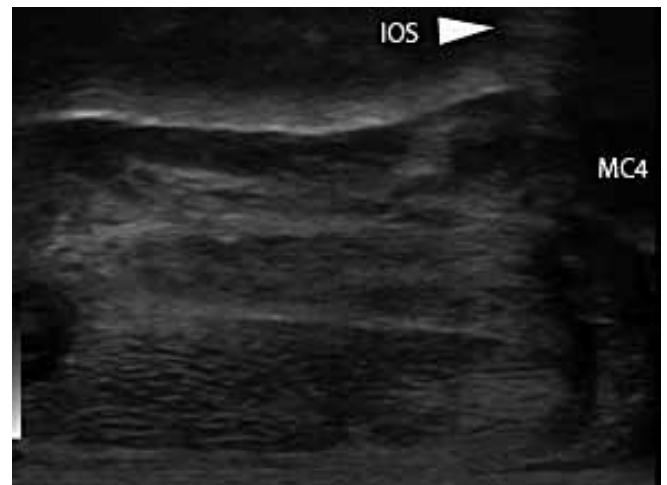
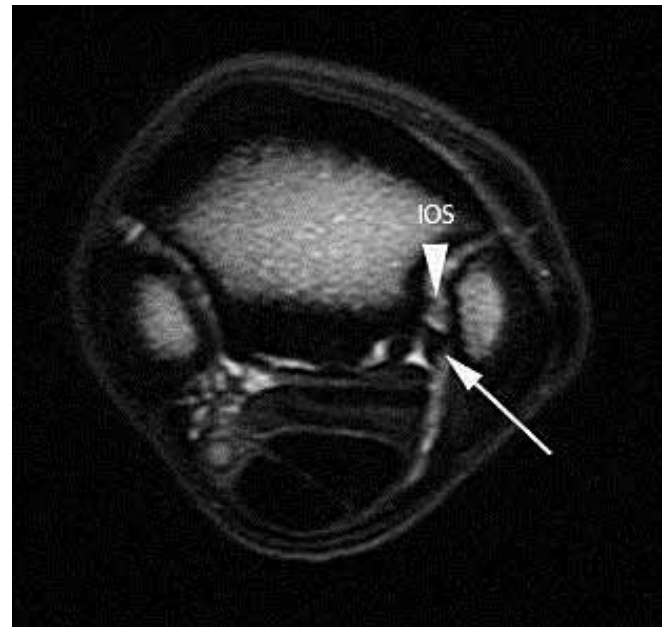


## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease



**Figure 5.** Circumferential area of the ligament and the different tissue types in the ligament averaged over the measurement levels. Circ area, circumferential area; tis, tissue type; 1=circumferential area of the suspensory ligament, 2=circumferential area of the muscle, 3=circumferential area of the adipose tissue, 4=circumferential area of the ligament fibers.



**Figure 6.** Paired MR and US image 2 cm distal to the carpometacarpal joint (dorsal is on the top and medial is to the left). There is a focal area of fibers extending from the fourth metacarpal bone (arrow). These fibers are located at the palmar extent of the interosseous space (arrowhead) and are difficult to identify on the US image. On the US image the lateral extent the suspensory ligament appears axial to its actual lateral margin which is apparent on the MR image. Therefore, the circumferential area measure less at this level on the US image compared to the MR image.

## **Summaries: Focus 2**

### *Early Diagnosis of Bone and Joint Disease*

#### **A Technique for Performing Radiographic Guided Needle Placement Into the Collateral Ligaments of the DIP Joint**

##### **Take Home Message**

Radiographs can be used to guide needle placement in the distal aspect of the collateral ligaments of distal interphalangeal joints. If clinically indicated this technique can be used to facilitate intra-lesional therapy for treatment of collateral ligament injury.

##### **Introduction**

The diagnosis of abnormalities in the collateral ligaments of the distal interphalangeal (DIP) joint distal to the coronary band is occurring with increased frequency. This is due to use of magnetic resonance imaging (MRI) as a diagnostic tool in cases with foot lameness. Collateral ligament (CL) injury proximal and immediately distal to the coronary band can be diagnosed using ultrasound<sup>1</sup>. Osseous lesions of the distal phalanx at the CL insertions can be diagnosed on radiographs. However, visualization of abnormalities in the collateral ligaments of the DIP joint at the level of the insertion on the distal phalanx can only be diagnosed with MRI or computed tomography (CT)<sup>2</sup>. Lesions identified in the collateral ligaments of the DIP joint diagnosed on MR images consist of core lesions with fiber disruption, diffuse injury without fiber disruption, margin tears, and complete ligament disruption.<sup>3-4</sup>

Intralesional therapy, such as platelet rich plasma (PRP) and stem cells, is one of the possible treatment options in cases with a diagnosis of soft tissue injury. Imaging modalities can be used to facilitate needle placement for injection of therapeutic agents. Soft tissue is best visualized with MR and ultrasound (US). Therefore, these modalities are often selected to facilitate needle placement into soft tissue lesions. CT can be used in a soft tissue window in conjunction with osseous landmarks for needle placement into soft tissue injuries.<sup>5</sup> MRI and CT require general anesthesia, while US and radiography can be performed in the standing horse. Radiography is commonly used to facilitate needle placement when reliable bony landmarks are available, such as injection of the navicular bursa using the navicular bone flexor surface as a landmark.<sup>6</sup>

The collateral ligaments of the DIP joint insert on the fossae of the distal phalanx. The fossae of the distal phalanx can be reliably identified on radiographs and recognized on multiple views. A paper, which was presented at AAEP, was done by Drs. Werpy and Frisbie with Lauren Farrington to provide a description of how to use radiography to facilitate needle placement into the distal aspect of the collateral ligaments of the distal interphalangeal joint for injection of intra lesion therapy.

##### **Methods and Materials**

This technique is performed following diagnosis of a lesion in the distal aspect of the collateral ligaments of the DIP joint using high or low field MRI. The MR images are used to determine the desired site of needle placement based on relative distance from the margins of the fossa and whether the lesion is dorsal, palmar or centrally located within the ligament. A 3 ½ inch, 18-gauge needle placed into the fossa and can be performed with the horse standing or under general anesthesia. It can also be performed in conjunction with a MRI exam or other procedure. This report will provide a description of the procedure in a standing sedated horse, but can certainly be adapted to a patient under general anesthesia.

Prior to performing the procedure, an abaxial nerve block is performed and the hair is clipped from the injection site. During the procedure, sedation is used at the discretion of the veterinarian to prevent movement of the limbs. To approximate the location of the collateral ligaments for clipping, the coronary band can be palpated for areas of dense tissue at the 10 o'clock and 2 o'clock positions on the dorsal aspect of the foot. If the collateral ligaments are not palpable due to diffuse swelling, ultrasound can be used to locate them. A region of approximately 10 cm extending from the coronary band centered over the collateral ligament of interest is clipped. Properly placing a needle in the collateral ligament fossa is dependent on being able to identify the fossa margins

on radiographs. Three radiographic views that are useful in locating the collateral ligament fossae include the lateromedial (LM), horizontal or weight-bearing dorsopalmar (DP), and dorso 60°proximal - palmarodistal (D60°P) views (Fig. 1). Therefore, the horse will need to be positioned with both front feet on foot blocks to allow proper radiographs to be taken once the needle is placed.

When taking radiographs it is important that proper safety precautions be taken and protective equipment is worn. In general, sequential radiographs are taken with continued needle advancement toward the fossa. We use a LM view followed by horizontal DP view and then a D60°P view. However, the sequence of radiographs could be adjusted as needed. When first learning this technique it is helpful to estimate the required proximal to distal angle of the needle prior to beginning needle placement. Before taking radiographs, a capped spinal needle can be positioned at the estimated proper angle and then held in place with tape (Fig. 2a). A lateral radiograph can be taken to view the needle placement in relation to the fossa. At this point, the capped needle can be adjusted accordingly until the person performing the procedure is confident it is properly angled. Once the appropriate angle is determined on a lateral radiograph this angle can be marked on the limb with correction fluid, pen or white tape so it can be repeated following sterile preparation of the injection site (Fig. 2b). Prior to removing the capped needle, a DP radiograph can be taken to approximate the medial to lateral angle needed to direct the needle towards the fossa. This initial process will reduce the need for repositioning the needle once it has been inserted through the skin. Once proficiency at this technique is obtained, initial marking of the limb for needle placement is often not necessary. However, this could depend on how exact the needle placement must be based on the size and shape of the lesion. The area of ligament injury is taken into account when positioning the needle. The lesion location in the ligament as identified on MR images should be taken into consideration when planning this procedure. The needle can be inserted dorsal, palmar,

or peripheral (abaxial) to the proximal aspect of the collateral ligament. This will avoid passing the needle through the proximal aspect of the ligament and allow placement of needle in the affected area of the ligament.

Following sterile preparation of the injection site, the needle is inserted approximately 3 cm toward the fossa. Angles determined by the LM and horizontal DP radiographs, if previously obtained, are used to direct the needle (Fig. 3). Once the needle has been advanced approximately 3 cm, LM and horizontal DP radiographs should be taken to confirm that the needle is at the correct angle to enter the fossa. If needed, the needle can be readjusted by retracting it 1-2 cm and redirecting it (Fig. 4). If the angle and placement are correct, the needle should be advanced 1-2 cm and then a D60°P radiograph is taken to evaluate needle position. Once the correct angles have been obtained, the needle can be advanced until it contacts the fossa of the distal phalanx (Fig. 5). Once the spinal needle has reached the fossa, any additional views needed to confirm proper needle placement should be taken. Intralesional therapy is administered once correct needle placement has been confirmed. Once the needle is removed, the injection site is bandaged.

### **Results**

This technique was performed initially on cadaver limbs using MRI compatible needles. The needle placement was confirmed on MR images. Subsequently, this technique was performed using spinal needles with contrast administration. The contrast agent was detectable on radiographs and MR images confirming placement of the contrast into the collateral ligaments. This technique has been used on 18 horses for administration of intralesional therapy following diagnosis of a collateral ligament lesion located in the distal aspect of the ligament. Lesions were diagnosed on MR studies from high and low field systems. No adverse effects from this technique have been observed. Stem cells and platelet rich plasma have been administered using this technique. Recheck examination six months following

## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease

treatment has been performed in three cases, which demonstrated clinical improvement as well as improvement in the appearance of the ligament on MR images.

#### Discussion

The technique provides a method for intralesional therapy of distal collateral ligament lesions with equipment commonly available in veterinary practice and without the use of general anesthesia. Proficiency can be achieved with knowledge of anatomy and radiographic technique as well as practice on a limited number of cadaver limbs. Using multiple radiographic views is necessary to ensure proper needle placement. Additional views, such as lateral oblique radiographs may also provide helpful landmarks. These views should be performed at the veterinarians discretion during the procedure such that needle placement is adequately confirmed. However, the needle could be consistently placed in the distal aspect of the collateral ligament using the three described views as was confirmed on MR images.

Fine tuning needle placement into specific areas identified on MR images, such as axial, abaxial, dorsal, or palmar aspects of the ligament can be achieved, but requires further practice (Fig. 6-7). Contrast can be used to confirm needle placement when practicing this technique on cadaver limbs. However, the effect of contrast material on most intralesional therapies is not known so contrast use is not recommended when performing this technique on clinical cases.

Axial lesions are the most challenging for needle placement, as they are in close proximity to the distal interphalangeal joint. The needle will enter the distal interphalangeal joint if it is placed axial to the ligament at the level of the joint or immediately distal to it. Often axial margin tears communicate with the distal interphalangeal joint. Furthermore, thinning of the ligament often occurs on the axial margin of the ligament. This space is often occupied by synovial membrane and fluid extending from the joint,

increasing the possibility of needle placement in the joint. However, these characteristics of the lesion will be apparent on the MR images and can be considered when planning the needle path. In these cases placing the needle abaxial to the peripheral margin of the tear is necessary to prevent placing the needle into the joint.

#### Conclusions

Radiographic guided needle placement into the distal aspect of the collateral ligaments of the DIP joint can be used to facilitate injection of intra lesion therapy.

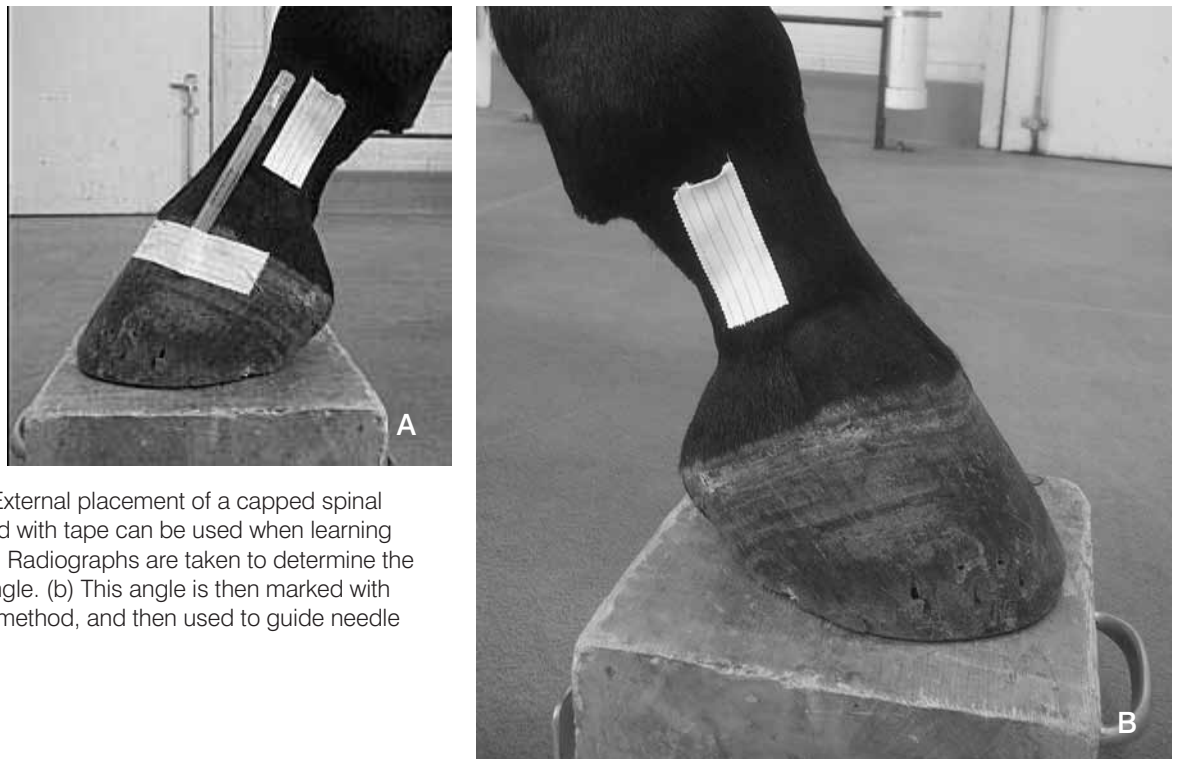
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**Summaries: Focus 2**  
*Early Diagnosis of Bone and Joint Disease*



**Figure 1.** Lateromedial and DP radiographs of the bones of the distal limb with wire (arrow) marking the fossa of the distal phalanx at the insertion site of the medial collateral ligament of the distal interphalangeal joint. These landmarks are used for needle placement.



**Figure 2.** (a) External placement of a capped spinal needle secured with tape can be used when learning this technique. Radiographs are taken to determine the appropriate angle. (b) This angle is then marked with tape, or other method, and then used to guide needle placement.



## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease

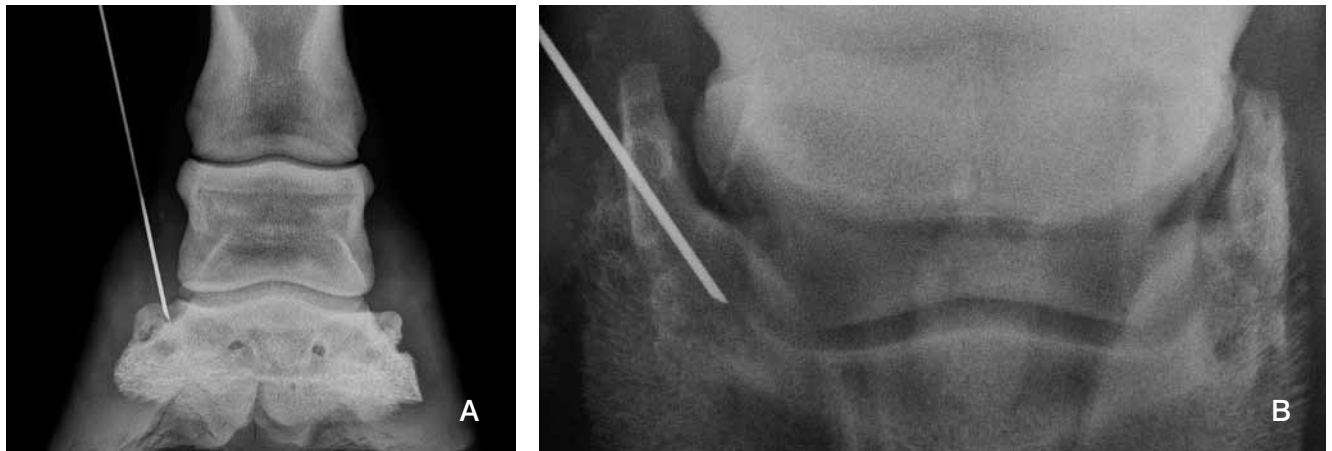


**Figure 3.** (a) Lateral radiograph showing initial needle placement. (b) The needle has been inserted approximately 3cm. The needle is correctly positioned and can be advanced toward the fossa. At this angle the needle will be placed in the dorsal aspect of the fossa.



**Figure 4.** Horizontal DP radiograph of initial needle placement. This view allows visualization of the medial to lateral angle of the needle. The distal aspect of the needle is angled too far axially or towards midline. Further advancement would place the needle in the DIP joint. The needle should be repositioned placing the hub closer to the limb, at an angle demonstrated by the arrow. This will allow advancement of the needle into the center of the fossa.





**Figure 5.** (a)Dorsopalmar and (b)D60°P views of the needle at the correct angle advanced into the center of the fossa.



**Figure 6.** Proton density transverse image at the proximal aspect of the distal phalanx. A focal area of fiber disruption is present in the palmar aspect of the medial collateral ligament of the distal interphalangeal joint (arrow) with an associated osseous cyst like lesion (arrowhead). Although abnormalities are present in the dorsal aspect of the ligament, the palmar aspect is most severely affected. Based on the MR image the needle would be directed into the palmar aspect of the ligament for administration of intralesional therapy.

## Summaries: Focus 2

### Early Diagnosis of Bone and Joint Disease



**Figure 7.** Three radiographic views of needle placement into specific areas of the collateral ligament for injection of intralesional therapy in a lesion diagnosed on MR images. The location of the lesion was determined on MR images and the needle was placed in the region of injury. (a) The lateral view shows an additional angle which places the needle into the palmar aspect of the ligament. (b) A D60 □P view of the needle in the dorsal aspect of the ligament. (c) A D60 □P view of needle placed in the abaxial aspect of the ligament, just inside the margin of the fossa.

**Development of an *in Vitro* Model of Injury Induced Osteoarthritis in Adult Equine Cartilage Using Single Impact Compressive Overload****Take Home Message**

To develop an *in vitro* model of cartilage injury using adult full thickness equine tissue that can be used to simulate the *in vivo* disease and aid in therapeutic screening.

*Introduction:* *In vitro* models of cartilage injury enable the investigation of the cellular responses and structural changes of cartilage to traumatic injury in a highly controlled and reproducible manner. *In vitro* models also allow for high through-put and inexpensive preliminary testing of therapeutics to treat and prevent the progression of Osteoarthritis (OA). The purpose of the investigations presented here were to develop an *in vitro* model of cartilage injury using full thickness cartilage (containing the superficial, middle, and deep zones) extracted from cadaveric stifles from healthy adult horses. The project was a collaborative one done by Drs. Lee and Frisbie of the ORC and Dr. Alan Grodzinsky of MIT. Using a previously characterized computer controlled single impact model of unconfined compression<sup>1-4</sup> samples were compressed to either 50, 60, 70, and 80% strain. Our objective was to determine which strain was sufficient to reproducibly induce pathologic change in cartilage that mimics the early stages of OA *in vivo*.

**Materials and Methods**

Cartilage explants were injured by single impact uniaxial compression to 50, 60, 70, or 80% strain at a rate of 100% strain/second, or left uninjured (control). Samples were subsequently cultured for twenty-eight days and evaluated histologically for characteristics of injury and early stages of osteoarthritis including: articular surface damage, chondrocyte cell death, focal cell loss, chondrocyte cluster formation, and loss of extracellular matrix molecules aggrecan, as well as types I and II collagen.

**Results**

Compared to controls, total proteoglycan content was significantly reduced in samples compressed to 60% ( $p<0.0001$ ), 70% ( $p<0.0001$ ), and 80%

strain ( $p<0.0002$ ), and type II collagen content was significantly reduced in samples compressed to 60% strain ( $p<0.003$ ). Compression to 60% strain or greater induced a significant increase in chondrocyte cell death, focal cell loss, and chondrocyte cluster formation compared to both controls and samples compressed to 50% strain. Independent of injury, severity of OA pathology differed significantly by region. Total proteoglycan content was most severely reduced in the superficial region ( $p<0.0008$ ). Chondrocyte cell death was most severe in the superficial region ( $p<0.0020$ ), while focal cell loss was most pronounced in both the superficial and deep regions ( $p<0.0009$ ). Chondrocyte cluster formation was also most severe in the deep region ( $p<0.0001$ ). Compression to all strain values induced some degree of pathologic changes seen in clinical equine OA *in situ*; however, only injury to 60% strain induced significant changes both morphologically and biochemically in the extracellular matrix (Figure 1).

**Discussion**

Injury to adult articular cartilage often leads to the degeneration of articular cartilage and the progression of OA. Previous research demonstrates mechanical injury induces matrix damage, chondrocyte death, and chondrocyte cluster formation, all of which are hallmark characteristics of OA. In this study full thickness adult equine cartilage explants were injured by single impact unconfined compression to 50, 60, 70, or 80% strain and evaluated after twenty-eight days in culture for histologic characteristics of OA. These data demonstrate the ability to produce histologic change consistent with previous cartilage injury models that are typical of OA *in vivo*<sup>4</sup>. For adult full thickness equine cartilage it was necessary to compress explants by at least 60% strain, resulting in peak stresses greater than  $14.68 (\pm 1.31)$  MPa, to induce significant pathologic change including proteoglycan loss, chondrocyte cell death, focal cell loss, and chondrocyte cluster formation.

## Summaries: Focus 3

### Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease

#### Conclusion

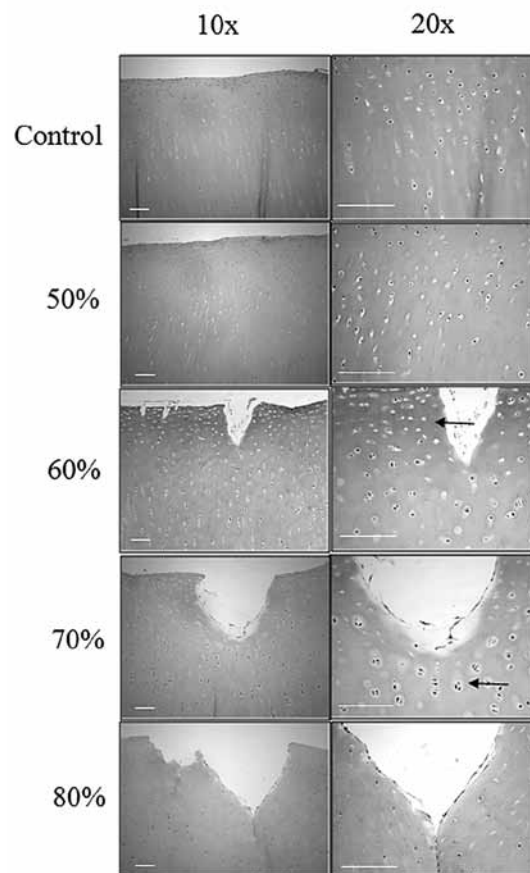
This model of cartilage injury to 60% strain at 100% strain/second should allow more accurate study of pathophysiologic changes and therapeutic interventions for osteoarthritis.

#### Acknowledgments

This research was supported in part by the Colorado State University College Research Council.

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**Figure 1.** Representative images of H&E stained sections from uninjured controls, or cartilage injured by 50, 60, 70, or 80% strain. The images at 10x resolution show tissue damage, such as fissures, in 60, 70, and 80% samples. Images at 20x resolution show cellular pathologies, such as chondrocyte cluster formation, in the 60 and 70% samples, and cell death in the superficial regions of the 60, 70, and 80% samples. Bar represents 100 $\mu$ m.

**Effects of Early Exercise on Metacarpophalangeal Joints in Horses****Take Home Message**

Exercising foals at an early age may help to improve their musculoskeletal tissue strength later in life, and in order to evaluate this theory, the investigators investigated the effects of early exercise on the metacarpophalangeal joints of horses. The results showed that the metacarpophalangeal joints of exercised horses were healthier and had denser subchondral bone than those horses that were not exercised.

**Introduction**

This study was a continuation of the collaboration between Drs. Kawcak and McIlwraith with Dr. Elwyn Firth at Massey University in New Zealand investigating the effects of early exercise on horses. The hypothesis was that early exercise of horses would lead to superior musculoskeletal tissues compared to horses that were reared in a pasture without additional exercise. The goal of this specific project was to provide a broad view of the effects of early exercise on the metacarpophalangeal joint of horses, including bone, articular cartilage, and synovial membrane.

**Methods**

Six horses that were conditioned from birth to 18 months of age were compared to six horses that had routine pasture turnout since birth. The metacarpophalangeal joints were evaluated for bone density distribution using computed tomography (CT), articular cartilage metabolism and histologic assessment of synovial membrane, articular cartilage, and subchondral bone (Fig. 1).

**Results**

Horses that were exercised since near birth had fewer gross lesions in the joints, greater bone fraction in the dorsolateral aspect of the condyle, and higher bone formation rate compared to non-exercised horses. However, there was less articular cartilage matrix staining in the dorsal aspect of the condyles in exercised horses.

**Conclusions**

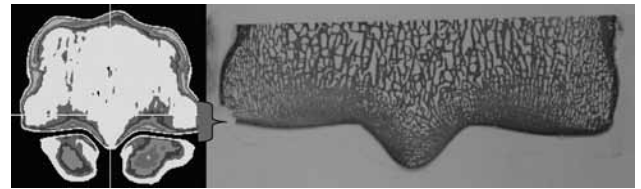
It was concluded from this study that exercise at a young age may be protective to the metacarpophalangeal joint as demonstrated through fewer gross lesions and healthier bone. However, there is a concern about the fact that there was less articular cartilage matrix staining in exercised horses. Further investigation is needed to further characterize these changes. Overall, the results of this study support previous conclusions that early exercise has a net positive effect on joints and can be recommended for strengthening joint tissues in horses.

**Acknowledgments**

This project was funded by the Marilyn Simpson Trust, the Horse Racing Betty Levy Board, Utrecht University and the New Zealand Equine Partnership for Excellence, and the New Zealand Racing Board.

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**Figure 1.** Metacarpophalangeal joints were evaluated for bone density distribution using computed tomography (CT), articular cartilage metabolism and histologic assessment of synovial membrane, articular cartilage, and subchondral bone.



## Summaries: Focus 3

*Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*

### ***Influence of Early Conditioning Exercise on the Development of Articular Surface Abnormalities in Cartilage Matrix Swelling Behavior in the Equine Middle Carpal Joint***

#### **Take Home Message**

Early conditioning of foals is thought to be needed in order to strengthen their musculoskeletal tissues for the rigors of exercise later in life. Through this study, the investigators found that there were no negative effects of early conditioning on the midcarpal joints of horses. In addition to previous work that showed a positive effect of early conditioning on chondrocytes, the authors concluded that early conditioning is safe and important for building early strong musculoskeletal systems.

#### **Introduction**

The investigators have worked for several years to determine the effects of early exercise on foals in the hopes of inducing stronger musculoskeletal tissues later in life. As a continuation of this project Drs. Kawcak and McIlwraith collaborated with Drs. Firth and Broom in New Zealand to determine the effects of early exercise on cartilage in the mid-carpal joint of horses. The purpose of this study was to specifically determine if exercise at an early age would lead to less gross lesions and better material properties of the articular cartilage in those joints.

#### **Methods**

Six horses that were conditioned from birth to 18 months of age were compared to six horses that had routine pasture turnout since birth. Midcarpal joints from each horse were evaluated, and gross defects were classified and mapped. Opposing surfaces of the radial carpal and third carpal bone surfaces were used to quantify swelling behavior of the articular cartilage extracellular matrix (Fig. 1).

#### **Results**

The investigators found that there was a wide range of gross defects on the joint surfaces, but that there were no significant differences in the number or severity of lesions in the midcarpal joint between the two groups. The investigators also found that there were no significant differences in material properties of the articular cartilage between both groups, as

reflected by the swelling behavior of the cartilage extracellular matrix.

#### **Conclusions**

The investigators concluded from this study that early conditioning had no negative effects on the articular cartilage in the midcarpal joint. This is important, because in an earlier study which was a collaboration between Drs. Kawcak and McIlwraith and Dr. Elwyn Firth, they found that early conditioning exercise had a beneficial effect on the chondrocytes in the articular cartilage of the metacarpophalangeal joint. Therefore, based on this and previous studies the investigators have concluded that early conditioning is a positive influence on articular cartilage and is safe to use.

#### **Acknowledgments**

This project was funded by the Grayson-Jockey Club Research Foundation, the Marilyn Simpson Trust, the Horse Racing Betty Levy Board, Utrecht University, the New Zealand Equine Partnership for Excellence, and the New Zealand Racing Board.

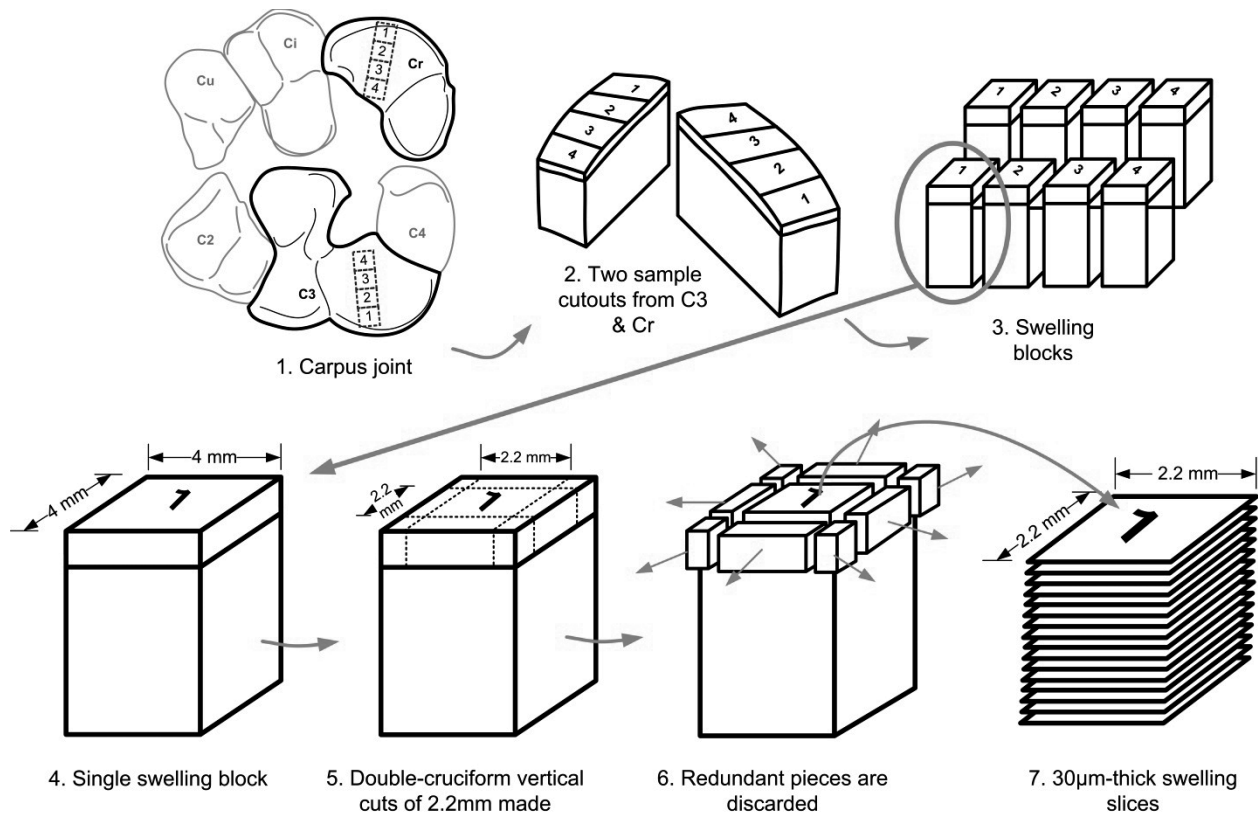
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### Summaries: Focus 3

#### *Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*



**Figure 1.** Map showing the orientation and collection of samples for assessment of articular cartilage swelling.

## Summaries: Focus 3

### *Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*

#### **Racing Surfaces White Paper**

##### **Take Home Message**

Although optimization of surfaces alone will never eliminate catastrophic injuries, and may not even be a primary factor in most injuries, the absence of well-accepted characterization methods and basic science of racing surfaces is a significant obstacle to improve performance and safety. A critical aspect of the effort to improve surfaces is looking at the factors which control the performance of racing surfaces in the context of the relative biomechanics, the different types of surfaces, and potential testing and maintenance strategies.

##### **Introduction**

This white paper was drafted as a collection of published scientific papers and data<sup>1</sup>. It is considered a work in progress and we update it as new scientific studies and track data become available. It is a collaborative effort between Dr. Mick Peterson and Miss Christy Mahaffey of the University of Maine, USA; Dr. Lars Roepstorff, Swedish University of Agricultural Sciences, Sweden; Dr. Jeffrey J. Thomason, University of Guelph, Canada; and Dr. Wayne McIlwraith at Colorado State University. The white paper is based on the recommendation of the Welfare Safety Summit (WSS) Racing Surface Sub-Committee supported by the Jockey Club. Its content has been reviewed by the WSS Racing Surface Committee. This white paper is available on line.

##### **Preface**

Racing surfaces have received a great deal of attention in the popular and fan coverage of horse racing. Additionally track surfaces have recently been a topic of discussion in the scientific literature. Three general areas of inquiry have emerged: (1) characterization of the interaction of the hoof and the ground, (2) *in-situ* testing of the surface and (3) specific characterization of the materials used in the racetrack. A general understanding of the hoof ground interaction has been facilitated by dynamic horseshoe studies over the last decade and loading of the ground and hoof has been reviewed.

Injury, in particular catastrophic injury, is a multi-factorial event that involves the complex interaction of a number of risk factors including but not limited to medication, genetics, and training. The full scope of the problem is summarized in Fig. 1, in which track-surface properties are isolated as the focus of this paper, from among several other known risk factors for injury. Given that the overwhelming majority of catastrophic injuries show clear evidence of preexisting disease, improved racing surfaces have the potential to result in an improvement in the safety of horse racing for both riders and horses (Fig. 1).

##### **Background**

A safe surface is one for which the surface properties have been designed to prevent injury. Current evidence indicates that consistency of each surface and limited variability among surfaces seen by each horse are more important than the exact values of each property. Factors that have been considered include:

- *Horse-hoof-track interaction*
- *Loading on specific anatomical structures*
  - Energy from the shock of contact with the ground, and the forces owing to changing the momentum of the legs and body are transferred through the hoof loading on specific anatomical structures – the static and dynamic load on the legs stress the materials of each anatomical structure in the leg including each bone, muscle, tendon, and ligament
- *Causes of injury* – Injuries can principally occur in two different ways, either as a catastrophic injury due to acute overload or as degenerative changes due to repeated minor overload.
- *Features of injuries* – Accurate diagnosis of injuries, together with information on their location, severity, and frequency of occurrence, provide valuable information in combination with the categories of data described in the preceding 3 subsections.

- *Linking track properties to injury* – Achieving the goal of making a direct connection between track properties and specific injuries (not simply injuries in general) is a major step in minimizing the effect of the track on the occurrence of injury.

#### **Interaction of the Horse and Hoof With the Track**

At racing speeds reaching 38 mph (17 m/s), the hoof hits the track approximately 150 times a minute, remaining on the ground in the stance phase for a sixth of a second each time. The short duration of the stance hides from our eyes several stages that are distinctive in their mechanical characteristics (Fig. 2), there is primary impact when the hoof impacts the ground and a secondary impact as it slides and stops.

The resistance of the track to the impact and loading of the hoof determines the rate of loading of the leg and thus the acceleration and forces accounted in the joints of the horse. The track influences how quickly the foot is decelerated and then the stiffness of the track when the load is being applied. The rate of deceleration controls the strain which is transferred to the leg and results in higher peak loads for stiffer surfaces. It is reasonable to expect that above a critical strain and strain rate, there lies potential for propagation of damage to the bone. The horizontal response of the surface also plays a key role in loading of the leg. In addition to these mechanical properties, rough deformable surfaces increase the variance of vertical forces at the hoof and positioning of the load in the hoof.

#### **Types of Racing Surfaces**

To date, little formal discussion has been given to design of the racing surface. The design of surfaces is addressed in detail in this white paper.

#### **Testing of Racing Surfaces**

The characterization of the racing surface materials is the best understood and most common type of racing surface monitoring. While important to the overall performance of the surface, material is just one aspect of developing an appropriate racing or training surface. Tracks can be tested for composition and a racing testing and a surface testing laboratory has been set up for looking at the composition of both dirt tracks

and synthetic tracks. However it is to be recognized that composition testing has a distinct limitation that it only describes the material not the results which occur from the composition of the material. In addition to this in-situ performance testing needed to describe the overall performance, the material when combined with the design of the track including shear strength, compaction, impact absorption and energy return and, moisture sensitivity.

#### ***In-Situ* Testing of Racetrack**

The first author of the white paper has designed a mechanism for *in-situ* testing. Outcome parameters include moisture content, depth, consistency, temperature, geometry, as well as *in-situ* performance testing. The equipment developed by the first author is a progression from previously used techniques including Clegg Hammer, dynamic penetrometer, agricultural penetrometer, and Going Stick. The Biomechanical Hoof Tester is a system that has been developed to make it possible to load the track at the rate and loads are applied by a horse at a gallop (Peterson, et al., 2008).

#### **Understanding Racing Surface Safety**

Beyond the tools needed for monitoring of racing surfaces, there is a need to understand what is done to the surface and how these surfaces are used. The condition of a racing or training surface is a result of maintenance, material, weather, and usage. A complete understanding of the surface can only be obtained if these factors are all included to understand the outcome in terms of the resulting surface performance.

Examination of climate and design, monitoring of the racing surface (composition, maintenance, weather and usage, performance) are critical and the authors of this white paper have been involved in a number of published studies looking at the various factors.

#### **Safety and the Epidemiological Literature**

The next step in this process is correlation of measurement and maintenance of track surface with epidemiologic database to define real world effectiveness. Correlation is critical in the assessment of engineered racing surfaces.

Summaries: Focus 3

Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease

Acknowledgments

Funded by Grayson-Jockey Club Research Foundation.

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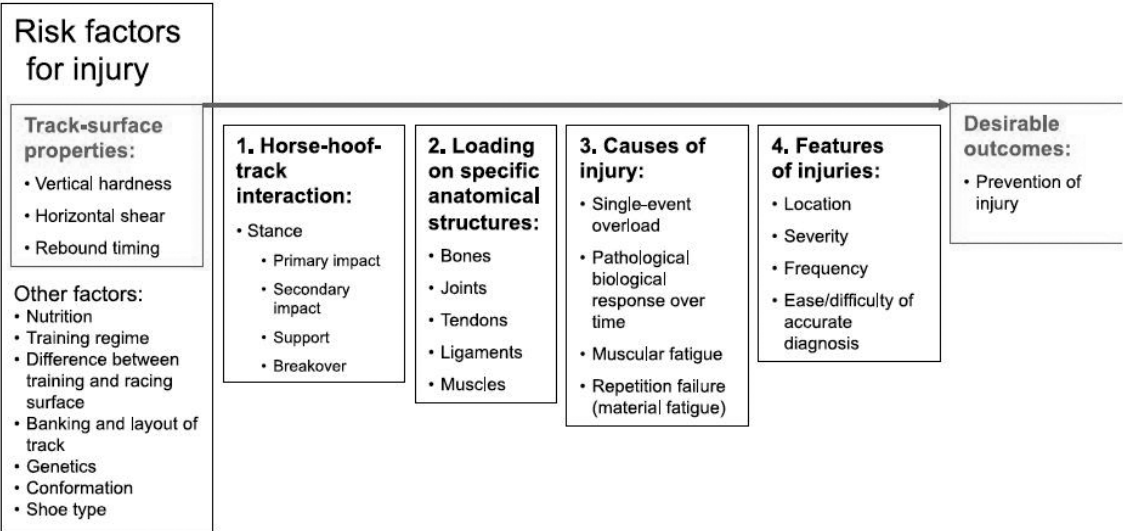


Figure 1. A pathway from track properties as a risk factor to the desirable outcome of prevention of injury, via the postulated mechanical underpinnings of the causes of injury, and relevant feature of injuries once they occur.

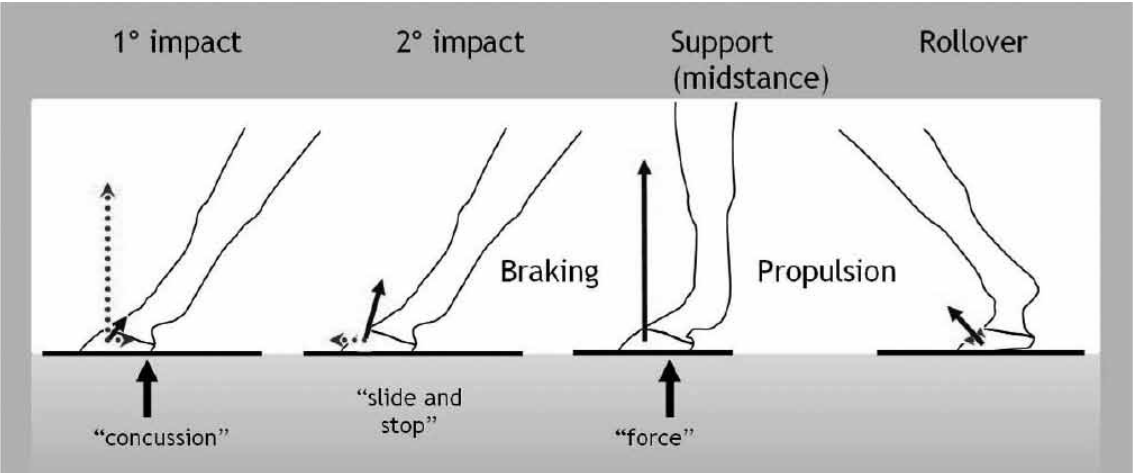


Figure 2. Stages of the stance showing the differences in acceleration (red) and GRF (blue) among the stages. When the GRF arrow is tilted, that indicates that both vertical and horizontal components of GRF are present. The arrow shows the direction in which the ground is pushing the horse.

**Effects of Platelet-Rich Plasma Composition on Anabolic and Catabolic Activities in Equine Cartilage and Meniscal Explants****Take Home Message**

Clinical preparations of platelet-rich plasma (PRP) from autologous blood are created using commercial kits, of which there are numerous options that produce different platelet and growth factor doses. It is not known which PRP preparation is most appropriate for applications to cartilage and the meniscus. Therefore, we evaluated the response of equine cartilage and meniscal explants to single or double spin PRP preparations, the latter of which is necessary to obtain a high density of platelets. In general, protein and proteoglycan synthesis for single spin PRP was higher than double spin PRP. Gene expression of the proteoglycan catabolic enzyme ADAMTS-4 was lowest for single spin PRP. This study suggests that single-spin PRP preparations may be the most advantageous for intra-articular applications, and that double-spin systems should be considered with caution.

**Introduction**

Platelet-rich plasma represents an accessible and inexpensive source of growth factors that has received considerable attention for the treatment of musculoskeletal tissues, although few studies have evaluated the effect of PRP on articular cartilage and the meniscus (1). An important factor in determining the potential of PRP to heal joint tissues is the dose of platelets that best stimulates cartilage or meniscal growth. PRP containing less than a two-fold increase in platelet concentration relative to blood may be obtained by a single spin protocol (2). To further concentrate the platelets, a second higher force spin may be used (3). Given that higher platelet concentrations translate into a higher growth factor dose, we hypothesized that double spin PRP preparations will stimulate higher extracellular matrix synthesis without a concomitant increase in catabolic activities in cartilage and meniscal explants than single spin protocols. This project was a collaborative study by Drs. Kisiday, Frisbie, and McIlwraith of the ORC with Drs. Rodkey and Steadman of the Steadman Philippon Research Institute.

**Methods**

Samples were obtained from five healthy two- to five-year-old horses. *Blood and tissues.* Blood was drawn into anticoagulant citrate dextrose at a ratio of 10:1. Articular cartilage and menisci were retrieved from the femorotibial joint. Full-thickness (1-2 mm) sections of articular cartilage were removed from the femoral condyle and divided into 5 mm by 5 mm explants. Three mm thick meniscal explants were harvested from the femoral articulating surface of the avascular portion of medial menisci. *PRP.* PRP was prepared using Arthrex ACP™ according to the manufacturer's single protocol (referred to as Single Spin Kit). PRP from Harvest Smart Prep 2™ was prepared according to the manufacturer's double spin protocol (referred to as Double Spin Kit). Laboratory PRP was created by centrifuging blood at 200 g for 18 minutes and harvesting the plasma. A portion of this PRP (referred to as Single Spin Lab), was set aside for experimentation. The remaining PRP was centrifuged at 1000 g for 15 minutes. The platelet-poor plasma (PPP) supernatant was removed, and PPP was added back to the centrifuged platelets in reduced volumes that resulted in 3x, 6x, and 9x concentrations of platelets relative to Single Spin Lab PRP. Hereafter, these PRP preparations are referred to as Double Spin 3x, 6x, and 9x, respectively. *Culture medium:* PRP was mixed with equal volumes of low glucose DMEM plus ascorbate and antibiotics. Half of the cultures received 10 ng/ml of recombinant human IL-1 $\beta$  resuspended to produce a proinflammatory environment (4). *Evaluation of extracellular matrix synthesis:* Explants were evaluated for <sup>35</sup>S-sulfate and <sup>3</sup>H-proline incorporation as measures of proteoglycan and protein synthesis, respectively. These data were normalized to total DNA (5). *Gene expression of catabolic enzymes:* RNA was extracted from explants and reverse transcribed to cDNA using random hexamers. cDNA and TaqMan Gene Expression Master Mix were mixed with primer/probes for the catabolic enzymes ADAMTS-4, ADAMTS-5, MMP1, MMP13, and the housekeeping gene GAPDH, and expression levels were determined using semi-quantitative real-time PCR. Gene expression was



## Summaries: Focus 4

### *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse*

normalized to GAPDH. (6). **Data analysis:** The analysis of cartilage and meniscal explants was repeated for blood and tissue samples from five donor horses, with blood and tissues paired in an autologous fashion. All data were analyzed using a mixed model analysis of variance, with the donor animal used as a random effect. Individual comparisons were made using least square means procedure. Individual comparisons of main effects or interactions were indicated based on a protected f-test. p-values less than 0.05 were considered significant. Data are reported as mean +/- sem.

#### Results

**Cellular content of PRP preparations.** The concentration of platelets in Single Spin Lab PRP (317 +/- 22 thousand/ $\beta$ l), was not significantly different from Single Spin Kit PRP (276 +/- 8 thousand/ $\beta$ l) ( $p=0.20$ ). Double Spin Kit PRP contained 2.3 and 2.6-fold higher platelet concentrations (725 +/- 95 thousand/ $\beta$ l) relative to Single Spin Lab and Single Spin Kit PRP, respectively. White blood cell counts in Single Spin Lab PRP (0.04 +/- 0.001 thousand/ $\beta$ l) were not significantly different from Single Spin Kit PRP (0.03 +/- 0.01 thousand/ $\beta$ l) ( $p=0.98$ ). Double Spin Kit PRP contained at least a 400-fold higher concentration of white blood cells (14.8 +/- 3.0 thousand/ $\beta$ l) relative to Single Spin Lab and Single Spin Kit PRP.

#### **Extracellular matrix synthesis. Cartilage (Fig. 1):**

**<sup>3</sup>H-proline** – In the absence of IL-1 $\beta$ , <sup>3</sup>H-proline incorporation in Single Spin Lab cultures was not significantly different from Single Spin Kit cultures ( $p=0.22$ ). <sup>3</sup>H-proline incorporation in Single Spin Lab PRP was 31-64% higher than Double Spin 3x, 6x, and 9x, and Double Spin Kit cultures. In Single Spin Kit cultures, <sup>3</sup>H-proline incorporation was 31% and 20% higher than Double Spin 3x and Double Spin Kit cultures, respectively. In Double Spin Kit cultures, <sup>3</sup>H-proline incorporation was not significantly different from Double Spin 3x PRP ( $p=0.25$ ). No significant differences were found among Double Spin 3x, 6x, and 9x cultures ( $p=0.07-0.58$ ). <sup>3</sup>H-proline incorporation decreased with the addition of IL-1 $\beta$  for Single Spin Lab cultures only. In IL-1 $\beta$  medium,

<sup>3</sup>H-proline incorporation was not significantly different among Single Spin Lab, Double Spin 3x, and Single and Double Spin Kit cultures. ( $p=0.22-0.98$ ). <sup>3</sup>H-proline incorporation in Double Spin 6x and 9x cultures was approximately 15% lower than Single and Double Spin Kit cultures. **<sup>35</sup>S-sulfate** – In the absence of IL-1 $\beta$ , <sup>35</sup>S-sulfate incorporation in Single Spin Lab cultures was not significantly different from Single Spin Kit cultures ( $p=0.28$ ). <sup>35</sup>S-sulfate incorporation in Single Spin Lab cultures was 32-72% higher than Double Spin 3x, 6x, and 9x, and Double Spin Kit cultures. <sup>35</sup>S-sulfate incorporation in Single Spin Kit cultures was 38-50% higher than higher than that in Double Spin 3x, 6x, and 9x cultures. No significant differences were detected among Double Spin 3x, 6x, and 9x PRP ( $p=0.47-0.97$ ). <sup>35</sup>S-sulfate incorporation in Double Spin Kit cultures was not significantly different from Single Spin Kit and Double Spin, 3x, 6x, and 9x PRP cultures ( $p=0.07-0.27$ ). <sup>35</sup>S-sulfate incorporation decreased with the addition of IL-1 $\beta$  for Single Spin Lab, Single Spin Kit, and Double Spin Kit cultures. In IL-1 $\beta$  cultures, <sup>35</sup>S-sulfate incorporation among all media was not significantly different ( $p=0.47-0.99$ ). **Meniscus (Fig. 2): <sup>3</sup>H-proline** – Interactions between PRP preparations and IL-1 $\beta$  were not significant ( $p=0.78$ ). When considering the effect of PRP independent of IL-1 $\beta$ , <sup>3</sup>H-proline incorporation in Single Spin Lab and Double Spin 3x cultures was not significantly different ( $p=0.46$ ). <sup>3</sup>H-proline incorporation in Double Spin 6x and 9x cultures were not significantly different ( $p=0.15$ ), and both were significantly lower than Single Spin Lab and Double Spin 3x cultures. Single Spin Kit cultures were not significantly different from all other cultures ( $p=0.06-0.35$ ) except for Double Spin 9x PRP. <sup>3</sup>H-proline incorporation in Double Spin Kit cultures was less than Single Spin Lab and Double Spin 3x cultures only. **<sup>35</sup>S-sulfate** – In the absence of IL-1 $\beta$ , <sup>35</sup>S-sulfate incorporation was not significantly different among Single Spin Lab, Double Spin 3x, and Single Spin Kit cultures ( $p=0.28-0.67$ ). <sup>35</sup>S-sulfate incorporation in Double Spin 6x and 9x, and Double Spin Kit cultures was not significantly different ( $p=0.40-0.74$ ). <sup>35</sup>S-sulfate incorporation in Double Spin 6x and 9x, and Double Spin Kit cultures was approximately 40% less than



Single Spin Lab, Double Spin 3x, and Single Spin Kit cultures.  $^{35}\text{S}$ -sulfate incorporation decreased with the addition of IL-1 $\beta$  for Single Spin Lab, Double Spin 3x, and Single Spin Kit PRP. IL-1 $\beta$  cultures,  $^{35}\text{S}$ -sulfate incorporation among all media was not significantly different ( $p=0.30-0.89$ ).

#### **Gene expression of catabolic enzymes. Cartilage:**

Interactions between PRP formulation and IL-1 $\beta$  were not significant for MMP1 ( $p=0.47$ ), MMP13 ( $p=0.12$ ), and ADAMTS-5 ( $p=0.19$ ). In the absence of IL-1 $\beta$ , ADAMTS4 expression in Double Spin 6x cultures was 3.4- to 8.9-fold higher than Single Spin Lab, Single Spin Kit, and Double Spin 3x cultures. ADAMTS-4 expression in Double Spin Kit cultures was not significantly different from Double Spin 6x cultures ( $p=0.78$ ), and significantly higher than all other IL-1 $\beta$ -free cultures. The addition of IL-1 $\beta$  to the culture medium increased ADAMTS-4 expression for Double Spin 9x cultures (11-fold) only. In IL-1 $\beta$  medium, ADAMTS expression was not significantly different between Single Spin Lab and Double Spin 3x cultures ( $p=0.97$ ). ADAMTS-4 expression increased approximately 5-fold for Double Spin 6x and 22-fold for Double Spin 9x cultures. ADAMTS-4 expression in Single Spin Kit cultures was not significantly different from all laboratory preparations ( $p=0.16-0.18$ ) except for Double Spin 9x. ADAMTS-4 expression in Double Spin Kit cultures was 2.1-fold higher than Single Spin Kit, approximately 5-fold higher than Single Spin Lab and 3x, and not significantly different from Single Spin 6x ( $p=0.68$ ) cultures. **Meniscus:** Expression levels of MMP1 and 13 were not analyzed as many samples resulted in expression levels near the PCR detection limit. Interactions between PRP formulations and IL-1 $\beta$  were not significant for ADAMTS-4 ( $p=0.48$ ) and ADAMTS-5 ( $p=0.33$ ) (data not shown).

#### **Conclusions**

This study evaluated PRP formulations across a range of platelet concentrations as an indicator of whether differences exist among the various PRP kits that are commercially available. These data reject the hypothesis that increases the concentration of

platelets in PRP stimulates ECM synthesis in cartilage and the meniscus; furthermore, these findings suggest that high platelet concentrations for intra-articular injection should be considered with caution. When projecting the results of *in vitro* studies to clinical applications, certain limitations of laboratory models must be considered. While the dilution of PRP by 50% was intended to mimic the dilution of an intra-articular injection in synovial fluid, tissue culture does not allow for clearance of the growth factors (7) as may occur in joints. Therefore, the laboratory model may overestimate the concentration of growth factors that is present *in vivo*. Importantly, the acute response of cartilage and meniscal explants does not necessarily recapitulate the response that occurs over weeks of clinical healing. *In vivo* testing will be necessary to address these limitations.

#### **Acknowledgments**

Funded by the Steadman Philippon Research Institute, Vail, Colo., and discretionary funds from the Orthopaedic Research Center at Colorado State University. We thank Dr. David Karli of the Steadman Clinic for his technical assistance.

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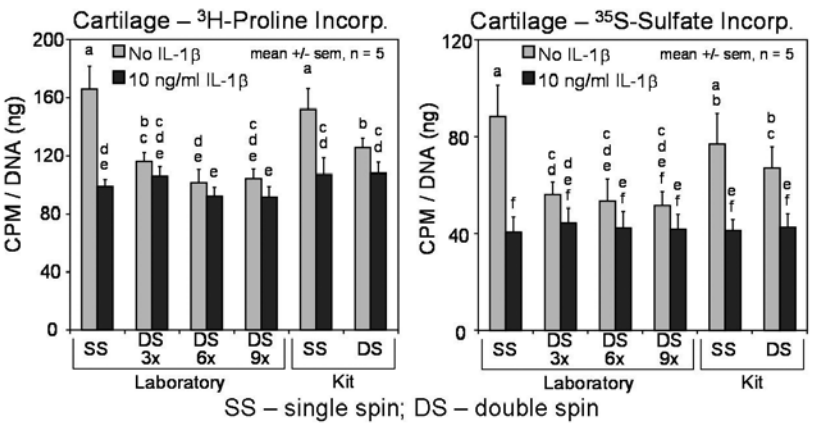


Figure 1: Cartilage – Radiolabel Incorporation

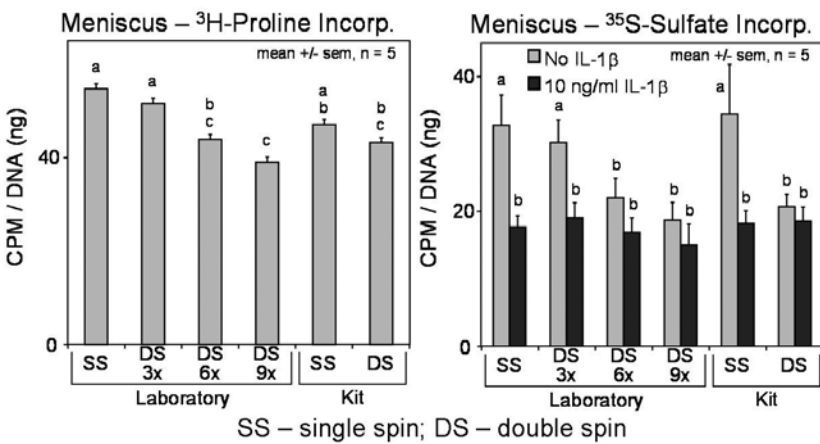


Figure 2: Meniscus – Radiolabel Incorporation

**Gene Therapy Approach to Treat Osteoarthritis Using AAVIRAP****Take Home Message**

Gene therapy vectors were used to deliver IL-1ra to joint tissues and appeared to significantly increase protein production in equine joint tissues. When scAAVIL-1ra was injected into metacarpal phalangeal and middle carpal joints of horses, IL-1ra was produced for 23 and 183 days, respectively. No evidence of intra-articular toxicity was noted throughout the study and horses remained sound for the duration of the testing periods.

**Introduction**

Osteoarthritis (OA) is a chronic and incurable disease that affects over 40 million Americans per year with estimated costs of over \$100 billion yearly. Gene therapy offers a radically different approach to the treatment of osteoarthritis. Interleukin receptor antagonist protein (IRAP) is antiarthritic due to its ability to effectively block or antagonize interleukin-1 (IL-1) which is a cytokine present in OA joint fluid and considered pivotal in the induction of osteoarthritis. An effective AAVIRAP vector would result in long-term abrogation of IL-1 and its devastating effects in the joint. This collaborative study was done by Nikki Phillips with Drs. Goodrich and McIlwraith of CSU, along with Drs. Samulski and Foti of the University of North Carolina, with the objective of constructing an AAVIRAP vector that would inhibit inflammation within the joint. Our hypothesis was that this vector would produce high levels of functional IRAP protein in the joint for up to six months.

**Materials and Methods**

An equine optimized IL-1ra gene was cloned into a mammalian expression vector, and transfected into AD293 cells. The vector was then cloned into an adeno-associated viral vector containing a CMV promoter. The scAAVIRAP vectors were tested for functionality against IL-1 stimulation in synoviocytes, and evaluated with a PGE2 ELISA. Two equines were evaluated for a pilot study in which joints were dosed with scAAVIRAP, scAAVGFP or saline. Synovial fluid was collected prior to dosing, two weeks after dosing,

every week thereafter for 14 weeks, and every other week until termination of the study. IRAP levels were evaluated with an equine ELISA kit. White blood cell counts and total protein values were also measured.

**Results**

The equine optimized IL-1ra gene produced IRAP in transfected 293 cells, with levels greater than 15ng/ml two days post-transfection. The scAAVIRAP vector produced similar levels in transduced synoviocytes (12 ng/ml), and was able to reduce the response of synoviocytes to IL-1 stimulation by 90%. Levels of IRAP protein were consistently high in the scAAVIRAP dosed joints of the pilot study animals with a peak occurring around week 8 (Fig. 1). Levels were between 400 and 1200 ng/ml and stayed above 400 ng for at least four months following injection. Study animals are ongoing and will continue to be monitored for at least two additional months. White blood cell count and total protein values rose only slightly in the scAAVIRAP or scAAVGFP injected joints and values stayed below that which is consistent with inflammation. Elevations of IRAP in saline injected joints were not detected.

**Discussion**

Our results suggest that the scAAVIRAP vector will produce extremely high levels of IRAP protein in the cells of joints and cause minimal to no toxicity in these tissues. The functional assay reveals that the IRAP protein produced effectively ameliorates the inflammatory cascade as evidenced by extreme reduction in PGE2. The viral vectors (scAAVGFP or scAAVIRAP) injected into the joints were well tolerated and didn't result in pain or joint swelling. This is the beginning of a gene therapy protocol that will be tested in horses with induced osteoarthritis to reveal if OA can be reduced or halted. Our objectives of this study were met and our hypothesis that scAAVIRAP would produce high levels of functional IRAP in the joint was proven. Further studies will define whether this gene therapy approach can result in the effective treatment and prevention of OA.

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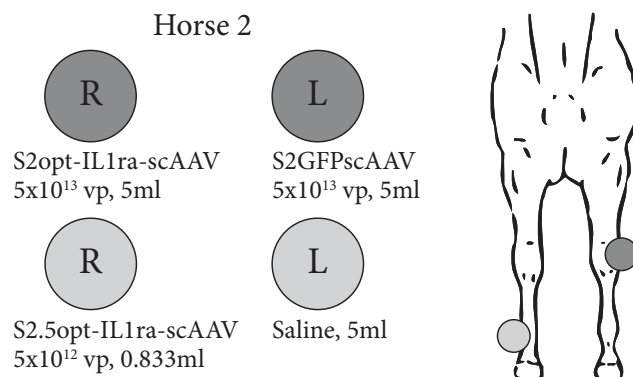
### *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse*

#### Acknowledgment

Funded by the National Institute of Health (NIH) National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), and the Grayson-Jockey Club Research Foundation.

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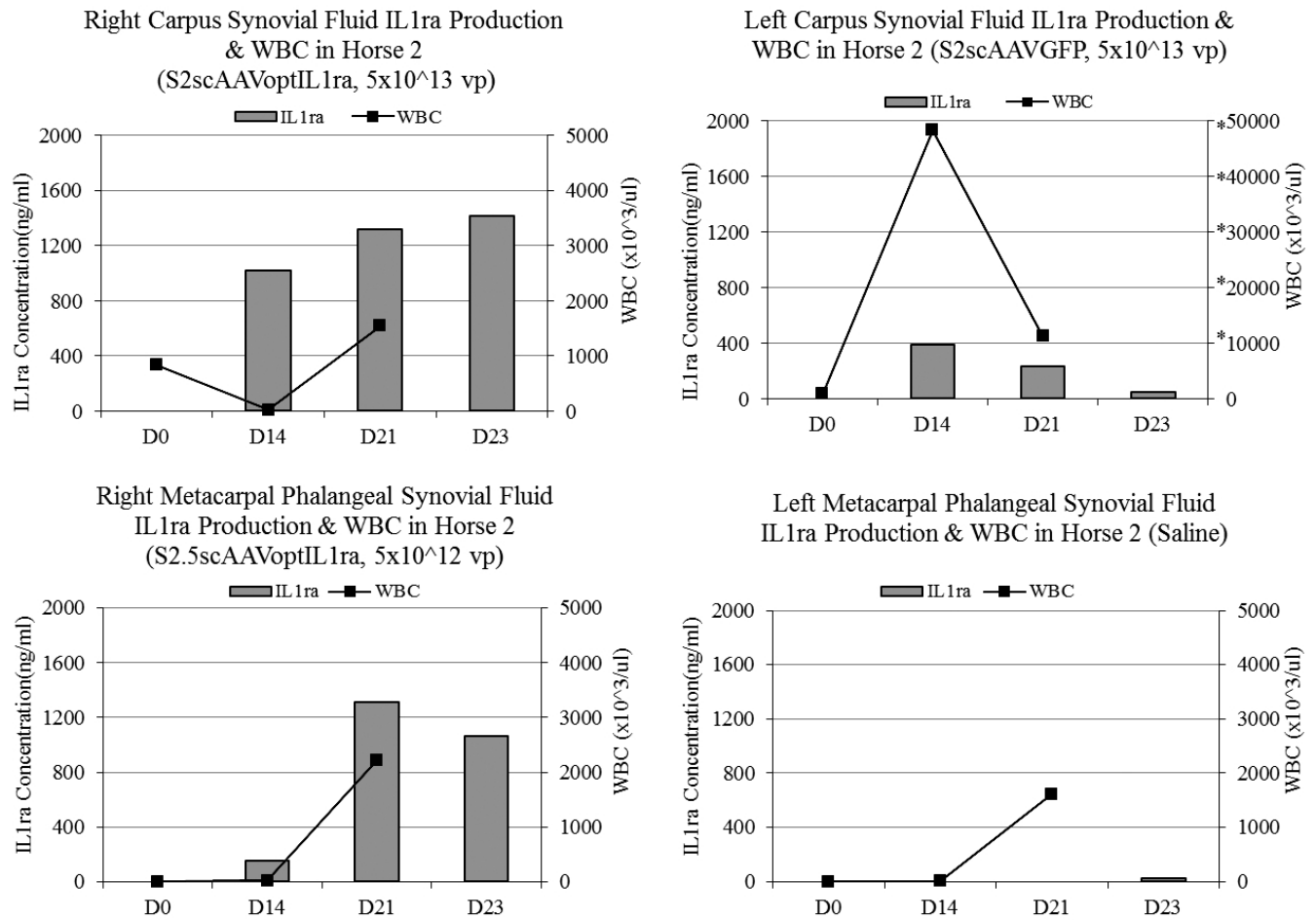
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**Figure1A.** Clinical design for the dosing of Horse 2. The blue circles correspond to the carpal joints, and the green circles correspond to the metacarpal phalangeal joints. ScAAV constructs were given at specific concentrations per joint, whereas 5 ml of saline was administered into the joint receiving that treatment.

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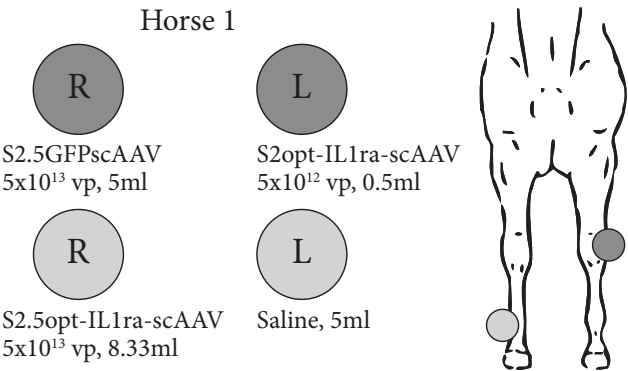
### Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse



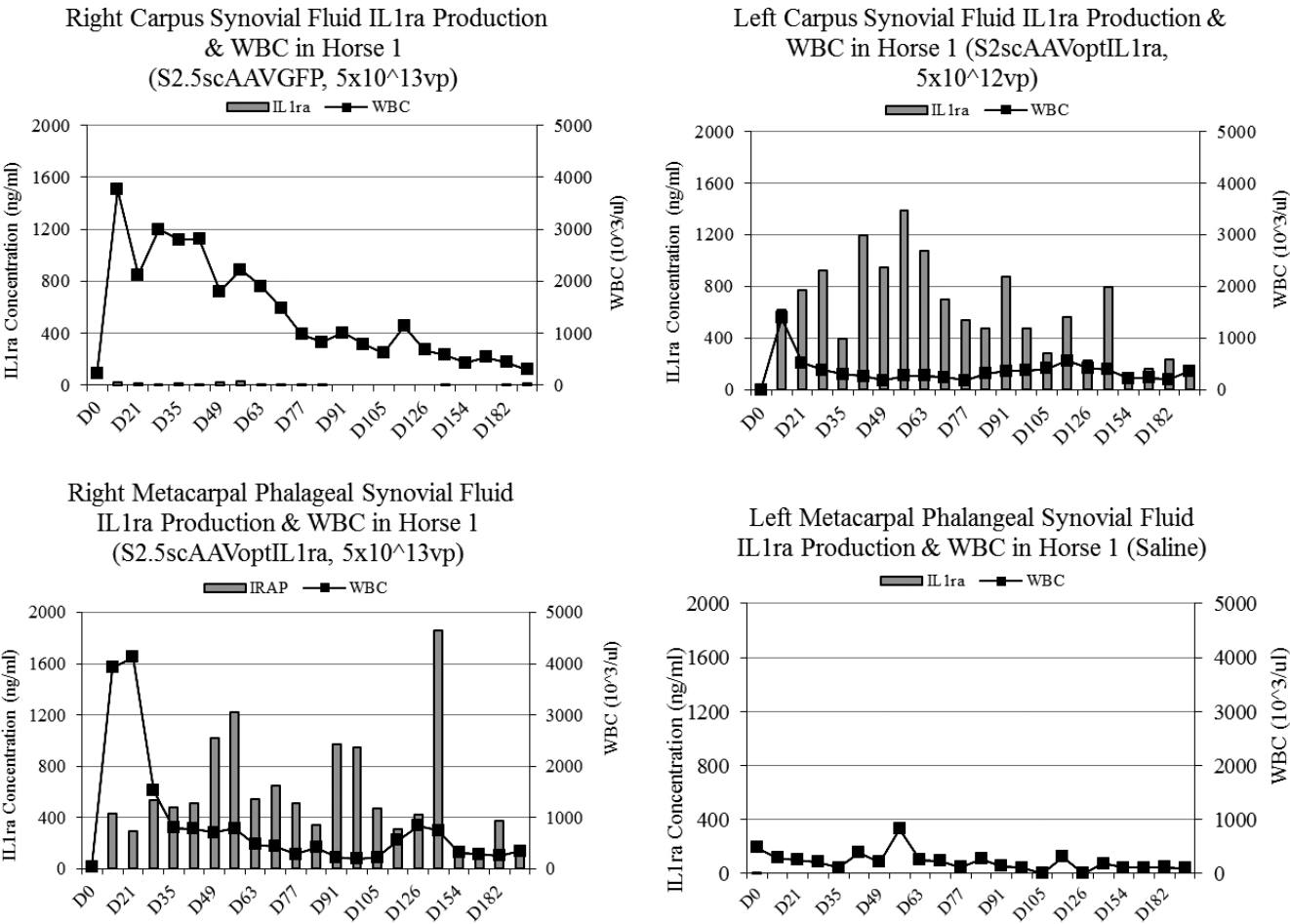
**Figure 1B.** IL1ra expression and WBC counts ( $10^3/\mu\text{l}$ ) in the synovial fluid of the carpus and metacarpal phalangeal joints of Horse 2. Each joint is graphed separately over time. IL1ra levels were assessed with an equine IL1ra ELISA kit. Note: the WBC numbers on the top right graph are higher due to a higher WBC count in that joint. WBC counts were not determined at D23.

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**Figure 1C.** Clinical design for the dosing of Horse 1. The blue circles correspond to the carpal joints, and the green circles correspond to the metacarpal phalangeal joints. ScAAV constructs were given at specific concentrations per joint, whereas 5 ml of saline was administered into the joint receiving that treatment.



**Figure 1D.** IL1ra expression and WBC counts (10<sup>3</sup> /ul) in the synovial fluid of the carpus and metacarpal phalangeal joints of Horse 1. Each joint is graphed separately over time. IL1ra levels were assessed with an equine IL1ra ELISA kit.



**Influences of Age on scAAV Transduction of Chondrocytes and Synoviocytes****Take Home Message**

Age does not appear to significantly influence the efficacy of gene transduction using scAAV in synoviocytes and chondrocytes in horses. Because the adult population may be more prone to receiving gene therapy than neonatal or young animals this is valuable information.

**Introduction**

Self-complementary adeno-associated viral vectors (scAAV) have shown promising results for gene delivery to cells of joint tissues. To test these vectors *in vitro*, it is most common to utilize cell cultures from skeletally immature animals due to the increased metabolic activity and recovery rate from cryopreservation of these cells compared to those from older animals.[1, 2] While initial investigation of transduction in neonatal tissue is relevant, it is important to determine if transduction of scAAV vectors is similar in adolescent and adult tissue since this is the population that would most frequently require gene therapeutic measures. This study was done by Nicki Phillips, Drs. Goodrich and McIlwraith of CSU and Dr. Jude Samulski of the Gene Therapy Center, University of North Carolina, and examined the transduction efficiency of scAAV in joint tissues from animals that represent a range of clinically applicable ages. Viral transduction efficiency was measured along with protein production from chondrocytes and synoviocytes of equine neonates (0-3 mth), weanlings (4-8 mth) and adults (2-5 yr). We hypothesized that scAAV transduction efficiency in chondrocytes and synoviocytes would decrease with age.

**Methods**

**Cell Culture:** Cartilage and synovial samples were taken from normal cadaveric stifle joints of neonates, weanlings, and adult equines within 4 hours of death. Age groups consisted of three to five individuals per group. Isolated chondrocyte cultures were plated immediately for use at passage 0 (P0), while synoviocytes were expanded to P2 to ensure a pure population of cells prior to experimental use. ScAAV

**Transduction:** Chondrocytes and synoviocytes were plated to 50% confluency 48 hours prior to transduction with scAAV constructs expressing GFP and IL1-ra at a concentration of 6000 viral particles per cell (vpc). Transductions were carried out for three hours and evaluated at four and eight days post transduction. **GFP Evaluation:** GFP (green fluorescent protein) expression was determined with a spectrophotometer and samples were normalized against the negative controls to account for auto fluorescence. **Equine IL1-ra ELISA Evaluation:** Culture media samples were collected at four and eight days post-transduction. Non-transduced samples and those transduced with IL1-ra were evaluated using a commercially available Equine IL1-ra ELISA kit (R&D Systems). Cultures expressing IL1-ra were normalized to their negative controls. **Cell Scoring:** Cultures were scored on a scale 0-5 based on the number of cells exhibiting abnormal morphology and complete cell death. A score of 0 had no abnormalities, 1 = 1-20% abnormality, 2 = 20-40%, 3 = 40-60%, 4 = 61-80% and 5 = >80%.

A one way ANOVA was performed to detect difference between age groups. A p value of <0.05 was considered significant.

**Results**

There were minimal differences observed in the transduction efficiency of synoviocytes between age groups at day 4 (data not shown) and day 8 post-transduction (Fig. 1). Fluoroscopic analysis revealed a gradual increase in fluorescence of chondrocytes with age and this trend was further emphasized by fluorescent microscopy (Fig. 2, C and D). However; this increase was not seen with IL1-ra protein production (Fig. 2). Transduction of synoviocytes with scAAVGFP did not change with age group.

Similarly, there were minimal differences in the associated protein production of synoviocytes between age groups at day 4 (data not shown) and day 8. Protein production in weanling chondrocytes were

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markedly reduced compared to those of neonates and adults (with similar production) (Fig. 3).

Cell morphology scores for adult synoviocytes were considerably higher (worse) at day eight, with minimal changes observed between chondrocytes and synoviocytes for neonates and weanlings (data not shown).

**Discussion**

Our results suggest that age does not significantly decrease scAAV transduction and associated protein production. Surprisingly, transduction with scAAVGFP actually increased in chondrocytes in adults although this was not demonstrated in IL1-ra production. This data is important when considering gene therapeutic protocols in adult animals since this population is more likely to receive gene therapeutic

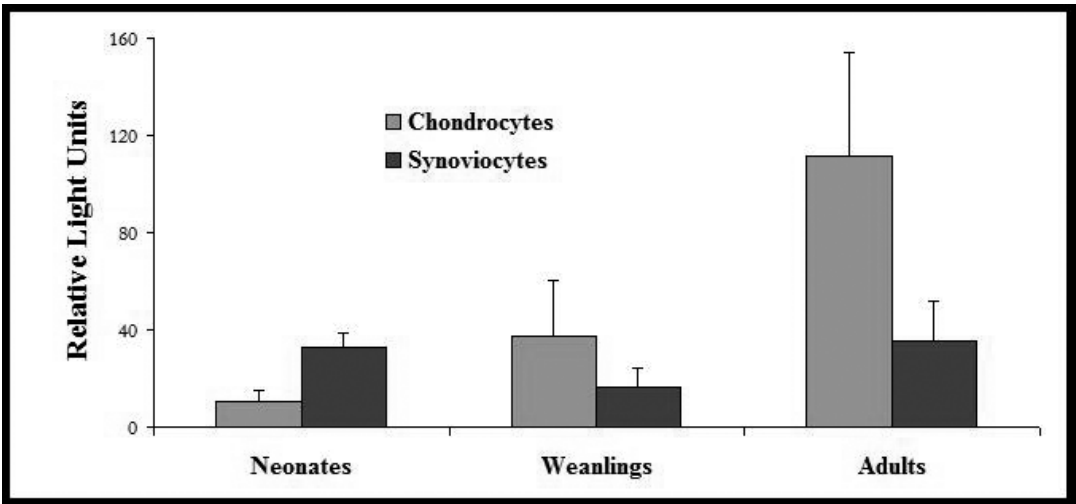
measures to treat joint disease. To our knowledge, age differences of scAAV transduction has not been investigated. This information will be important to clinicians choosing viral dosages for *in vivo* gene therapy trials.

**Acknowledgments**

Funded by NIH grant K08AR054903-01A2.

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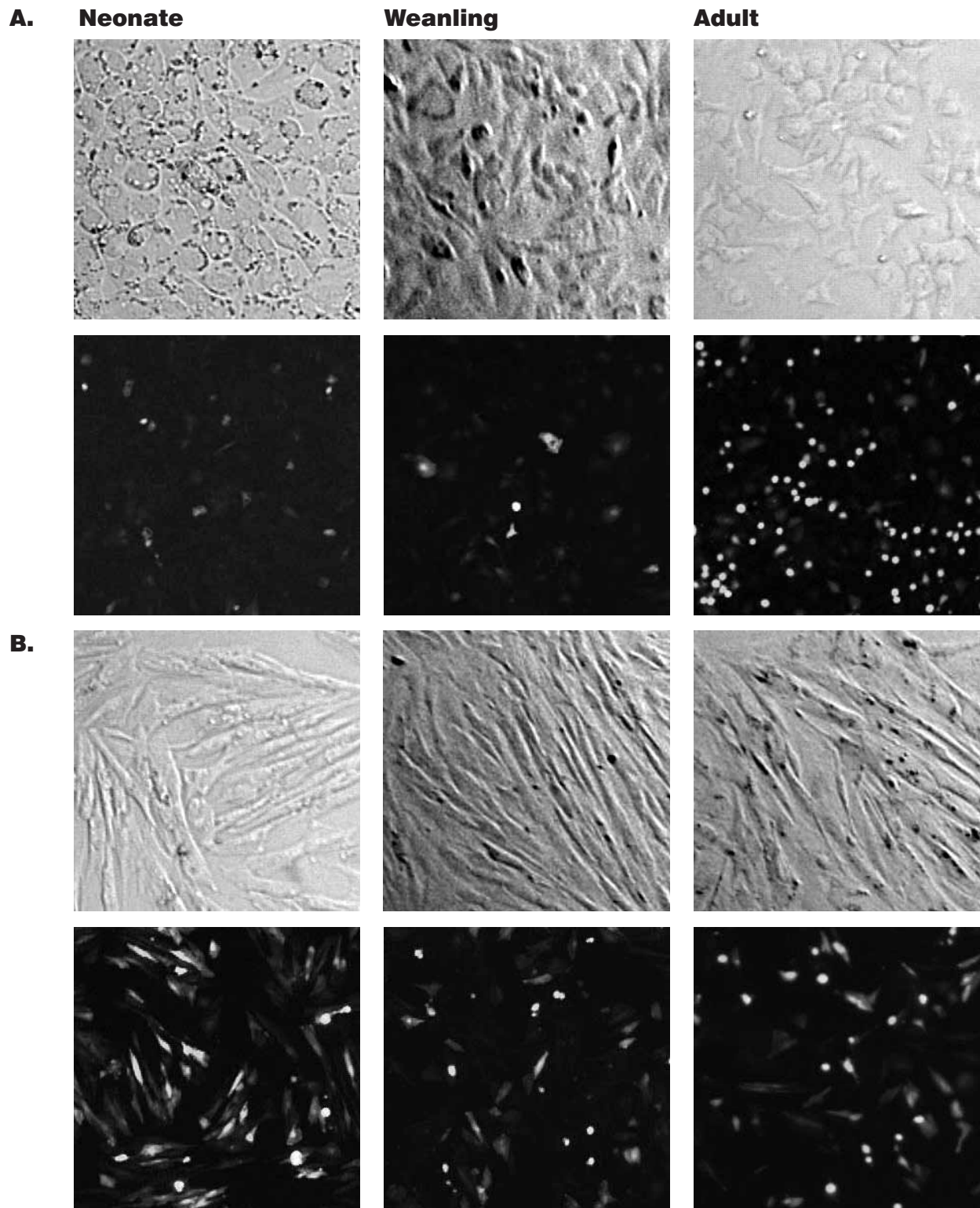
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**Figure 1.** D8 GFP expression in chondrocyte and synoviocyte cultures taken from neonates, weanlings, and adults.

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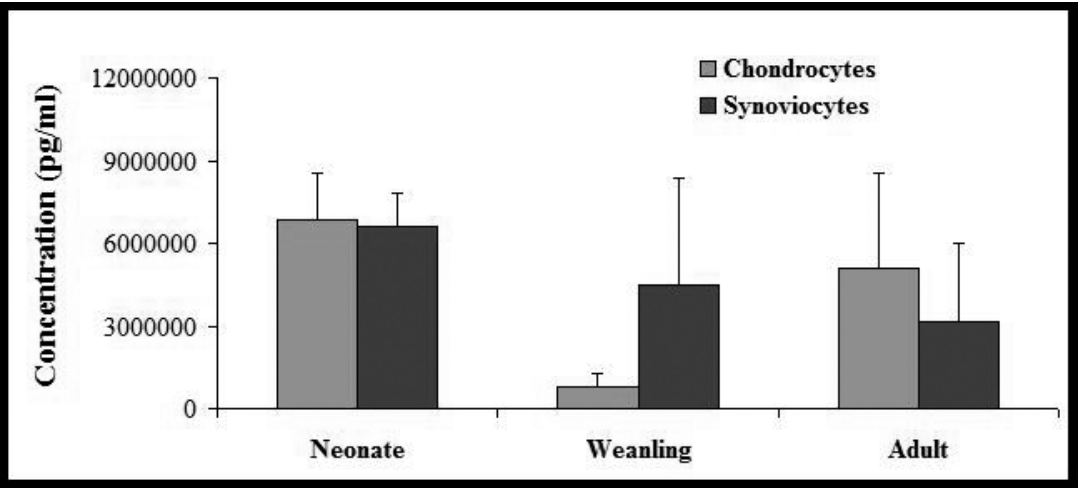
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**Figure 2.** Fluorescent microscopy of chondrocytes (A) and synoviocytes (B) 8 days post-transduction with IRAP (top) and GFP (bottom) scAAV.

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**Figure 3.** D8 IL1-ra expression in chondrocyte and synoviocyte cultures taken from neonates, weanling and adults.

**Mesenchymal Stem Cell Proliferation and Expression of Growth Factors in Response to Extracorporeal Shock Wave Therapy Treatment****Take Home Message**

While extracorporeal shock wave therapy (ESWT) has been shown to enhance the therapeutic potential of certain cell types, its effect on equine mesenchymal stem cells (MSCs) is not known. We report that ESWT does not enhance MSC proliferation *in vitro*, and that gene expression of selected growth factors is not affected. For proliferation cultures, the first application of ESWT decreased growth relative to unshocked MSCs, although subsequent ESWT treatments did not affect MSC proliferation. These data suggest that it may be possible to administer multiple ESWT treatments to defects containing MSCs without harming the repair response of transplanted MSCs.

**Introduction**

Extracorporeal shock wave therapy has been widely used to treat tendonitis in human (1) and veterinary medicine (2, 3). For equine patients, the use of ESWT on proximal suspensory desmitis has been suggested to improve the prognosis in the hindlimb (4). Similarly, promising evidence of increased collagen fibril accumulation (5) and neovascularization (2) in experimental models of equine tendonitis has been demonstrated with ESWT. A second promising therapy for equine tendonitis is injections of bone marrow MSCs (6, 7). When injected into tendon and ligament core lesions, MSCs have led to a higher return to work and lower reinjury rate than would be expected without treatment (7-9). To date ESWT or MSCs have been separately to treat tendonitis, although it remains possible that the combination of ESWT following MSC injection into core lesions may further enhance repair over the individual treatments. As a first measure of the effect of ESWT on equine MSCs, Drs. Kisiday, Frisbie, and McIlwraith evaluated MSC proliferation following ESWT using cell culture techniques that are similar to those used to prepare MSCs for clinical applications (7). In addition, we explored the effect of ESWT on MSC gene expression of growth factors that may influence healing of diseased tendon.

**Methods**

**Proliferation:** The effect of ESWT on MSC proliferation in monolayer culture was evaluated over a ten day timecourse. Equine MSCs were plated at a concentration of 12,000 MSCs/cm<sup>2</sup> and maintained in baseline media containing 10% FBS. Half of the cultures were fed baseline media, while a second group was fed baseline media plus 2 ng/ml fibroblast growth factor 2 (FGF). Control groups received no additional treatment. Experimental groups were subjected to 500 pulses using energy level E2 prior to the first plating and in between passages. ESWT was administered to MSCs in suspension within 2 ml of baseline medium held within 15 ml centrifuge tubes. For each passage, MSCs were allowed to grow for two days, a point where control cultures in FGF-2 medium reached near confluence. At this point the cells were lifted using trypsin, counted, and replated at a concentration of 12,000 MSCs/cm<sup>2</sup>. The cultures were expanded as such five times, resulting in five passages over 10 days. At each passage, the cell yield was divided by the number of cells plated at the start of the experiment to obtain a fold-change in cell number. The log<sub>2</sub> of the fold-change was calculated to determine the number of population doublings. This experimental protocol was repeated for MSCs from three animals.

**Gene expression of growth factors:** ESWT was administered to MSCs in suspension as previously described, and then seeded at 20x10<sup>3</sup> cells/cm<sup>2</sup> in monolayer culture in alphaMEM with or without 10% FBS. Control cells that did not receive ESWT were seeded in parallel. After four hours, RNA was harvested from monolayer cultures and isolated using the RNeasy Mini Kit according to the manufacturer's instructions. RNA was reverse transcribed to cDNA in the presence of random hexamers. Semiquantitative real time PCR was performed for vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and transforming growth factor beta (TGFβ) using the Applied Biosystems 7000 system and TaqMan Universal



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PCR Master Mix. For each sample, expression was normalized to 18S threshold values.

*Statistical Analysis:* Proliferation assays were conducted using MSCs from three donor horses, while gene expression analysis was evaluated using MSCs from four donor horses. Log transformed data were analyzed using a mixed model analysis of variance, with the donor animal used as a random effect. Individual comparisons were made using least square means procedure. For MSC proliferation through the first five passages, ESWT, FGF, and passage were considered main effects as well as their interactions. For cumulative proliferation through the first five passages, ESWT and FGF were considered main effects as well as their interactions. For gene expression analysis, ESWT and FBS were considered main effects as well as their interactions. Individual comparisons were made when main effects or interactions resulted in an *f*-value less than 0.05. For individual comparisons, *p*-values less than 0.05 were considered significant. Data are reported as mean  $\pm$  standard error of the mean.

## Results

*Proliferation:* Interactions among ESWT, FGF, and passage were not significant ( $p = 0.91$ ). When considering the effects of ESWT and passage independent of FGF, at passage one, ESWT cultures ( $0.71 \pm 0.17$  population doublings) experienced 0.95 fewer population doublings than control cultures ( $1.66 \pm 0.17$ ) ( $p < 0.001$ , Fig. 1). For passages two through five, the number of population doublings in ESWT cultures were not significantly different from controls for each passage ( $p = 0.24$ - $0.95$ ). When considering the effect of FGF independent of ESWT and passage, FGF+ cultures ( $1.86 \pm 0.09$ ) averaged 0.59 more population doublings compared to FGF- cultures ( $1.27 \pm 0.09$ ) ( $p < 0.001$ , data not shown). For the cumulative population doublings over the first five passages, interactions between ESWT and FGF were not significant ( $p = 0.32$ ). When considering the

effect of ESWT independent of FGF, ESWT cultures ( $7.35 \pm 0.40$ ) resulted in 0.90 fewer population doublings than control cultures ( $8.25 \pm 0.40$ ) ( $p < 0.005$ , data not shown). For MSCs from FGF+ cultures that were expanded through a sixth passage, the number of population doublings by MSCs that received ESWT throughout the first five passages was not significantly different from that in control cultures that did not receive ESWT ( $p = 0.87$ ) (data not shown). MSCs from control cultures that were subjected to ESWT for the first time prior to seeding into passage six experienced approximately 0.3 fewer population doubling than cultures treated with ESWT throughout the six passages and untreated controls ( $p < 0.05$ ).

*Gene expression of growth factors:* For IGF-1, TGF $\beta$ , and VEGF, interactions between ESWT and FBS were not significant ( $p = 0.67$ - $0.79$ ) (data not shown). When considering the main effect of FBS, gene expression of IGF-1 in FBS-free conditions were 4.3-fold higher than FBS cultures, while gene expression of TGF $\beta$  and VEGF in serum-free cultures conditions was 2.1- and 9.3-fold lower than controls ( $p < 0.05$ ). When considering the main effect of ESWT, gene expression of IGF-1, TGF $\beta$ , and VEGF were not significantly different ( $p = 0.45$ - $0.92$ ).

## Conclusion

ESWT and MSCs are used separately to treat equine tendonitis, and it is not known how ESWT applied following the injection of MSCs may affect healing. This *in vitro* study did not demonstrate a benefit of ESWT on MSC proliferation or gene expression of growth factors; however, our data suggest that the only negative effect of ESWT is a short-term decrease in MSC proliferation following the initial treatment only.

## Acknowledgments

Funded by Pulse Veterinary Technologies, Alpharetta, Ga.

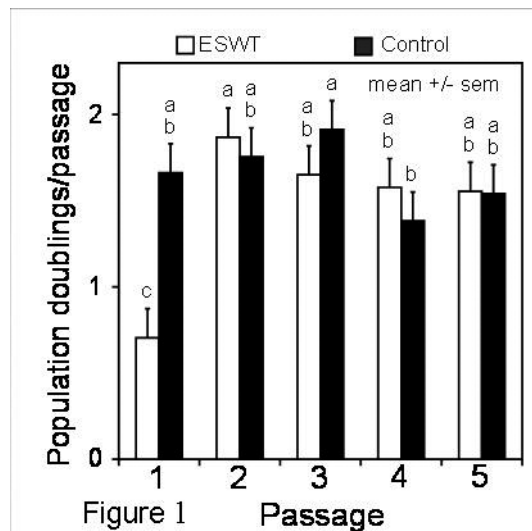


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### Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse

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**Figure 1.** Nis non rati dicium rest, optur rem untius percipi enemque autem expligenis delitiumquis poreprem que volenim odipsunte pa que peria velentur molores volor simperi onectec eperest ibusdam qui quatur?

## Summaries: Focus 4

*Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse*

### **Review of the Scientific Basis for Use of Intra-Articular Corticosteroids in the Horse**

#### **Take Home Message**

Intra-articular corticosteroids are potent anti-inflammatory agents with prolonged effectiveness for traumatic joint disease in the horse. Generalizations about harmful effects of intra-articular corticosteroids are inappropriate, and research has defined differences in beneficial and deleterious effects. There is also no evidence that intra-articular corticosteroids cause harm to subchondral bone or promote catastrophic injury. Sound evidence linking laminitis to corticosteroid injection of normal doses of corticosteroids is lacking.

#### **Introduction**

The Thoroughbred horse-racing industry has come under renewed scrutiny in recent years in the United States because of horses suffering from catastrophic injury during high-profile events. Consequently the public outcry from these events has led to Congressional oversight and investigation into the industry, which has led to significant scrutiny of medications that are used in sport. Anabolic steroids were banned in 2009 and now, other medications are also being critically reviewed. Intra-articular use of corticosteroids has recurred as a focus of attention. Proponents of intra-articular corticosteroid treatment argue that such therapy is needed to decrease inflammation and musculoskeletal pain, which is common in Thoroughbred racehorses, and more importantly, to avoid overloading of the other limbs, possibly leading to catastrophic injury. However, opponents of corticosteroid use feel that it is merely masking pain and results in joint deterioration. Much of this perception of harm is based on opinion and has been passed down through the literature.<sup>1</sup>

This paper was a review of the clinical use and scientific basis of intra-articular corticosteroid administration that was generated by a request to Dr. McIlwraith by the American Association of Equine Practitioners Racing Committee and the Racing Medication and Testing Consortium (RMTTC) to critically review the scientific data regarding intra-articular corticosteroids. After doing this review Dr.

McIlwraith has published a full review in *Equine Vet Journal*<sup>2</sup> and it was also presented at the Annual Meeting of AAEP in December 2010.<sup>3</sup>

#### **Summary of Important Points**

- The use of hydrocortisone in the treatment of a variety of musculoskeletal conditions in 94 horses and cattle was first reported in 1955 by Wheat. The author cited profound improvements in clinical signs in most cases but also cautioned treated animals should be rested after injection to allow healing of affected animals.
- This report was followed by multiple investigations that showed positive effects by Van Pelt and colleagues.
- The first paper indicting corticosteroids as harmful in the horse was written in 1968 and was based on an anonymous paper in the human literature. The statement, “an endless destructive cycle is set into motion which, if continued, will produce a steroid arthropathy which can render the horse useless” was referenced and the reference was an abstract written by an anonymous author. In a subsequent textbook chapter on drugs in the performance horse a number of statements were made regarding corticosteroids and their harmful effect on joints.
- Early studies evaluating the effect of methylprednisolone acetate (MPA) injected into normal joints showed deleterious effects.
- Based on observations of the lack of secondary cartilage damage when doing arthroscopic surgery for joints with osteochondral fragmentation, research projects were commenced at the ORC at Colorado State University.
- The initial project was done with betamethasone esters and there were no harmful side effects observed in an experimental model in which a chip fragment was created and horses exercised on the treadmill.
- Subsequent evaluation of MPA showed definite deleterious side effects using the osteochondral fragment model that was modified and has been

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### *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse*

used in multiple studies since then. This led to recommendation of caution using MPA.

- Triamcinolone acetonide (TA) was then evaluated using the osteochondral fragment model and protective effects to the cartilage as well as good clinical effects in lowering inflammation were found.
- There had been a long fear of the use of TA potentiating development of laminitis. Early work had recommended that a total body dose of 18 mg not be exceeded. Subsequent to that a clinical paper showed use of doses up to 40 mg were not associated with laminitis. More recently a report of intra-articular injection of corticosteroids in 2,000 cases revealed laminitis being associated with this treatment in three cases. The majority of the time TA was used at higher dose rates ranging from 20-45 mg.
- A traditional concept has been that a load is better not to use MPA in high motion joints, but its use in low motion joints (such as the distal tarsal joints) is appropriate. There certainly has been decreased use of TA by sport horse veterinarians even in low motion joints, but the use persists in racehorses but there is no evidence either way that this practice needs to be continued. While MPA is believed to be longer acting there is questionable logic to selective harm to articular cartilage.
- There is no association of intra-articular use of triamcinolone acetonide or methylprednisolone acetate causing harm to the bone and predisposition to fracture and catastrophic injury based on controlled studies in horses.
- Anti-inflammatory actions of corticosteroids are mediated through cytoplasmic receptors which lead to gene-regulation changes and consequent

changes in protein expression through signaling pathways. The anti-inflammatory effects are quite long acting and the pharmacologic presence in the joint. There are a number of pharmacokinetic studies being done with corticosteroids but it is difficult to directly transpose pharmacokinetic data into biologic potency or activity. Measuring gene expression using pharmacogenomic methods provides the potential of more global assessment or pharmacodynamics responses after intra-articular corticosteroid injection.

- There is no current evidence that combination therapy with HA can mitigate the negative effects associated with MPA but recent work shows that HA does have chondroprotective effects in combination therapy with TA or betamethasone esters is appropriate.

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## Summaries: Focus 4

Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse

### scAAV Transduction Efficiencies in Joint Tissue Monolayer and Explant Cultures and the Effects of Synovial Fluid Neutralization

#### Take Home Message

Gene transduction of chondrocyte and synovial monolayer is significantly different than cartilage and synovial explants and this study highlights the necessity to test gene therapeutic tissue *in situ*. Furthermore, neutralizing antibodies exist in both the synovial fluid and serum which can reduce efficient gene transduction. It is important to determine which serotypes have antibodies that exist in the horse.

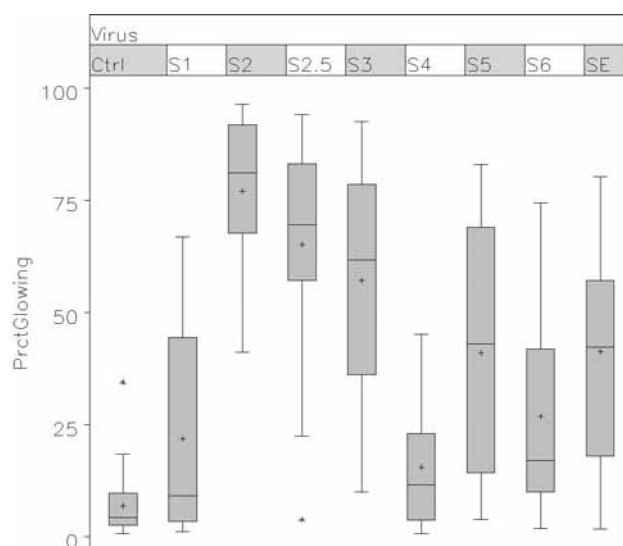
#### Introduction

Within the joint there are two major tissues currently targeted by gene therapy vectors to treat osteoarthritis. Synoviocytes are within the synovial membrane which has fimbria that extend into the synovial fluid. Chondrocytes are surrounded by matrix in the cartilage layer. Although transduction efficiency has been well quantified in chondrocyte and synoviocyte monolayers, transduction of explants of cartilage and synovium has not been thoroughly investigated. Although efficient transduction of synoviocytes and chondrocytes is important to *ex vivo* gene therapy, it is important to determine if self-complimentary adeno-associated viral (scAAV) vectors can transduce cells *in situ*. scAAV is currently the vector of choice being investigated to carry therapeutic genes to both the synovium and cartilage.<sup>1</sup> There are many serotypes of AAV each with specific and variable tissue tropisms. Furthermore, neutralizing antibodies have been identified in the synovial fluid of humans which results in decreased scAAV transduction of tissue.<sup>2</sup> The purpose of this study done by Daniel Hemphill together with Drs. McIlwraith and Goodrich of the ORC and Dr. Jude Samulski of the University of North Carolina was to compare scAAV transduction in monolayer to explant tissue of synovium and cartilage and to investigate whether the presence of synovial fluid inhibits efficient transduction in joint tissue. Our goal was to identify which serotype of AAV leads to efficient transduction in both synovium and cartilage in the horse model. We hypothesized that monolayer transduction efficiency is similar to

explant transduction efficiency and that neutralizing antibodies exist in synovial fluid that will decrease transduction efficiency.

#### Methods

Synovium from the femoropatellar joint capsule and cartilage from the patella were aseptically harvested post-mortem from three horses 2-5 years of age. Each tissue was cut into pieces of similar size for explants with the remainder digested overnight in collagenase type II solution. The isolated chondrocytes and synoviocytes were expanded one passage and then plated in 48 well plates at 50,000 cells/cm<sup>2</sup> for synoviocytes and 100,000 cells/cm<sup>2</sup> for chondrocytes. After two days all explants and monolayers were transduced with scAAV-GFP at 8000 virus particles per cell for four hours at 37C. Fluorescent microscopy pictures were taken every three days and flow cytometry was performed 35 days after the transduction. Multivariable ANOVA was performed on the data and results with  $p < 0.05$  chosen as statistically significant.



**Figure 1.** Explant experiment transduction efficiencies for serotypes averaged across all tissue types. On left showing the percentage of cells expressing GFP at significant levels and on right average intensity of fluorescence.

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### Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse

Synovial fluid was aseptically collected post-mortem from five horses 2-5 years of age. Synoviocytes and chondrocytes were plated in 48 well plates at the densities previously mentioned. Three days after plating, 4000 virus particles per cell were incubated with a 1:2 or 1:200 synovial fluid dilution for one hour at 37C. Immediately following, the cells were transduced with the virus and synovial fluid mixture for four hours at 37C. Plates were read using a fluorometric plate reader every two days. One way ANOVA was performed and  $p < 0.05$  chosen as statistically significant.

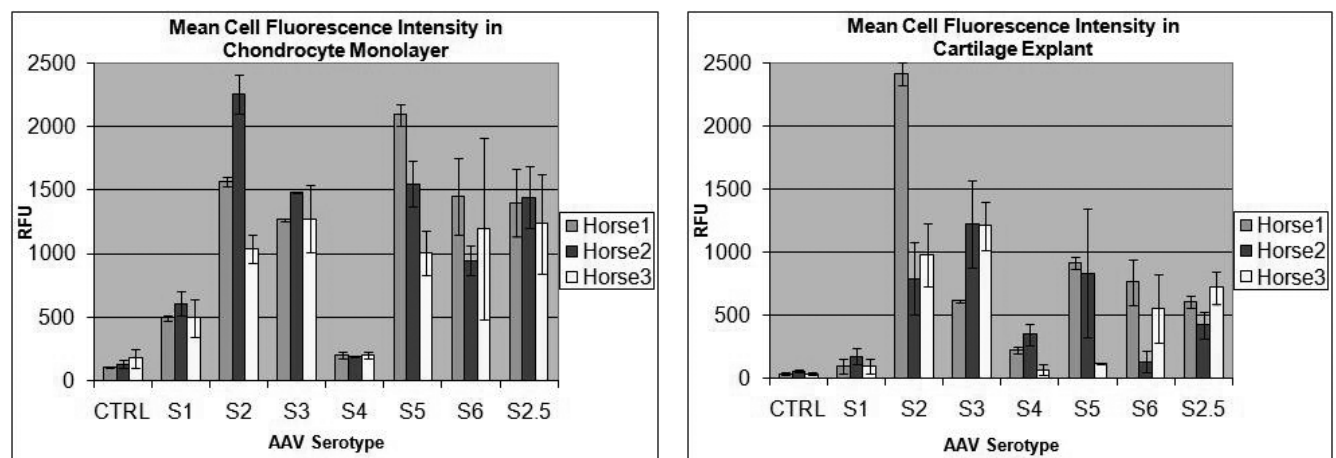
#### Results

*In vitro*, explants and monolayers were successfully transduced and there was no difference in transduction efficiency between monolayer and explants when analyzing the percentage of cells transduced. The mean fluorescence intensity of the vector gene expression revealed that monolayer cells appeared more fluorescent than explants cells. When percentage of cells transduced and mean expression intensity were combined, horses did not show statistical difference, but virus serotypes did show statistical differences with S2 > (S3, S5, S2.5, S6); only these serotypes were significantly different from the control. Furthermore, cartilage showed statistically higher transduction efficiency than synovium.

Virus incubation with synovial fluid decreased GFP production in synoviocytes for several serotypes of virus, although averaging over all serotypes showed no difference. While specifically testing individual serotypes: 2, 2.5, 5, and 6 revealed significant differences following synovial fluid incubation. There was no statistical difference between the five horses tested.

#### Discussion

The results of this study suggest that cell culture monolayer is a good indicator for actual tissue tropism of scAAV serotypes in the joint as tissue explants (both cartilage and synovium) are efficiently transduced with various serotypes. These results suggest that when scAAV vector is placed into the joint environment, vector can transduce chondrocytes and synoviocytes efficiently *in situ*. Furthermore serotype 2 appears to be the best option for total transduction of joint tissue. However, synovial fluid has a negative effect on transduction efficiency with AAV serotypes 2, 2.5, and 6. This suggests that, in addition to identifying the optimal serotype for transducing joint tissues, some attention must be paid to identifying a serotype that will not be inhibited by synovial fluid; or the removal of synovial fluid through joint lavage should be considered in a treatment. Despite monolayers and explants having similar transduction efficiencies, the intensity of gene



**Figure 2.** Transduction efficiency as mean cell GFP intensity from chondrocyte monolayer and cartilage explants.

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### *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse*

S2	No SF > 1:200 > 1:2
S2.5	No SF > (1:200 , 1:2)
S3	(No SF , 1:200 , 1:2)
S5	1:200 > (No SF , 1:2)
S6	(No SF , 1:200) > 1:2

**Table 1.** Synovial fluid dilution experiment – Comparison of GFP expression in synoviocytes. “No SF” indicates the control in which virus was incubated with only media and no synovial fluid.

expression differed between the two and this deserves further investigation. The clinical significance of this study is that specific serotypes have been identified that will efficiently transduce synoviocytes and chondrocytes *in situ* and while neutralizing antibodies appear to exist, significant transduction can still occur.

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**Review Article – Trying to Shed Light on the Use of Stem Cells in Equine Orthopaedics****Take Home Message**

The field of mesenchymal stem cell research is an ever evolving science and much work is still needed in the clinical application of this treatment modality. Some good basic science studies have been done over recent years but equally importantly, clinicians need to continue communicate openly on the success and failures with this emerging modality under evidence-based (EBM) conditions.

Given the amount of information and misconception that has been introduced into the veterinary and lay press, Drs. Frisbie and Roger Smith of the Royal Veterinary College, London published a review article to try and clarify key points of the stem cell technology and research. The following is a summary of that publication.

The manuscript focused on the clinical use of mesenchymal stem cells (MSCs) in horses and the justification for, as well as issues surrounding, their use. Many of the early reports used bone marrow as a source of these cells, but other sources have been more recently demonstrated. For example, muscle, cartilage, and adipose tissue all have been shown to contain multipotent MSCs. Direct comparisons of the two most commonly used clinical sources of cells, bone marrow and fat derived, have shown superior cells from bone marrow when tested in a joint environment. Work to compare the best source for tendon is yet to be published; however, work done after the current publication suggests bone marrow may be better for tendon repair as well.

Various methods of preparing stem cells from bone marrow aspirates have been used. They range from injecting the raw aspirate to picking out stem cells and culture expanding them. While the direct injection of aspirate was first labeled as stem cell treatment it has been shown only 2,000 stem cells/ml

are contained in such a preparation, thus significantly below what is believed to be needed (1-5 million cells). More recently bone marrow concentration has been proposed. This method concentrated the stem cells found in the raw aspirate approximately 12 times however, given the initial volume limitations the final number of cells is usually in the hundreds of thousands, not millions. Thus culture expanding the stem cells to provide adequate stem cell numbers is still the gold standard. While some have looked at using allogeneic cells or cells derived from another horse, this process has not been approved by the Food and Drug Administration (FDA).

Research has been done looking at horses with experimental osteoarthritis, in this study published in the *Journal of Orthopaedic Research* a significant decrease in PGE2 (an inflammatory mediator) was seen in response to a single treatment with bone marrow derived stem cells. While this is encouraging, it is not clear given the cost of stem cell treatment that the improvement warrants the expense. Stem cells have also been used to treat clinical cases of joint disease. A group of horses that have undergone surgery to ascertain a definitive diagnosis as well as determine the degree of pathology were followed for almost two years. The majority of these horses had meniscal damage and were treated surgically, as well as with bone derived culture expanded stem cells. These horses were more likely to go back to work compared to a previously published group of horses that received surgery alone, especially in cases of severe damage. These results have provided encouragement for continued intraarticular treatment of horses with stem cells.

The publication also detailed reports of horses that sustained tendon or ligamentous injuries that were treated with rehabilitation and stem cells and then compared to those treated with rehabilitation alone. It

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### *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse*

has been shown that stem cells can help these horses go back to work and in flat racing horses decrease the incidence of re-injury after they have gone back to work, when compared to rehabilitation alone.

In conclusion, the field of stem cell research is an ever evolving science and much work is still needed in the clinical application of this treatment modality. It is

important for clinicians to continue to communicate openly on the success and failures with the emerging modality, under evidence-based (EBM) conditions.

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***The Role of Aquatic Therapy in Managing Equine Osteoarthritis*****Take Home Message**

Aquatic therapy has become increasingly popular for the rehabilitation of equine musculoskeletal injuries; unfortunately, there has been no scientific evaluation of its effectiveness for the treatment of OA and its associated alterations in musculoskeletal function in horses. The purpose of this review is to define the proposed mechanisms of action of aquatic therapy and to determine its efficacy in the treatment of equine OA.

**Introduction**

Osteoarthritis (OA) is one of the most debilitating musculoskeletal disorders among equine athletes.<sup>1</sup> It is a common cause of poor performance, early retirement, and reduced life expectancy. Medical and surgical management of equine associated lameness disorders cost more than \$700 million within the United States in 1998.<sup>2</sup> Osteoarthritis is a progressive disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and a progressive deterioration of articular cartilage.<sup>3</sup> The ensuing disease process affects not only the articular cartilage but also the surrounding articular tissues, including subchondral bone, joint capsule, synovial membrane, and periarticular soft tissues.<sup>3</sup> As joint disease progresses, characteristic pathologic changes occur including fibrosis and thickening of the joint capsule, articular cartilage fibrillation and erosion, and osteophyte formation, which all lead to functional impairments.<sup>3</sup> Unremitting joint pain and inflammation often cause adaptive muscle guarding and altered weight bearing to protect the affected limb from further discomfort and injury.<sup>4</sup> Compensatory muscular adaptations are characterized by inefficient muscle activity leading to muscle weakness, joint instability, and altered limb loading.<sup>5</sup> Maladaptive musculoskeletal responses may produce additional gait alterations and predispose other articulations to an increased risk of injury (i.e., compensatory lameness).<sup>6</sup> In humans, compensatory changes in posture and movement exacerbate the initial joint injury, which cause further alterations in limb biomechanics and contribute to the progression

of OA.<sup>5</sup> Similar compensatory mechanisms such as delayed muscle activation, muscle weakness, restricted joint range of motion, and a redistribution of limb loading are likely to also occur in horses.

Physical rehabilitation has become an effective treatment option for improving deficiencies and weaknesses associated with primary joint injuries, as well as reducing harmful compensatory gait abnormalities in humans.<sup>7</sup> Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Therapeutic aquatic interventions can be used to optimize the treatment of sensory and motor disturbances in order to achieve the functional restoration of full athletic performance. Aquatic therapies, such as underwater treadmill exercise have been reported in humans to increase cardiovascular endurance, improve muscle strength and timing, decrease limb edema, improve range of motion, decrease pain, and reduce mechanical stresses applied to the limb.<sup>8</sup> Exercising in water provides a medium in which the effects of increased buoyancy, hydrostatic pressure, and the viscosity of water along with the ability to alter both temperature and salinity combine to play an important role in musculoskeletal rehabilitation (Fig. 1). The increased resistance and buoyancy inherent in aquatic exercise increases joint stability and reduces weight bearing stress on muscles and joints.<sup>9-11</sup> The immersion of the distal limb in water applies a circumferential compression of equal magnitude that increases with depth below the surface of the water, increasing extravascular hydrostatic pressure, which in turn promotes circulation and reduces edema. Hydrotherapy can also aid in decreasing pain through the application of either cold or warmer water conditions. Warm water causes blood vessels to dilate increasing circulation and decreasing muscle spasms, while colder water acts to reduce inflammation by restricting blood flow. Aquatic conditions with higher solute concentrations provide an osmotic effect, which ultimately reduces edema and decreases pain. Aquatic therapy is a versatile treatment modality, capable of producing a

## Summaries: Focus 5

### *Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease*

wide variety of therapeutic effects and therefore is an effective method of rehabilitation for individuals with OA that have difficulty with weight bearing associated with land exercise<sup>12</sup> (Table 1).

#### *Effectiveness of aquatic therapy in human OA patients*

Rehabilitation protocols used to address OA co-morbidities often focus on improving muscle function and joint biomechanics.<sup>13-15</sup> Aquatic therapy is frequently prescribed for rehabilitation of orthopaedic injuries in humans, with the goal of improving the overall function of the affected limb and preventing further musculoskeletal injury.<sup>16</sup> Following aquatic rehabilitation, human patients have demonstrated normalized muscle activation patterns and improved joint stability, joint range of motion, and proprioception.<sup>7</sup> Joint replacement and arthroscopic surgery patients are also commonly referred postoperatively for aquatic therapy intervention. Aquatic exercise decreases the weight bearing stresses applied to the operated joint, which allows an earlier and more intensive but less painful rehabilitation program without risk of overloading the injured joint.<sup>17</sup> Human patients undergoing surgical reconstruction of their anterior cruciate ligament demonstrate improved knee range of motion and quadriceps strength following aquatic therapy, compared to traditional clinic based rehabilitation programs.<sup>18</sup>

#### *Aquatic therapy in dogs*

Although aquatic therapy is widely used in rehabilitation programs for humans; there are few investigations into the benefits of this form of exercise for equine patients. However, several studies on aquatic therapy have been conducted in dogs that primarily demonstrate significant improvements in joint range of motion following aquatic exercise. Aquatic therapy in dogs post cranial cruciate ligament reconstruction produces significant increases in joint range of motion, not only in the operated stifle, but also in the non-operated stifle.<sup>19</sup> A similar canine study demonstrated improved pelvic limb biomechanics, with no difference in peak vertical force or vertical impulse, as measured by force platform analysis between the repaired and contralateral limb at six-months follow up.<sup>20</sup> Kinematic analysis of dogs

walking in an underwater treadmill demonstrated that joint flexion is maximized when the depth of the water is maintained above the joint of interest.<sup>21</sup> Clinical outcome measures of thigh circumference and stifle joint range of motion were assessed in cranial cruciate ligament-deficient dogs after tibial plateau osteotomy.<sup>22</sup> Underwater treadmill exercise improved stifle passive joint range of motion and increased thigh circumference, compared to cage rest and controlled walking.<sup>22</sup> Six weeks after surgery, there was no difference in thigh circumference and joint range of motion between the affected and unaffected limbs in the aquatic therapy group.<sup>22</sup> In contrast, the cage rest and controlled walking group demonstrated continued progression in joint stiffness and muscle atrophy.<sup>22</sup>

#### *Aquatic therapy in horses*

Unlike the canine studies, equine investigations into aquatic therapy focus mainly on the horse's cardiovascular and respiratory responses to exercising in water.<sup>23-25</sup> Equine swim training programs report an improvement in cardiovascular function, a reduction in locomotor disease, and an increase in the development of fast-twitch, high-oxidative muscle fibers, reflecting improved aerobic capacity.<sup>26,27</sup> In addition, fine-wire EMG electrodes have been used to demonstrate the increased muscle intensity of the equine thoracic limbs during a pool swimming exercise program compared to overground walking.<sup>28</sup> A recent equine study assessed changes in stride parameters while walking in various depths of water.<sup>29</sup> Underwater treadmill walking with water at the level of the ulna resulted in a longer stride length with a reduced stride frequency, compared to walking in water at the level of the pastern joint.<sup>29</sup> To date there are no objective studies that have determined the ability of underwater treadmill exercise to improve muscle activation patterns or limb biomechanics in the horse. There are also no studies that have objectively assessed the effects of aquatic therapy on diminishing the progression of equine OA.

## Conclusion

Osteoarthritis in horses is typically managed with conventional therapies aimed at reducing

inflammation. There are a limited number of therapies that have demonstrated disease-modifying effects however; no one therapeutic agent has effectively eliminated the progression of joint disease. Any form of treatment that can retard the progression of OA is of great importance, both for continued athletic performance and for quality of life. From the human and canine literature and limited equine studies reviewed, aquatic therapy has a beneficial effect on pain reduction and related musculoskeletal outcome measurements. Similar results such as improving joint range of motion, motor control, and joint stability as well as decreasing pain and soft tissue swelling following aquatic therapy are expected to occur in our equine patients.

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## Summaries: Focus 5

### Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

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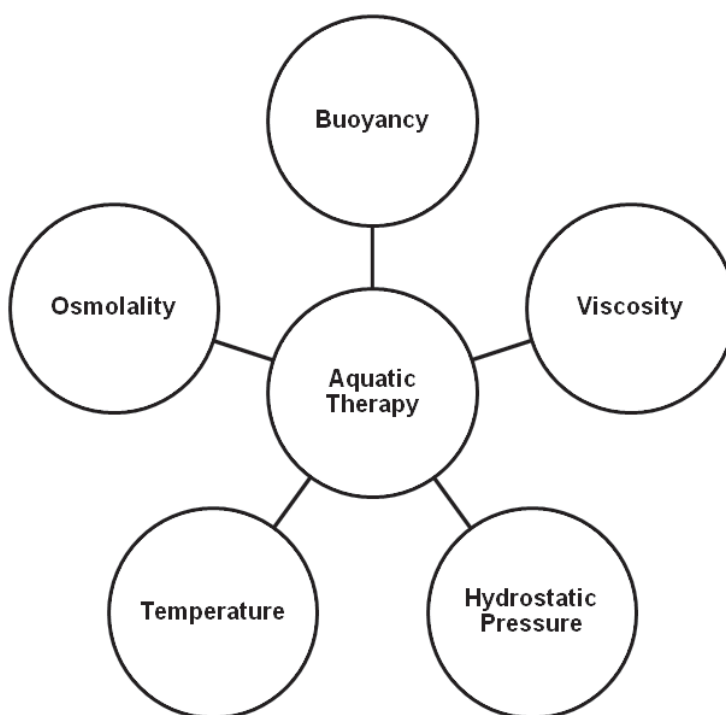


Figure 1: Graph illustrating the combined variables involved in aquatic therapy.



**Table 1.** Summary of the reported therapeutic effects of aquatic therapy.

<b>Aquatic Therapy Variables</b>	<b>Reported Benefits</b>
Buoyancy	<ul style="list-style-type: none"> <li>• Reduces weight bearing stresses on joint and soft tissue structures</li> <li>• Improves joint range of motion</li> </ul>
Viscosity	<ul style="list-style-type: none"> <li>• Increases muscle activity</li> <li>• Enhances neuromuscular control</li> </ul>
Hydrostatic Pressure	<ul style="list-style-type: none"> <li>• Reduces edema</li> <li>• Increases joint range of motion</li> <li>• Decreases pain</li> </ul>
Temperature	<ul style="list-style-type: none"> <li>• Increases soft tissue perfusion and lymphatic drainage (warm)</li> <li>• Reduces blood flow and decreases inflammation and pain (cold)</li> </ul>
Osmolality	<ul style="list-style-type: none"> <li>• Improves mechanical nociceptive thresholds</li> <li>• Reduces edema</li> </ul>

## Summaries: Focus 5

### *Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease*

#### **Use of an Equine Back Profiling System for Objectively Measuring Trunk Contours**

##### **Take Home Message**

Current saddle fitting methods do not provide a quantifiable or standardized method for assessing three-dimensional trunk morphometry. The Equine Back Profiling System (EBPS) was able to reliably capture a wide variety of dorsal trunk contours of horses ridden under English dressage or jumping saddles as well as Western saddles. The EBPS provides a significant advancement as we are now be able to better communicate findings or changes in dorsal trunk contours related to saddle fit to colleagues or owners and trainers.

##### **Introduction**

The goal of saddle makers and fitters is to match the size and shape of a specific saddle as closely as possible to an individual horse's dorsal trunk contour in an effort to maximize surface area and to provide uniform, low-pressure distribution patterns without limiting performance.<sup>1</sup> However, current saddle fitting methods do not provide a quantifiable or standardized method to assess three-dimensional morphometry, which means that saddle manufacturers are unable to produce a consistent product that is able match the wide variability present in different body types and trunk morphologies. Even after centuries of applying saddles to ridden horses, there is no established industry-wide standard for assessing the size or shape of a horse's trunk in an effort to improve saddle fit and reduce associated back pain.<sup>2</sup> Unfortunately, the majority of descriptions of equine trunk shape and size are nondescript and not clinically useful.<sup>3</sup> Morphometric features (i.e., height, length, depth, and width) of a horse's trunk can provide objective measures of body size or shape that are required to quantify specific segmental characteristics or general body conformations.<sup>4,5</sup> However, it continues to be difficult to capture and quantify surface contours, which are considered critically important for establishing uniformity between the contours of the ventral surface of the saddle and the dorsal surface of the trunk of the horse.<sup>6,7</sup>

The EBPS consists of a series of durable plastic cards with cut-out contours that are positioned at several predetermined anatomical sites along the dorsal trunk in an effort to provide an objective assessment of conformation and contours relevant to proper saddle construction and fitting. The cards are reportedly easy to use and provide a potential method for quantifying the dorsal trunk morphology across a wide-variety of body types and breeds of horses. The EBPS may provide a standardized method to quantify the shape of a horse's trunk, which would be a significant advancement in improving saddle construction, standardizing saddle fit, and reducing the prevalence of trunk pain in ridden horses. The objective of this study was to assess the ability of the EBPS to readily quantify the contours of the dorsal trunk in several breeds of horses used in different athletic disciplines.

This project was completed by Drs. Kevin Haussler, Ashley Hill, Wayne McIlwraith, and Chris Kawcak with Sierra L. Blauvelt and Jodi Callison at Colorado State University.

##### **Methods**

Each EBPS transverse card contained three or four cut-out contours that were able to capture a total of 33 different transverse contours of the dorsal trunk (Figure 1). The cards were placed transversely at six specified dorsal trunk locations that included the following sites: 1) widest portion of the withers, 2) highest point of the withers, 3) caudal edge of the scapula, 4) base of the withers (i.e., where the slope of the withers transitions into a more horizontal curvature of the trunk), 5) lowest point of the trunk, and 6) the thoracolumbar junction (Figure 2). These six vertebral locations were judged to be the minimum number of sites needed to optimally capture potential variations in the dorsal trunk contours across different breeds of horses using both English and Western saddles.

The EBPS also contains two cards used to capture the parasagittal contours of the dorsal trunk in the

region of typical Western saddle tree bar or English panel placement (Figure 3). The parasagittal cards were positioned 10-cm lateral to the dorsal midline of the trunk over the left and right epaxial musculature to assess bilateral longitudinal contours. Chi-squared analysis was used for nominal (unordered) data and Spearman Rank correlations was used for ordered and continuous data comparisons. Kruskal-Wallis one-way analysis of variance was used if one variable consisted of continuous data and post-hoc analysis was done using Bonferroni adjustment.

### Results

The EBPS readily quantified the dorsal trunk contours in these horses. The transverse contours at the widest point of the withers were mostly narrow and 35% (n=73) of cards were judged to have good fit ( $\leq 0.5$  cm). At the highest point of the withers, 39% (n=82) of transverse cards had a good fit. The widest and highest points of the withers were located at the same site in 64% (n=135) of horses. At the caudal aspect of the scapula, 89% (n=188) of transverse cards had a good fit and only 4% of regions had a gap height  $> 0.5$  cm. At the base of the withers, 77% (n=163) of cards had a good fit and 5% of regions had a gap height  $> 0.5$  cm. At the lowest point of the trunk, 97% (n=205) of cards had a good fit and 1% of regions within a card had a gap height  $> 0.5$  cm. At the thoracolumbar junction, 91% (n=191) of cards had a good fit and 2% of regions within a card had a gap height  $> 0.5$  cm. The transverse contour measurements at the three cranial vertebral locations (i.e., widest, highest and caudal scapula sites) were not significantly different from each other; however, the caudal three vertebral locations (i.e., base, lowest and thoracolumbar sites) were all significantly different from the cranial vertebral locations and each other.

Over the left epaxial musculature, 89% (n=187) of parasagittal cards had a good fit and 2% of quadrants had a gap height  $> 0.5$  cm. Over the right epaxial musculature, 92% (n=195) of cards had a good fit and 2% of quadrants had gap heights  $> 0.5$  cm. There were significant associations between the parasagittal card sizes and breed and athletic discipline. Warmbloods had flatter longitudinal contours and Morgans had

more epaxial muscle concavity. For use, hunter-jumpers had the least amount of longitudinal epaxial muscle concavity and Morgans had the most muscular concavity.

### Discussion

The objective of this study was to assess the ability of the EBPS to readily quantify the contours of the dorsal trunk in several breeds of horses used in different athletic disciplines. Using the three transverse locations and the left parasagittal site recommended by the EBPS manufacturer, the overall success rate was 92% as defined by overall gap heights  $\leq 0.5$  cm within a card. The parasagittal cards had similar high rate of success (89-92%) as did the transverse cards at the manufacturer's recommended sites for measuring dorsal trunk contours. Across all four recommended vertebral locations, there was a 72% percent chance that all four cards had a good fit with gap heights  $\leq 0.5$  cm within an individual horse. The EBPS was easy to use and was able to quantitatively capture subtle differences in trunk contours across horses of significantly different ages, wither heights, body weights, breeds, and uses of horses judged to have diverse trunk conformations. This suggests that the EBPS can be used to reliably capture the dorsal trunk contours in horses in an effort to improve saddle fit and construction in ridden horses.

### Acknowledgment

Supported in part (or total) by the PVM student grant program in the Center for Companion Animal Studies at Colorado State University.

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## Summaries: Focus 5

### Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

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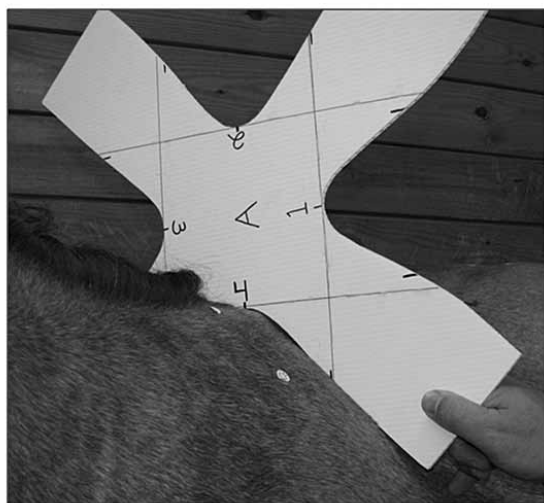


Figure 1.

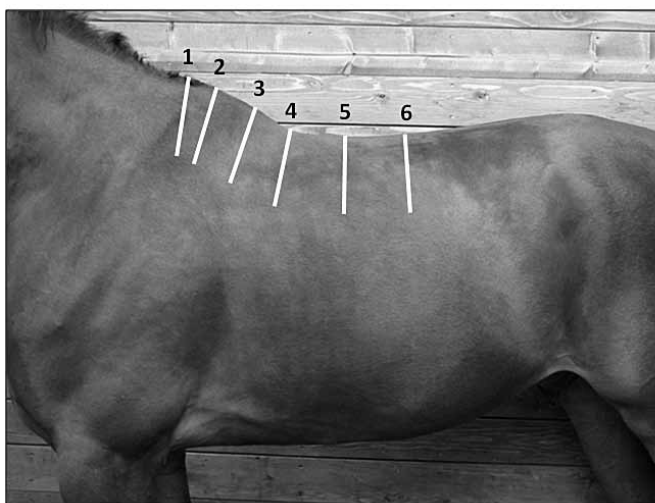


Figure 2.

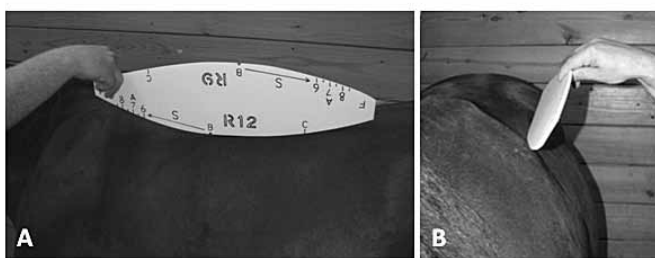


Figure 3.









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