ORTHOPAEDIC RESEARCH CENTER

2017-2018 REPORT

Colorado State University

MISSION

TO INVESTIGATE THE PATHOGENESIS, DIAGNOSIS, TREATMENT, AND PREVENTION OF MUSCULOSKELETAL DISEASE AND INJURY FOR THE BETTERMENT OF BOTH ANIMALS AND HUMANS.

PREFACE

"Our principal focus continues to be solving the significant problems in equine musculoskeletal disease"

It is my pleasure again to present our 2017-2018 report from the Musculoskeletal Research Program which consists of the Orthopaedic Research Center (including the Orthopaedic Bioengineering Research Laboratory), as well as, the Preclinical Surgical Research Laboratory and Orthopaedic Oncology at Colorado State University. At the Orthopaedic Research Center, 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 increase our investigation of comparative problems and questions relevant to human joint disease and joint injury including techniques and devices for human osteoarthritis and articular cartilage repair when the technique can potentially benefit the horse. The increased number of translational projects and funding support from the National Institute of Health (NIH) and human orientated industry partners support our mission of helping both horses and humans.

As part of that evolution in June 2017 we broke ground on the new Translational Medicine Institute (TMI) and on the 6th of May 2019 (it might be noted that I am writing this Preface sometime after the 2017-2018 years that we are reporting on!) we moved into the TMI. As some of you will remember, the TMI was initially described as the Institute of Biological Translational Therapies but people were having difficulty with this name and understanding what we did. In March 2016 we completed the matching challenge from our lead donors John and Leslie Malone of one half the cost of the building with a $10 million commitment from Colorado State University (per then President Dr. Tony Frank) and $20 million from Abigail K. Kawananakoa and moved forward quickly from then.

As I write this preface for our 2017- 2018, we have settled into the TMI. It is an incredible facility that is providing the unique abilities for research, education and entrepreneurial development of biologic therapies. The mission of the TMI is to improve the lives of animals and humans through biologic therapies created via the collaborative work of leading scientists and clinicians; to expedite the availability of these therapies, and to promote education related to these goals. Our vision is, leading the way in discovery and implementation of the body's therapeutics to improve the lives of animals and their humans. This vision that was developed by a TMI Steering Committee led by Dr. Dave Frisbie and supported by our lead donors John and Leslie Malone and by our matching donor Abigail K. Kawananakoa has a focus on investigating the next generation remedies based on living cells and their products including patient derived stem cells to treat musculoskeletal disease and other ailments and to literally be able to carry basic science discoveries in the TMI all the way to bedside with entrepreneurial and regulatory abilities within the TMI.

The work and accomplishments of the excellent team we developed at the Orthopaedic Research Center (ORC) led to this vision and the Institute. The expertise we established in analyzing and developing medical treatments for animal patients, and then providing knowledge gained to boost

human medical advancements, goes along with the concept of translational medicine and is successful because of similarities in animal and human physiology and disease. The funding and building of the TMI in a two- and one-half year period is truly transformational and will certainly take us to a higher level. It is also an endorsement of what we have achieved already. Those achievements have come from a combination of ingenuity and work of our faculty, research associates, graduate students, veterinary students and undergraduate students as well as the critical help of our donors.

Our continued evolution has been greatly strengthened by the addition of other principal investigators and programs to the ORC and Orthopaedic Bioengineering including, the Preclinical Surgical Research Laboratory led by co-directors Jeremiah Easley and Howard Simon as well as the programs of Drs. Steve Dow and Mike Lappin. These additions have brought additional talent along with postdoctoral researchers, graduate students and research associates. Each of our components continue to grow. The Orthopaedic Research Center will continue to be a major component of the TMI, but our other partners give us considerable strength and Reports such as this will continue to evolve as one major entity. I would like to acknowledge our partners Tetrad, the developers for the building together with their partners Clark Ennison Architects and JE Dunn the contractors. Dr. Dave Frisbie worked closely with Tetrad in all aspects of the building and what we have in this remarkable facility. Dave deserves much credit for what we have in this facility in terms of appropriateness of what we have as he and, as mentioned previously the TMI Steering Committee was also critical to the process. We continue to evolve as a facility particularly with the large step up in continuing education (CE), and imaging, as well as research. Achieving what we envisioned with the TMI is continuing to evolve with the Scientific Advisory Board (SAB) selecting projects for support based on their potential to find new therapies and advances.

Two other major items that are critical to achieving our goals in advanced equine health are the Gail Holmes Equine Orthopaedic Research Center transitioning to a Sports Medicine and Rehabilitation Center under the leadership of Dr. Melissa King as well as funding of phase 1 of a new Equine Veterinary Teaching Hospital under the leadership of, Director of Equine Clinical Services, Dr. Chris Kawcak. Currently our Transitional Leadership Team is working carefully to evaluate what we need for optimal progress in the TMI. The step up in both facility and management of our added programs, has been a learning curve as one continues to grow, I have handed over my leadership roles and have the pleasure of looking back on 39 years of progress that would not have been possible without the terrific support of the faculty and staff as well as the support of our research funders and donors.

Best wishes,

Wayne McIlwraith

CONTENTS

FOCUS 5

VALIDATION OF REHABILITATION AND PHYSICAL THERAPY TECHNIQUES FOR MUSCULOSKELETAL DISEASE

RESEARCH FOCUSES OF THE ORTHOPAEDIC RESEARCH CENTER

Including the Orthopaedic Bioengineering Research Laboratory

MUSCULOSKELETAL TISSUE HEALING

This focus addresses articular cartilage, tendon, ligament, and menisci healing.

EARLY DIAGNOSIS OF MUSCULOSKELETAL DISEASE

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 MUSCULOSKELETAL DISEASE (INCLUDING NEW MODELS)

These investigations use 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

This focus includes evaluation of biologic inhibitors of critical mediators in joint disease, novel protein therapies, including platelet-rich plasma (PRP), gene therapy techniques, and mesenchymal stem cell therapies.

VALIDATION OF REHABILITATION AND PHYSICAL THERAPY TECHNIQUES FOR MUSCULOSKELETAL DISEASE

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.

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, renewed in 2008, 2012, and again in 2014).

THE MUSCULOSKELETAL RESEARCH PROGRAM COVERS ALL ORTHOPAEDIC RESEARCH AT COLORADO STATE UNIVERSITY AND INCLUDES:

1. Orthopaedic Research Center, including Orthopaedic Bioengineering Research Laboratory

2. Preclinical Surgical Research Laboratory

3. Orthopaedic Oncology

SCHOOL OF BIOMEDICAL ENGINEERING

Most of the faculty within the Musculoskeletal Research Program are also faculty in the 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. Drs. Christian Puttlitz, Tammy Donahue, Wayne McIlwraith, David Frisbie, Chris Kawcak, Seth Donahue, Laurie Goodrich, Kevin Haussler, Kirk McGilvray 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 bachelor of science (B.S.), master of engineering (M.E.), master of science (M.S.), and doctor of philosophy (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.

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1

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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 American College Veterinary Surgeons, Diplomate European College Veterinary Surgeons, Diplomate American College Veterinary Sports Medicine and Rehabilitation, University Distinguished Professor, Barbara Cox Anthony University Chair in Orthopaedics, Director of Musculoskeletal Research Program; Department of Clinical Sciences

Research Interests: Equine orthopaedic surgery and joint disease (arthritis), musculoskeletal biomarkers, cartilage repair and novel biologic treatments including stem cells

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 is 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 co-author of five textbooks: Techniques in Large Animal Surgery (two editions): Equine Surgery: Advanced Techniques (two editions); Arthroscopic Surgery in the Horse (four editions); Joint Disease in the Horse (second edition just published); and Equine Welfare. He has authored or co-authored over 450 refereed publications and textbook chapters, and has presented more than 600 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 U.K. 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; Dr. vet. med. (honoris causa), Royal Veterinary College, University of London, 2010; Life Member, New Zealand Equine Veterinary Association, 2011; Jacob Markowitz Award, Academy of Surgical Research, 2013; Marshall R. Urist M.D. Award for Excellence in Tissue Regeneration Research, Orthopaedic Research Society, 2014; American Association Equine Practitioners Distinguished Service Award, 2014.

Myra Barrett

D.V.M., M.S., Diplomate ACVR, Assistant Professor of Radiology, Department of Environmental and Radiological Health Sciences

Research Interests: Equine musculoskeletal imaging and comparative imaging

Dr. Barrett earned her D.V.M. from Colorado State University. After graduating, she completed a year-long internship at Oakridge Equine Hospital in Edmond, Okla.

Dr. Barrett underwent a non-conforming radiology residency in order to particularly focus on equine diagnostic imaging. The residency was based at CSU, but included training with multiple equine imaging experts in the U.S. and internationally. At the same time, Dr. Barrett obtained a master's degree through the ORC. She remained at CSU and is currently an assistant professor of radiology. Dr. Barrett works closely with the Equine Surgery and Sports Medicine services. She has spoken at multiple large national meetings and is regularly involved in continuing education courses. Dr. Barrett is dedicated to the advancement of the specialty of equine diagnostic imaging and is currently the president-elect of the Large Animal Diagnostic Imaging Society, a subgroup of the American College of Veterinary Radiology.

Erin Contino

D.V.M., M.S., Diplomate American College Veterinary Sports Medicine and Rehabilitation, Assistant Professor, Department of Clinical Sciences

Research Interests: Equine musculoskeletal imaging, diagnostic analgesia, lameness, and performance issues in equine athletes

Dr. Erin Contino joined our faculty in 2014 as a Fellow in Equine Imaging and as a Clinical Instructor in Equine Sports Medicine. She was promoted to Assistant Professor of Equine Sports Medicine and Rehabilitation in 2015. Erin graduated with a D.V.M. from Colorado State University in 2010 and completed a 1-year internship at Pioneer Equine Hospital in California. She then returned to CSU for a three-year Sports Medicine and Rehabilitation Residency and became a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation in 2014. Before and during her time as a D.V.M. student, Erin also completed a Master's Degree in Equine Radiology at the Orthopaedic Research Center. She is a passionate 3-day event rider.

Nicole Ehrhart

D.V.M., M.S., Diplomate ACVS, Professor, Ross M. Wilkins, M.D. Limb Preservation University Chair in Musculoskeletal Oncology and Biology; Department of Clinical Sciences

Research Interests: Stem cell therapy, tissue engineering, guided bone regeneration, allograft healing, limb preservation, bone substitutes

Dr. Ehrhart is one of 30 fellowship-trained veterinary surgical oncologists in the world. She is a full 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 Laboratory of Comparative Musculoskeletal Oncology and Traumatology and has been actively involved in limb preservation research, regenerative medicine, tissue engineering, and sarcoma research for the last sixteen years. She has been an invited speaker at various venues for MD researchers in translational research, both nationally and internationally.

She holds joint faculty positions in the School of Biomedical Engineering, the Cell and Molecular Biology program, the Gates Regenerative Medicine Center at the University of Colorado, and The University of Colorado Cancer Center. In addition to her research, she has held several prestigious positions in the American College of Veterinary Surgeons (Scientific Program Chair, Residents Forum Chair, and Examination Committee) and Veterinary Orthopedic Society (President). She has authored numerous publications on limb preservation and translational cancer research. She is currently the director of the Musculoskeletal Oncology section of the University-wide Cancer Supercluster.

David D. Frisbie

D.V.M., M.S., Ph.D., Diplomate American College of Veterinary Surgeons, Diplomate American College of Veterinary Sports Medicine and Rehabilitation, Professor, Director of Research, Orthopaedic Research Center, Interim Director of Operations, Translational Medicine Institute; Department of Clinical Sciences

Research Interests: Treatment and diagnosis of musculoskeletal disease with an emphasis on biologics.

Dr. Frisbie began his professional career after obtaining both a B.A. in biochemistry and a 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 CSU, 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 Equine Surgery in the Department of Clinical Sciences in 1999, was promoted to associate professor (with tenure) in 2007, and then to professor in 2013. He is also a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation and a Founding Fellow of ACVS Minimally Invasive Surgery (large animal orthopaedics). Dr. Frisbie has served on the American Association of Equine Practitioners Board of Directors as well as held the position of Secretary on the Board of Directors for the American College of Sports Medicine and Rehabilitation. His current areas of research include musculoskeletal diagnosis and treatment. He has evaluated the therapeutics such as Adequan, corticosteroids (Vetalog and Depo-Medrol), Orthokine (IRAP) and other biologics such as stem cells. As well as looking at novel platforms for diagnosing musculoskeletal disease such as joint and tendon issues and he has developed other diagnostic tools such as standing arthroscopy of the equine stifle.

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., Diplomate ACVS, 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, Va. 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. Since commencing her position at CSU, Dr. Goodrich has focused on gene therapy and regenerative medicine for musculoskeletal disease in joint and bone repair. Specifically, her main focuses have included using IGF-I, IL-1ra, and BMP gene therapy to enhance cartilage repair, reduce inflammation in osteoarthritis, and improve bone repair, respectively. Further, she has investigated stem cell therapy applications for enhancement of cartilage repair. She is now a Professor in equine surgery and lameness. Dr. Goodrich's clinical interests include arthroscopy, joint disease, fracture repair, lameness and pain management.

Honors include: Orthopaedic Research Society, New Investigator Research Award, Semi-Finalist, 2006; Recipient five-year NIH KO8 Training Grant Award, 2008-2013; Clinician of the Year Award for Teaching Excellence, 2011; Elastikon Equine Research Award, 2011, AOSSM Cabaud Research Award, 2017.

Kevin K. Haussler

D.V.M., D.C., Ph.D., Diplomate American College of Veterinary Sports Medicine and Rehabilitation, Associate 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 B.S. 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. 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 associate professor with continued research interests in objective assessment of musculoskeletal pain and spinal dysfunction, and evaluation of rehabilitation approaches in horses.

Christopher E. Kawcak

D.V.M., Ph.D., Diplomate ACVS, Diplomate American College of Veterinary Sports Medicine and Rehabilitation, Professor, Iron Rose University 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 a Professor in the Iron Rose Ranch Chair in the ORC, and is Director of Equine Clinical Services in the James L. Voss Veterinary Teaching Hospital. His collaborations with the Biomedical Engineering Program at CSU, the Southwest Research Institute in San Antonio, Texas, The I-STAR Laboratory at Johns Hopkins University, 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 the effects of exercise on the incidence of musculoskeletal injury, the development of computerized models of joints and joint diseases, and development of a new standing computed tomography machine for 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, the American College of Veterinary Surgeons, and the American College of Veterinary Sports Medicine and Rehabilitation.

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.

Melissa King

D.V.M., Ph.D., Diplomate American College of Veterinary Sports Medicine and Rehabilitation, Assistant Professor, Department of Clinical Sciences; Lead Clinician, Equine Sports Medicine and Rehabilitation Service

Research Interests: Equine sports medicine and rehabilitation

Dr. Melissa King received her D.V.M. from CSU in 1997 and then completed an internship at Rood & Riddle Equine Hospital in Lexington, Ky. Upon completion of her internship, Dr. King returned to northern Colorado to begin her career as an equine ambulatory clinician focusing on equine sports medicine. In 2011, Dr. King completed a Ph.D. at the ORC assessing the efficacy of underwater treadmill exercise to diminish the progression of carpal osteoarthritis. Currently, Dr. King is an assistant professor and the lead clinician for the Equine Sports Medicine and Rehabilitation Service at CSU. Dr. King is actively involved in clinical research to advance the quality and effectiveness of rehabilitation for the equine athlete.

John Kisiday

Ph.D., Associate Professor, Department of Clinical Sciences

Research Interests: Mesenchymal stem cell chondrogenesis; cellular therapies for treating orthopaedic injuries

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. He is now an associate professor in Clinical Sciences. Dr. Kisiday is currently involved with research projects evaluating the potential of bone marrow mesenchymal stem cells to heal orthopaedic injuries, with an emphasis on cartilage repair. He has collaborated with ORC faculty to bring autologous mesenchymal stem cell treatments to the clinic. In the laboratory, he is investigating factors that influence mesenchymal stem cell differentiation with the goal of increasing the effectiveness of clinical treatments.

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

Valerie Moorman

D.V.M., Ph.D., Diplomate ACVS, Assistant Professor, Equine Surgery and Lameness

Research Interests: Early detection of musculoskeletal injury and methods of quantitative lameness detection

Valerie Moorman graduated from North Carolina State University with a B.S. in Animal Science in 2000. She graduated from North Carolina State University College of Veterinary Medicine in 2004. She then completed an internship in large animal medicine and surgery at Auburn University in June 2005 and continued as a large animal ambulatory clinical instructor through June 2006. She then completed a combined equine surgery residency and master's program at Oklahoma State University in July 2009. She became a Diplomate of the American College of Veterinary Surgeons in March 2010, and in July 2009, she began a Ph.D. program at the Orthopaedic Research Center at CSU, where she worked to develop a hoof-mounted motion analysis system. From July 2009 until June 2012, she also provided afterhours surgical emergency coverage at the CSU James L. Voss Veterinary Teaching Hospital. From July 2012 until July 2013, she served as staff veterinarian at the ORC. In July 2013, she was named an Assistant Professor of Equine Surgery and Lameness in the Department of Clinical Sciences at Colorado State University.

Kelly Santangelo

D.V.M., Ph.D., Diplomate ACVP Assistant Professor, Department of Microbiology, Immunology, and Pathology

Research Interests: Cartilage biology, osteoarthritis (OA) pathogenesis, rodent models of primary and post-traumatic OA

Following completion of a doctoral degree in veterinary medicine from Cornell University, Dr. Santangelo completed an equine surgery and anesthesia fellowship at a top referral hospital in Ohio. Her next educational phase focused her efforts on achieving a Ph.D. in comparative and translational medicine at The Ohio State University. This work predominantly revolved around pre-clinical, clinical, and industry-sponsored studies that focused on musculoskeletal disorders, including bone fracture healing, tendinopathies, and arthropathies. Dr. Santangelo was then awarded an NIH F32 NRSA Post-Doctoral Fellowship to investigate the role of interleukin-1β mediated signaling in a guinea pig model of spontaneous osteoarthritis. She subsequently received a competitive GlaxoSmithKline and ACVP/STP Coalition Award to fund a veterinary pathology residency combined with pharmaceutical industry exposure. This latter experience focused on all aspects of proprietary high through-put drug development and screening, and has molded her scientific perspective to include industry-inspired research and business tactics. Hired as an Assistant Professor at Colorado State University in July of 2013, she currently has a predominantly research appointment while actively maintaining high clinical service and teaching commitments. Dr. Santangelo's long-term professional goal is to systematically characterize molecular factors that contribute to the generation and progression of OA and identify novel treatment options. Her research utilizes a multi-disciplinary approach to medical science, which integrates molecular techniques, high resolution imaging, and computer-aided gait analyses to provide a comprehensive depiction of OA in multiple species. Dr. Santangelo is also Co-Director of the Experimental Pathology Facility at CSU, an emerging core focused on providing anatomic and clinical pathology support to local and national researchers.

Honors Include: NIH F32 NRSA, 2006; PEO International Foundation – Scholar Award for Women, 2009; GlaxoSmithKline/ACVP/STP Coalition Training Award for Residency in Veterinary Pathology, 2009; AVMA and Merck-Merial – Young Investigator Award, 2009; ACVP Pathology Resident of the Year, 2011; OARSI World Congress – Top Abstract and Plenary Talk, 2017; Boettcher Foundation – Webb-Warring Biomedical Research Award, 2017

Katie Seabaugh

D.V.M., M.S., Diplomate American College of Veterinary Surgeons, Diplomate American College Veterinary Sports Medicine and Rehabilitation, Assistant Professor, Department of Clinical Sciences, Staff Veterinarian, Equine Orthopaedic Research Center

Research Interests: Equine lameness, intra-articular therapies and diagnostic analgesia.

Dr. Seabaugh joined our faculty in 2016. She obtained her Doctorate in veterinary medicine from Washington State University in 2007. The following year she completed an internship at a specialty equine referral practice in Oakdale, California. Following the path she set for herself, she obtained and completed a large animal surgical residency at Colorado State University and subsequent board certification in the American College of Veterinary Surgeons in 2013. Also, in 2013 she took a faculty position at the University of Georgia and began pursuing board certification in the American College of Veterinary Sports Medicine and Rehabilitation. She achieved this certification in January 2015. She is joined in Fort Collins by her husband, who is an equine radiologist and faculty member at CSU. Together they have two kids, Beckett and Calder, two dogs, a cat and a horse.

Kurt Selberg

M.S., D.V.M., M.S., Diplomate American College of Veterinary Radiology, Equine Diagnostic Imaging

Dr. Selberg received his training in diagnostic imaging from Colorado State University and is a Diplomate of the American College of Veterinary Radiologists. Following his residency, he completed fellowship in advanced imaging with training from Colorado State University and from Musculoskeletal Radiologists in Fort Collins, CO. He accepted a position in equine diagnostic imaging at the University of Georgia for 4 years before returning to Colorado State University in September of 2016. Most recently, he was the on-site imaging consultant for the 2018 World Equestrian Games. He is also an FEI treating veterinarian. Aside from equine radiology, he also enjoys skiing, jiu jitsu, spending time with good friends and family, his lovely wife Katie and two children, and two yellow dogs.

Richard Slayden

Ph.D., Associate Professor of Microbiology, Executive Director and founding member of the Center for Environmental Medicine at CSU

Dr. Slayden has 14 years of drug discovery and genomics experience with bacterial pathogens (F. tularensis, Burkholderia pseudomallei, Y. pestis, M. tuberculosis) and mouse models of infection. In the last several years, Dr. Slayden has employed Next Generation Sequencing techniques and metagenomics strategies to perform systems-based transcriptional studies to investigate molecular marks and metabolic tendencies of complex biological systems, including animal models of infection. During this time, Dr. Slayden has formed multi-disciplinary collaborations in the areas of microbiology, infectious disease, mathematics, and computational modeling to study host-pathogen interactions. Using this approach, Dr. Slayden has successfully characterized the host response to different infections and the unique in vivo transcriptional patterns and metabolism of bacterial pathogens.

Melinda Story

D.V.M., Diplomate ACVS, Diplomate American College of Veterinary Sports Medicine and Rehabilitation, Assistant Professor, Department of Clinical Sciences

Research interests: Assessment and treatment of axial skeletal dysfunction and pain; clinical research interest in the areas of acupuncture and chiropractic therapy

Dr. Melinda Story is a native of Colorado and joined CSU's Equine Sports Medicine team in 2013. She earned her B.S. in microbiology from CSU, and following a year at Texas A&M University in biomedical research, Dr. Story returned to CSU to obtain her D.V.M. in 1999. She completed an internship at Rood and Riddle Equine Hospital in Lexington, Kentucky. She then completed her residency training program in equine surgery at Kansas State University and became a diplomate of the American College of Veterinary Surgeons in 2004. Dr. Story joined the staff at Littleton Equine Medical Center with interests in surgery and sport horse lameness. In 2006, Dr. Story became certified in Veterinary Medical Acupuncture, and in 2011, she became certified by the International Veterinary Chiropractic Association. Dr. Story became a diplomate of the American College of Veterinary Sports Medicine and Rehabilitation in 2014

Susan P. James

Ph.D., Professor and Head, Department of Mechanical Engineering; Professor, School of Biomedical Engineering

Research Interests: Biomaterials for orthopaedic, cardiovascular, and ocular applications, including permanent implants and tissue engineering

Dr. Susan James joined the CSU Mechanical Engineering faculty in 1994 as an assistant professor. She is now the Head of Mechanical Engineering Department at CSU, and was the founding director of the School of Biomedical Engineering. She received her Ph.D. in polymers from MIT and her B.S. in metallurgical engineering and materials science from Carnegie Mellon. Professor James' research focuses on characterization and development of biomaterial solutions to health care problems. These include orthopaedic, cardiovascular, and ocular applications, as well as regenerative medicine and tissue engineering. She and her students invented the BioPoly® materials, now in clinical use in partial resurfacing knee implants (http://www.biopolyortho.com/). Much of her current work is on hyaluronan-enhanced plastics, which do not cause blood clotting and platelet activation like most synthetic plastics. In collaboration with several faculty, students, and researchers, she is working on developing hyaluronan-enhanced flexible leaflets for heart valve prostheses. Her group is also researching new materials for small diameter vascular grafts, and contact and intraocular lenses. Dr. James is committed to giving back and has been involved with many organizations over the years, including Africa Higher Education Partnerships (AAHEP), Women and Minorities in Engineering Program (WMEP), and SWE. She has also performed countless outreach programs for young girls to get them interested in engineering careers. Dr. James was awarded the prestigious Margaret Hazaleus award this year for her strong commitment to mentoring and helping women.

Kirk McGilvray

Ph.D., Colorado State University

Dr. Kirk McGilvray is currently working as an Assistant Research Professor and severs as one of the Principal Investigators (PI) at the Orthopaedic Bioengineering Research Laboratory (OBRL). He is a Colorado native and received his B.S., M.S., Ph.D., and Post-doctoral education at CSU. His research efforts focus on comparative animal studies investigating pathways to enhance both soft tissue and bone healing following surgical intervention or trauma. He is also responsible for directing much of the day-to-day operations within the biomechanical testing center at the OBRL, which includes mentoring students in research techniques. Kirk's overreaching goals are to develop advance in vitro and in vivo measurement techniques that can be used to assess biological tissue in both its normal and diseased states.

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 and his team have global interests in how engineering mechanics can be applied towards solving orthopaedic related problems, including both experimental and computational modeling to better under-stand the underlying tissue-level mechanobiology. Dr. Puttlitz and his colleagues have leveraged well-known orthopaedic hardware systems to functionally isolate the ovine metatarsus to develop a Haversian bone model of microgravity. The model will be used to simulate the fracture healing cascade that is expected to occur during deep space flight. In addition, the model will be used as an evaluation platform for emerging technologies that seek to enhance fracture healing in microgravity environments. These experiments are complemented by a computational effort that merges musculoskeletal and finite element models of the ovine hindlimb in an attempt to span numerous length scales and relate the observed biological response to the localized (i.e., tissue-level) mechanics. Dr. Puttlitz received his B.S. in material science and engineering mechanics from Michigan State University, his M.S. in bioengineering from Clemson University, and his Ph.D. in biomedical engineering from the University of Iowa. Dr. Puttlitz became a Postdoctoral Fellow in the Orthopaedic Bioengineering Research Laboratory at the University of California, San Francisco. He joined the Department of Orthopaedic Surgery faculty at UCSF as an assistant professor in 2001, and directed the Orthopaedic Biomechanics Laboratory at the San Francisco General Hospital. In 2005, he accepted a faculty position at CSU in the Department of Mechanical Engineering and is currently appointed as an associate professor. He also holds secondary appointments in the School of Biomedical Engineering and the Department of Clinical Sciences.

Honors include: Monfort Professorship, May 2011; 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.

Raoul F. Reiser, II

Ph.D., Associate Professor, Department of Health and Exercise Science

Research Interest: Musculoskeletal biomechanics

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 CSU. The emphasis of his dissertation was the biomechanics of recumbent 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, and was promoted to associate professor with tenure in 2008. His current research is mainly associated with the foot-surface interface, understanding how the surface and athlete interact and implications on injury risk and performance. This research not only includes humans, but also horses. He also continues to explore bilateral asymmetries of the lower extremities and how they relate to both injury risk and performance.

Honors include: Elected Fellow, American College of Sports Medicine, 2007; CSU College of Engineering's Outstanding Research Assistant, 2000; GAANN Three-Year Fellowship, 1997; CSU Graduate Fellowship, 1997; NSCA Challenge Scholarship, 1996.

Katie Sikes

Ph.D.

Dr. Katie Sikes received her Bachelor of Science in Biomedical Engineering from Rose-Hulman Institute of Technology in 2011 and her Doctor of Philosophy in Bioengineering from the University of Illinois at Chicago in 2016. As a Postdoctoral Fellow with Dr. Dave Frisbie at the Orthopaedic Research Center, Dr. Sikes utilizes a multi-discipline approach to study animal models of musculoskeletal disease, primarily a murine model of tendinopathy and rat model of myotendinous injury, where molecular analyses can be correlated with structural and functional properties to assess full-scale disease progression and healing.

Aimee Colbath

V.M.D. (University of Pennsylvania), M.S. (Colorado State University)

Dr. Aimee Colbath graduated from the University of Pennsylvania School of Veterinary Medicine in 2010 and became interested in stem cell research and biologic therapies during her general large animal internship at the University of Georgia, where she worked in Dr. Peroni's research laboratory. She then moved on to a surgical internship at the Tufts Cummings School of Veterinary Medicine, where she worked in the regenerative medicine laboratory studying the effects of shipping on stem cells. In 2015, Aimee earned her master's in clinical sciences and completed her surgical residency with CSU. In July of 2015, she began her Ph.D. in clinical sciences where she works closely with both the ORC and the Stem Cell and Regenerative Medicine Laboratory in the Animal Cancer Center. Since joining CSU, her research focus has been on the immunomodulatory effects of equine stem cells. In addition, Aimee has be-gun working with induced pluripotent stem cells (iPSC) and induced mesenchymal stem cells (iMSCs). In 2015, Dr. Colbath received the Grayson Jockey Club Career Development Award and an American Association of Equine Practitioners Research Fellowship Award.

Jodie Daglish

B.V.Sc, MRCVS

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

Katie Ellis

D.V.M.

Dr. Katie Ellis joined the Equine Sports Medicine and Rehabilitation residency program in July 2017 having finished a one-year Equine Diagnostic Imaging Internship with Dr. Myra Barrett and Dr. Kurt Selberg here at CSU. Dr. Ellis graduated from the University of Georgia College of Veterinary Medicine in 2009 and then went on to complete a Large Animal rotating internship, also at the University of Georgia in 2010. Dr. Ellis then worked as an equine ambulatory practitioner for Jacksonville Equine Associates until 2015. During that time, she became certified in veterinary acupuncture and chiropractics. She then completed an Equine Surgery and Sports Medicine internship at the University of Florida prior to coming to CSU.

Sherry Johnson

D.V.M.

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

Frances Peat

B.V.Sc

Dr. Peat joined the Equine Sports Medicine and Rehabilitation Services residency program in July 2013. She is the fifth resident in our program that remains unique as the only residency in Equine Sports Medicine and Rehabilitation. Dr. Peat is from New Zealand and received her veterinary degree (B.V.Sc.) from Massey University. She has also done a postgraduate clinical diploma at Massey and was in practice for five years at one of the leading equine practices in New Zealand, Matamata Veterinary Services.

Gustavo Miranda Zanotto

D.V.M., M.Sc.

Dr. Gustavo Zanotto is originally from Curitiba, Brazil, where he received a D.V.M. from Parana Federal University in 2007. Gustavo then moved to Sao Paulo where he completed a residency in large animal internal medicine and surgery, and received a master's degree in veterinary surgery at Sao Paulo University. For his master's degree, Gustavo evaluated chitosan hydrogel as a scaffold for equine stem cells. The main objective of this study was to improve the tissue engineering techniques for repair of osteochondral defects. From 2010 to 2013, Gustavo was an assistant professor of large animal internal medicine and surgery at Anhanguera Educational School of Veterinary Medicine. Currently, Gustavo is a visiting researcher at the ORC working with Dr. David Frisbie on a project to compare the freeze-dried and fresh platelet-rich plasma in injured tendon explants. Additionally, Gustavo is doing an internship with CSU's Veterinary Diagnostic Imaging Service focusing on equine musculoskeletal imaging under the supervision of Dr. Myra Barrett-Frisbie.

Alyssa Ball

M.S.

Alyssa graduated from CSU in 2013 with a B.S. degree in biochemistry and started her M.S. graduate program in the fall of 2013 under the direction of Dr. Laurie Goodrich. In 2014, Dr. Goodrich and Alyssa received CRC funding to explore the use of genetically modified stem cells in equine fracture repair. In 2015, Alyssa received a NIH-T32 Fellowship allowing her to take a year off of veterinary school and complete the final year of her master's. Alyssa returned to veterinary school at CSU in the fall of 2016. After completing veterinary school, Alyssa started a Ph.D. pursuing equine musculoskeletal research.

Aimee Colbath

V.M.D. (University of Pennsylvania), M.S. (Colorado State University)

Dr. Aimee Colbath graduated from the University of Pennsylvania School of Veterinary Medicine in 2010 and became interested in stem cell research and biologic therapies during her general large animal internship at the University of Georgia, where she worked in Dr. Peroni's research laboratory. She then moved on to a surgical internship at the Tufts Cummings School of Veterinary Medicine, where she worked in the regenerative medicine laboratory studying the effects of shipping on stem cells. In 2015, Aimee earned her master's in clinical sciences and completed her surgical residency with CSU. In July of 2015, she began her Ph.D. in clinical sciences where she works closely with both the ORC and the Stem Cell and Regenerative Medicine Laboratory in the Animal Cancer Center. Since joining CSU, her research focus has been on the immunomodulatory effects of equine stem cells. In addition, Aimee has begun working with induced pluripotent stem cells (iPSC) and induced mesenchymal stem cells (iMSCs). In 2015, Dr. Colbath received the Grayson Jockey Club Career Development Award and an American Association of Equine Practitioners Research Fellowship Award.

Jimmy Johnson

B.S.

Jimmy Johnson started in the Mechanical Engineering Ph.D. program at Colorado State University in 2017. His research focuses on understanding chronic rotator cuff degeneration in humans and generating ovine models that accurately emulates those changes. He received his Bachelors of Science in Mechanical Engineering from University of Wisconsin – Madison in 2014.

Gerardo Narez

B.S., Bioengineering, University of California, San Diego

Gerardo is currently working towards a Ph.D. in biomedical engineering under the guidance of Dr. Tammy Haut Donahue. His major area of study is testing the efficacy of drugs in orthopedic tissues of the knees, particularly the meniscus. The goal of this research is to couple the drugs with ACL reconstruction surgery to delay the progression of osteoarthritis in patients who have suffered of an ACL tear. He was awarded with the National Science Foundation Bridge to the Doctorate Fellowship to pursue his studies at CSU.

Holly Stewart

D.V.M.

Dr. Holly Stewart started in a Ph.D. program at the ORC in 2016. Holly graduated from the University of Pennsylvania School of Veterinary Medicine in 2012, and then completed an equine internship at Pioneer Equine Hospital in California, followed by a residency in large animal surgery at University of Pennsylvania's New Bolton Center. Holly's Ph.D. research focuses on application and optimization of computed tomography for assessment of equine bone injury, including detection of bone marrow edema. She is also part of the team that runs the cone-beam computed tomographic scanner for evaluation of clinical cases at the Veterinary Teaching Hospital.

Gustavo Miranda Zanotto

D.V.M., M.Sc.

Dr. Gustavo Zanotto is originally from Curitiba, Brazil, where he received a D.V.M. from Parana Federal University in 2007. Gustavo then moved to Sao Paulo where he completed a residency in large animal internal medicine and surgery, and received a master's degree in veterinary surgery at Sao Paulo University. For his master's degree, Gustavo evaluated chitosan hydrogel as a scaffold for equine stem cells. The main objective of this study was to improve the tissue engineering techniques for repair of osteochondral defects. From 2010 to 2013, Gustavo was an assistant professor of large animal internal medicine and surgery at Anhanguera Educational School of Veterinary Medicine. Currently, Gustavo is a visiting researcher at the ORC working with Dr. David Frisbie on a project to compare the freeze-dried and fresh platelet-rich plasma in injured tendon explants. Additionally, Gustavo is doing an internship with CSU's Veterinary Diagnostic Imaging Service focusing on equine musculoskeletal imaging under the supervision of Dr. Myra Barrett-Frisbie.

Christine Battaglia

M.S., Virginia-Maryland Regional College of Veterinary Medicine

Christine (Chrissy) began her appointment at the Orthopaedic Research Center as a Research Scientist/Lab Manager in January 2014. Chrissy attended St. Michael's College in Colchester, VT and obtained a B.S. in environmental science. She obtained an M.S. in bio-chemical toxicology from Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, VA in 2001. Shortly after, Chrissy moved to Fort Collins and began working at Colorado State University in the Environmental and Radiological Health Sciences. She has worked in a variety of research areas since her arrival at CSU, including the Center for Environmental Toxicology, Neurobiology and Radiation Cancer Biology. She looks forward to participating in the exciting research advancements being made at the ORC.

Britt Mactavish

B.S., Colorado State University

Britt is a Colorado native and graduated from CSU in 2002 with a B.S. in equine science. She managed horses for several equine operations in the area, including Chatellen Farm and Double Dove Ranch. In addition, she worked as a technician for Pilchuck Animal Hospital in Snohomish, WA and CSU's Equine Sports Medicine Service, and was a representative in the HR department of Starbucks Coffee Co. before joining the team as the Equine Operations Manager. Britt brings a balance of customer service experience and extensive equine industry connections to her new position. In her downtime, Madsen spends time at home in the garden with her daughter, Riley, and attempts to find time to ride one of her three horses.

Lynsey-Ann Bosch

B.S., Michigan State University

Lynsey graduated from Michigan State University (MSU) with a B.S. in Veterinary Technology, and worked at MSU's Large Animal Hospital as a veterinary technician throughout her education. After moving to Colorado, she worked as a lead technician at an equine practice and as a teacher at Bel-Rea Institute of Animal Technology. Lynsey joined the ORC in 2005 as a Research Associate and currently assists the PIs at the ORC with multiple tasks such as editing and submission of re-search articles, grant submission, presentation creation and project management. Additionally, Lynsey coordinates 3- and 4-day continuing education courses hosted by the ORC at CSU.

Cecily Broomfield

M.S., Colorado State University

Cecily received a B.S. in microbiology from California Polytechnic State University in 2000, and an M.S. in agriculture from CSU in 2006. She is currently working as a research associate for the Orthopaedic Bioengineering Research Lab (OBRL).

Jennifer Daniels

B.S., Colorado State University

Jen is originally from Altamont, Utah, and graduated from CSU in 2009 with a bachelor's degree in equine science and agricultural business. She started at the ORC on feed crew, and returned after graduation to work as an animal care technician. Jen joined the ORC full time as Research Trials Coordinator, Barn Manager and Volunteer Coordinator in June 2010. She was named the 2013 Technician of the Year, an award coordinated by the American Association for Laboratory Animal Science and the International Council for Laboratory Animal Science.

Whitney McMillan

B.S., Colorado State University

Whitney joined the Equine Sports Medicine and Rehabilitation service at the end of 2014 as a technician. She is a Georgia native and has a bachelor degree in Equine Science from CSU. She has been working in equine orthopedic research since 2005 and now brings her extensive experience to the Equine Sports Medicine team.

Mindy Meyers

M.S., University of Minnesota-Duluth

Melinda Meyers is a Research Associate with ten years of experience in the biomedical and biotechnology field. She received a B.S. from the University of Minnesota-Duluth and an M.S. in a focus on equine biotechnology, flow cytometry, and genetic preservation. Mindy is a research associate (laboratory) for the Orthopaedic Research Center.

Meredith Park

B.S., Virginia Tech

Meredith Park joined the Equine Sports Medicine and Rehabilitation service as a veterinary technician in November of 2015. Although originally from Louisiana, Meredith considers Virginia to be “home.” Growing up in Middle-burg, she was heavily involved in the fox hunting and racing community (flat and steeplechase). Meredith left Middleburg to attend Virginia Tech, graduating with a B.S. in Animal and Poultry Sciences in 2010. Following graduation, she returned to Northern Virginia to work for Spring Hill Farm – a world-class thoroughbred breeding and racing operation – foaling out mares, prepping yearlings for sales, and rehabbing layups off the track. After the dispersal of the farm, Meredith made her way to Virginia Equine Imaging, where she worked as a veterinary assistant and managed the farm for Drs. Kent Allen and Rae Stone be-fore making the move to Colorado.

Nikki Phillips

B.S., Tulane University

Nikki received her B.S. in cell and molecular biology in May 1997 from Tulane University. She has been at CSU 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 re-search associate to assist in the laboratory.

Heather Troyer

Quality Systems Coordinator

Preclinical Surgical Research Laboratory/Orthopaedic Bioengineering Research Laboratory

As a Research Liaison, Heather Troyer assists the PSRL and the OBRL in the planning, conduct and reporting of Good Laboratory Practice (GLP) and non-GLP preclinical research. She supports the quality system of multiple projects through study organization, coordination with quality assurance, and research data collection ongoing with the PSRL, OBRL, and their collaborators. Her responsibilities include providing quality control for GLP compliance and ensuring the integrity of study-conducted data and records. This includes collaborating with the Research Integrity and Compliance Review Office (RICRO), reviewing and updating Standard Operating Procedures, coordinating training, maintaining the Master Schedule for GLP projects, and acting as Archivist.

Kelly Zersen

Kelly was born and raised in Grand Island, Nebraska. She graduated from the University of California, Davis, School of Veterinary Medicine, and then completed an internship at Pioneer Equine Hospital. Kelly moved to Fort Collins in 2015, where she worked at the Gail Holmes Equine Orthopaedic Research Center performing equine general anesthesia prior to transitioning to her current role as the Anesthesia Coordinator at the C. Wayne McIlwraith Translational Medicine Institute. Outside of work, she enjoys golfing, college football, concerts, spending time with her wife, Kristin, and taking walks with their dog.

Candice Hastings

Business Officer

Candice is the business officer for the Department of Clinical Sciences, and in May 2011, she began managing the accounting activity for the ORC.

Lindsey McCormick

Equine Sports Medicine
Administrative Assistant

Lindsey grew up in Littleton, Colorado. She attended Colorado State University and graduated with an Equine Science degree in 2012. Before working for the ORC, she organized horse shows for the National Western Stock Show, as well as local Colorado Hunter Jumper Association shows. She rides horses and spends time with her dogs for fun.

Paula Vanderlinden

Program Coordinator

Paula joined the ORC in March 2007 as program coordinator and Dr. McIlwraith's personal assistant. Paula manages the development and publication of the annual ORC lab report and newsletter, prepares the PRSE reports and reapplications, as well as, other reports.

Brian Cole

M.D., M.B.A.; Professor, Department of Orthopedics; Chairman, Department of Surgery, Rush OPH; Shoulder, Elbow and Knee Surgery; Section Head, Cartilage Restoration Center at Rush; Team Physician Chicago Bulls and Chicago White Sox; Rush University Medical Center

Dr. Brian Cole is an orthopedic surgeon specializing in sports medicine at Midwest Orthopaedics at Rush and a Professor of Orthopedics and Anatomy and Cell Biology at Rush University Medical Center. He is the Associate Chairman of the Department of Orthopedics at Rush and the Section Head of the Cartilage Research and Restoration Center. Since 2011, he has served as Chairman of Surgery at Rush Oak Park Hospital and as the head of the Rush Orthopedic Master's Program. Dr. Cole's research interests include cartilage restoration, therapeutic biologics, and minimally invasive surgical techniques for the treatment of the knee, elbow, and shoulder. He has published more than 1,000 articles and 8 textbooks on orthopedic surgery and sports medicine. He received an M.D. and MBA from the University of Chicago, completed his orthopedic residency at the Hospital for Special Surgery at Cornell Medical Center, and a Sports Medicine fellowship at the University of Pittsburgh. His professional career outside of academia includes serving as team physician for the Chicago Bulls, co-team physician for the Chicago White Sox and team physician for DePaul University. He also co-hosts a weekly sports-medicine talk-show on ESPN radio.

Mark W. Grinstaff

Ph.D.; Distinguished Professor, Boston University, Boston, MA

Dr. Mark W. Grinstaff is the Distinguished Professor of Translational Research and a Professor of Biomedical Engineering, Chemistry, and Materials Science and Engineering, and Medicine at Boston University. Mark received his Ph.D. from the University of Illinois under the mentorship of Professor Kenneth S. Suslick and was an NIH postdoctoral fellow at the California Institute of Technology with Professor Harry B. Gray. Mark's awards include the ACS Nobel Laureate Signature Award, NSF Career Award, Pew Scholar in the Biomedical Sciences, Camille Dreyfus Teacher-Scholar, Alfred P. Sloan Research Fellowship, the Edward M. Kennedy Award for Health Care Innovation, and a Fellow of the National Academy of Inventors. He is an author or co-author on more than 200 peer-reviewed manuscripts, given more than 275 oral presentations, and an inventor or co-inventor on more than 200 issued patents or pending applications. His students and fellows have given more than 125 oral presentations and 350 posters at national and international meetings. He is a co-founder of four companies that are commercializing his ideas, and he has three products being sold and used in the clinic. His current research activities involve the synthesis of new macromolecules and biomaterials, self-assembly chemistry, imaging contrast agents, drug delivery, and wound repair.

Charles Ho

Ph.D., M.D., Director of Imaging Research, Steadman Philippon Research Institute, Consultant to the Steadman Clinic

Dr. Ho is experienced and active in musculoskeletal and orthopaedic sports medicine imaging and research, particularly in musculoskeletal Magnetic Resonance Imaging. He has been a member of the Radiological Society of North America, the American Roentgen Ray Society, the Society of Skeletal Radiology, 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 radiologic and orthopaedic literature, and presented numerous papers internationally in radiologic and orthopaedic 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.

Johnny Huard

Ph.D.; Distinguished Professor and Vice Chair for Research, Department of Orthopaedic Surgery, University of Texas Health Science Center at Houston Medical School, Houston, Texas; Director, IMM Center for Tissue Engineering and Aging Research Chief Scientific Officer; Director of the Center for Regenerative Sports Medicine, Steadman Philippon Research Institute, Vail, Colorado

Dr. Johnny Huard is a Professor in the Department of Orthopaedic Surgery at the University of Texas Health Science Center at Houston as well as being Chief Scientific Officer of the Steadman-Philippon Research Institute and Director of SPRI's Center for Regenerative Medicine. Prior to these two recent appointments, Dr. Huard was an endowed Professor and Vice Chair for the Department of Orthopaedic Surgery and Musculoskeletal Cellular Therapeutics at the University of Pittsburgh. He also served as the Director of the Stem Cell Research Center at the University of Pittsburgh School of Medicine. Dr. Huard completed his Ph.D. in neurobiology at Laval University in Quebec before earning two post-doctoral degrees in gene therapy, the first from McGill University in Quebec and the second from the University of Pittsburgh.

Dr. Huard is internationally recognized in the areas of gene therapy, tissue engineering and regenerative medicine application based on the use of muscle-derived stem cells (MDSCs). His primary areas of interest are in basic stem cell biology and their translation to clinic to aid in the healing and the regeneration of a variety of tissues. Dr. Huard's research has received multiple honors and awards nationally and internationally and he and his team have published over 300 peer reviewed papers and 82 book chapters. In addition, of significant international recognition in the form of major awards received from organizations in the field of orthopaedic medicine, Dr. Huard has received funding from the National Institutes of Health, the Department of Defense, and the Muscular Dystrophy Association.

William G. Rodkey

D.V.M., M.S.; Chief Scientific Officer and Senior Scientist, Director, Center for Translational and Regenerative Medicine; Research Chairman, Scientific Advisory Committee, Steadman Philippon Research Institute, Vail, Colo.

Dr. Rodkey has been chief scientific officer and director of the Center for Translational and Regenerative Medicine Research at the Steadman Philippon Research Institute in Vail, Colo., since 1990. He is also the chairman of the Scientific Advisory Committee. Dr. Rodkey's research is focused on tissue regeneration with scaffolds, and cellular therapy with an emphasis on articular cartilage, meniscus, and ligaments. Prior to joining Dr. Steadman in Vail, Dr. (Colonel, U.S. Army, retired) Rodkey was chairman of Military Trauma Research at Letterman Army Institute of Research in San Francisco and earned numerous awards and military decorations, including the United States of America Legion of Merit Medal, Meritorious Service Medal, U.S. Army Commendation Medal (with five oak leaf clusters), Humanitarian Services Medal, Order of Military Medical Merit, and the U.S. Secretary of the Army Research and Development Achievement Award. He has authored more than 200 published works and has made more than 450 presentations at national and international meetings. Dr. Rodkey has received numerous awards, including the Excellence in Research Award from AOSSM, the Cabaud Memorial Award from AOSSM twice, the Albert Trillat Award for Knee Research, and GOTS-Beiersdorf Research Award 2000. He received undergraduate and Doctor of Veterinary Medicine degrees from Purdue University and completed medical education and surgical and orthopaedic residency training at University of Florida. He is a member of AAOS, AOSSM, ISAKOS, ESSKA, ICRS, OARSI, EFORT.

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, and 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. President of American Society of Cell and Gene Therapy, 2012

Frank Barry, Ph.D., Professor of Cellular Therapy at the Regenerative Medicine Institute (REMEDI), National University of Ireland Galway.

Frank Barry directs a large group of researchers who focus on the development of new repair strategies in stem cell therapy and gene therapy in orthopaedics. Previously, he was Director of Arthritis Research at Osiris Therapeutics in Baltimore, Md., and a Research Fellow at Shriners Hospital for Children, Tampa, Fla. He has contributed to the fields of tissue engineering and regenerative medicine by developing innovative and successful cellular therapies for the treatment of acute joint injury and arthritic disease. This has included the generation of a large body of new data in groundbreaking preclinical studies, and has led to the first phase of clinical testing of mesenchymal stem cells in clinical trials for joint injury. In a career that has spanned both industry and academic research, he has been a driver in the development of cellular therapy as a biological repair strategy. It is his belief that the application of new technologies in regenerative medicine, including cellular therapy, gene therapy, growth factor augmentation, implantable scaffolds, and nanomaterials, will have a profound impact in Orthopaedics. Frank Barry was the recipient of the 2012 Marshall Urist Award for excellence in tissue regeneration research from the Orthopaedic Research Society.

Constance R. Chu, M.D., Professor and Vice Chair Research, Department of Orthopedic Surgery, Stanford University; Director of Joint Preservation Center and Chief of Sports Medicine, VA, Palo Alto

Dr. Constance R. Chu was previously the Albert Ferguson Professor of Orthopaedic Surgery at the University of Pittsburgh. She is a clinician-scientist who is both principal investigator of several projects funded by the National Institutes of Health, and who has been recognized as a Castle-Connelly/US News and World Report "Top Doctor" in orthopedic surgery, as well as on Becker's list of 125 Top Knee Surgeons in the U.S. Her clinical practice focuses on knee reconstruction, arthroscopy, ACL and meniscus surgery, and cartilage repair. She graduated from the U.S. Military Academy at West Point and earned her medical degree from Harvard Medical School. As director of the multi-disciplinary Joint Preservation Center structured to seamlessly integrate basic, translational and clinical research with clinical practice, Dr. Chu developed the center to advance the concept of early diagnosis and treatment of cartilage injury and degeneration as a strategy to delay or prevent the onset of disabling osteoarthritis. Towards this end, she is leading innovative translational research from bench to bedside in three main areas: quantitative imaging and biomarker development for early diagnosis and staging of joint and cartilage injury and degeneration; cartilage tissue engineering and stem cell based cartilage repair; and molecular and biological therapies for joint restoration and rejuvenation. Her research efforts have led to more than 30 professional awards and honors to include a Kappa Delta Award, considered to be the highest research honor in Orthopedic Surgery. Dr. Chu also regularly holds leadership and committee positions in major professional organizations such as the American Association of Orthopedic Surgeons (AAOS) and the American Orthopedic Association (AOA). In her subspecialty of Orthopedic Sports Medicine, she is a past president of the Forum Sports Focus Group, a member of the prestigious Herodicus Society of leaders in sports medicine, and immediate past Chair of the American Orthopedic Society for Sports Medicine (AOSSM) Research Council. She is alumnus of the highly selective AOA American, British, Canadian (ABC) Traveling Fellowship and the AOSSM Traveling Fellowship, opportunities enacted to recognize and promote careers of emerging leaders in orthopedic surgery and orthopedic sports medicine, respectively.

Lisa Fortier, D.V.M., Ph.D., Diplomate ACVS Lisa Fortier is a professor of surgery at Cornell University in Ithaca, N.Y.

She received her D.V.M. from Colorado State University and completed her Ph.D. and surgical residency training at Cornell University. She is boarded with the American College of Veterinary Surgeons and is an active equine orthopaedic surgeon at Cornell University and the Cornell Ruffian Equine Specialists Hospital at the Belmont race track in New York. Her laboratory studies the intracellular pathways involved in the pathogenesis of osteoarthritis, with particular emphasis on post-traumatic osteoarthritis. In addition, Lisa's research program investigates the clinical application of stem cells and biologics such as PRP for cartilage repair and tendonosis. She has received the Jaques Lemans Award from the International Cartilage Repair Society, the New Investigator Research Award from the Orthopaedic Research Society, and the Pfizer Research Award for Research Excellence from Cornell University. Lisa is the vice president of the International Veterinary Regenerative Medicine Society and past president of the International Cartilage Repair Society.

Alan J. Grodzinsky, Sc.D., Professor, Director of the Center for Biomedical Engineering, Departments of Biological Engineering, Mechanical Engineering, and Electrical Engineering and Computer Science, MIT

Dr. Grodzinsky is a professor in the departments of Biological, Electrical, and Mechanical Engineering at the Massachusetts Institute of Technology. He is also the director of the Center for Biomedical Engineering. Dr. Grodzinsky's 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.

Virginia Byers Kraus, M.D., Ph.D., Duke Molecular Physiology Institute

Dr. Virginia Byers Kraus is Professor of Medicine and Professor of Pathology and Professor in Orthopaedic Surgery at the Duke University School of Medicine. She is a practicing Rheumatologist with over 20 years' experience in musculoskeletal research focusing on osteoarthritis. She trained at Brown University (Sc.B. 1979), Duke University (M.D. 1982, Ph.D. 1993) and Duke University Medical Center (Residency in Internal Medicine and Fellowship in Rheumatology). Her career has focused on elucidating osteoarthritis pathogenesis and translational research into the discovery and validation of biomarkers for early osteoarthritis detection, prediction of progression, and monitoring of disease status. She served as the President of the Osteoarthritis Research Society International (OARSI, 2013-2015). In addition, she is a member of the Orthopaedic Research Society (ORS), American College of Rheumatology (ACR) and served as a member of the national board of directors of the Arthritis Foundation (2014-16). For work related to prevention of post-traumatic arthritis, she is a recipient of the 2015 Kappa Delta award from the American Academy of Orthopaedic Surgeons (AAOS) and ORS.

Christopher Little, B.Sc., B.V.M.S., M.Sc., Ph.D.; Diplomate ACVS; Professor and Director, Raymond Purves Bone & Joint Research Laboratories, Kolling Institute, Institute of Bone and Joint Research, University of Sydney at Royal North Shore Hospital

Professor Christopher Little is director of the Raymond Purves Bone and Joint Research Labs at the Kolling Institute and the SubDean of Research for Sydney Medical School (Northern) at Royal North Shore Hospital, Australia. 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 a 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 (U.K.), he was awarded an Arthritis Foundation of Australia Fellowship at the University of Melbourne. In 2004, he moved to his current position in the University of Sydney Faculty of Medicine. Chris's research interests focus on defining the biochemical and molecular mechanisms of joint pathology in OA, and tendon and intervertebral disc degeneration, and are based on the belief that it is only through a better understanding of the mechanisms that drive the initiation and progression of these diseases that new therapies can be developed. 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 and disc degeneration. Chris is recognized internationally for his expertise in the development and use of animal models of bone and joint disease. He has served as an Associate Editor of Osteoarthritis and Cartilage, and as leader of the OARSI international initiative to establish standardized methods for evaluation of animal models of OA. Chris received the 2010 Barry Preston Award from the Matrix Biology Society of Australia and New Zealand, presented to an outstanding leader in the field. He has authored/co-authored 112 scientific papers and seven book chapters.

Alan J. Nixon, B.V.Sc., M.S., 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 ORC at CSU, the combined use of interleukin receptor antagonist gene therapy to diminish degradation in arthritic joints.

Michael "Mick" Peterson, Ph.D., Professor, University of Maine

Dr. Peterson is a professor of mechanical engineering at the University of Maine. Prior to coming to the University of Maine, he was a faculty member at CSU 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 animal biomechanics, dynamic response of materials, and waves in solids.

Christopher B. Riley, B.Sc. (Physics), B.V.Sc. (Hons), M.Sc., Ph.D., Diplomate ACVS, PGCert Innovation Mgt, Professor, Chair and Service Chief, Equine Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerton North, New Zealand

Following military service in the Air Force, Dr. Riley received degrees in physics and veterinary medicine from the University of Melbourne, Australia. After 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 a Diplomate in the American College of Veterinary Surgeons. He joined the faculty at the Atlantic Veterinary College, Canada, in 1999 rising to the rank of professor, and completed an MBA course in Innovation Management in 2007 at the University of Melbourne. In 2010, he accepted an appointment as the inaugural professor and chair of Equine Health the University of Adelaide, establishing the equine curriculum, teaching and veterinary hospital facilities. He commenced his current position at Massey University in 2013 during the veterinary program's 50th Anniversary year. Dr. Riley has focused his research on the development of biomedical tests for animal diseases using the emerging technologies of infrared spectroscopy (FTIR), optoacoustics, and bioinformatics. He established the first FTIR laboratory of its kind in Canada, developed to investigate the veterinary potential biomedical infrared spectroscopy. He has continued this work with ~U.S. $6.7 million in funded projects to date. Dr. Riley has a special interest in biomarkers for orthopaedic disease, and humoral immunity, but is also interested exploring the full potential of emerging technologies as they apply to veterinary and comparative medicine. Dr. Riley partnered with the Orthopaedic Research Center and the Institute for Biodiagnostics, National Research Council of Canada, to develop the first FTIR test for equine traumatic arthritis and osteochondrosis. More recently, he has collaborated with Prof. Sheila Laverty at the University of Montreal and Prof. James Cook at the University of Missouri to examine and characterize this technology further in rabbit and canine models of orthopaedic disease. He looks further to continued collaboration and advances in this new field of research. Currently, he is continuing work with the carpal chip fracture model established at the ORS.

Roger K. W. Smith, M.A., VetMB, Ph.D., FHEA DEO, AssocECVDI, Diplomate ECVS MRCVS; Professor of Equine Orthopaedics, Royal Veterinary College, London, U.K.; RCVS and European Specialist in Equine Surgery (Orthopaedics); President, International Veterinary Regenerative Medicine Society

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 is also an Associate member of the European College of Veterinary Diagnostic Imaging and Fellow of the Higher Education Academy. 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 pathogenesis of tendinopathy 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.

Stephen B. Trippel, M.D., Orthopaedic Surgeon; Professor of Orthopaedic Surgery and Anatomy and Cell Biology, Indiana University School of Medicine

Dr. Stephen Trippel is an orthopaedic surgeon with a clinical focus on adult reconstructive surgery and a research focus on musculoskeletal repair. He is professor of Orthopaedic Surgery and of Anatomy and Cell Biology at Indiana University School of Medicine and is an advisory member of the graduate faculty at Purdue University. Dr. Trippel received his M.D. from Columbia University College of Physicians and Surgeons, and completed his orthopaedic residency in the Harvard Combined Orthopaedic Residency Program. He also completed a fellowship in orthopaedic research at Massachusetts General Hospital and a Pediatric Endocrinology research fellowship at the University of North Carolina, Chapel Hill. He served on the faculty of Harvard Medical School before joining the faculty of the Indiana University School of Medicine. Dr. Trippel's current research is focused on the development of new approaches to the treatment of articular cartilage damage, including tissue engineering and gene therapy. This includes an ongoing study with the ORC investigating a novel approach to articular cartilage repair in an equine stifle joint model.

René van Weeren, D.V.M., Ph.D., Diplomate ECVS, Royal Dutch Veterinary Association; Professor of Equine Musculoskeletal Biology, Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Paul Rene van Weeren (1957) graduated in 1983 from the Utrecht University Veterinary Faculty (The Netherlands). He became a staff member of the Department of General and Large Animal Surgery in that year and obtained his Ph.D. in 1989. From 1991-1993 he worked as a visiting professor at the Escuela de Medicina Veterinaria of the Universidad Nacional in Heredia, Costa Rica. He became a diplomate of the European College of Veterinary Surgeons in 1994. He was appointed as full professor to the chair of Equine Musculoskeletal Biology in 2007, and is now mainly involved in research with focus areas articular cartilage, tendons, and biomechanics. He became head of the Department of Equine Sciences of the Faculty of Veterinary Medicine of Utrecht University in 2012. Rene van Weeren has been a supervisor of 27 Ph.D. students, who have obtained their degree in the past years and currently supervises 10 Ph.D. students, who will be graduating within the next few years. He is an associate editor of Equine Veterinary Journal, member of the editorial board of The Veterinary Journal, and member of the scientific board of several others. He has been, or is, guest editor of various Special Issues or Supplements of a variety of scientific journals. He has been external examiner for Ph.D. students abroad at various occasions in Belgium, the U.K., France, Austria, Sweden, Norway, and Finland. He is author or co-author of more than 250 peer-reviewed scientific publications and has contributed various chapters to a variety of text books.

Tim Woodfield

Tim Woodfield is Associate Professor of Regenerative Medicine at the University of Otago Christchurch, New Zealand. He leads the CReaTE Group within the Department of Orthopaedic Surgery and is Director of the Otago Centre for Bioengineering & Nanomedicine. He holds a prestigious Rutherford Discovery Fellowship from the Royal Society of New Zealand, and is Principal Investigator within the Medical Technologies Centre of Research Excellence (CoRE). He holds an adjunct Associate Professor at Queensland University of Technology, Australia.

His research is investigating stem cell and biomaterial-based strategies for musculoskeletal tissue regeneration and their application in the clinical translation of orthopaedic medical devices and cell-based therapies. His research technology platform involves complex 3D Biofabrication and Additive Manufacturing of biomaterial scaffolds and medical devices applied to regenerative medicine of cartilage and bone, including: novel bio-ink/bio-resin development, advanced 3D tissue culture models and high throughput screening.

He has published over 105 peer reviewed journal articles, book chapters and published conference proceedings (h-index: 30), and acted as coordinator of a recent European Commission 'skelGEN' consortia project. He has attracted over NZ$23 million in competitive research funding as a Principal or Named Investigator through grants from the Royal Society of New Zealand, Ministry of Business Innovation & Employment, Health Research Council, AO Foundation.

He is past President of the Australasian Society for Biomaterials & Tissue Engineering (ASBTE) and is currently Executive Board member and Vice President of the International Society for Biofabrication (ISBF). He sits on the TERMIS-Asia Pacific Council, and is Editorial Board member for Biofabrication, APL Bioengineering, and Frontiers in Bioengineering & Biotechnology.

Student	Degree	Date Graduated	Current Position
Gayle W. Trotter	M.S.	1981	Formally Professor in equine surgery, Colorado State University now private practice Weatherford, TX
George Martin	M.S.	1983	Private practice, specialist equine surgeon
Alan Nixon	M.S.	1983	Professor in equine surgery, Cornell University
Kenneth Sullins	M.S.	1984	Professor, University of Virginia, Marion DuPont Scott Equine Center
Alicia Bertone	M.S., Ph.D.	1986, 1987	Professor and Truman Endowed Chair, Ohio State University
John Yovich	M.S., Ph.D.	1986, 1988	Vice Chancellor, Murdoch University (now retired)
Cathy Gibson	M.S.	1989	Regulatory veterinarian, Australia
Scott Gustafson	M.S.	1989	Associate Professor, University of Oregon, Corvallis, OR
Jeff Foland	M.S.	1992	Co-owner and specialist equine surgeon, Weatherford Equine Hospital, TX
Dan Steinheimer	M.S.	1995	Specialist radiologist, Veterinary Clinics of America, Loveland, CO
Rick Howard	M.S., Ph.D.	1993, 1996	Specialist surgeon private practice, Arizona Equine Medical, AZ
Fahd Al-Sobayil	M.S., Ph.D.	1998, 2002	Assistant Professor, King Saud University, Riyadh, Saudi Arabia
Abigail Dimock	M.S.	1997	Currently a Ph.D. student, Equine Nutrition (Orthopaedic Related), Rutgers University
JoAnne Engel-Fehr	M.S.	1997	Specialist equine surgeon, Pilchuck Veterinary Hospital, WA
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, Purina
Chris Kawcak	M.S., Ph.D.	1995, 1998	Professor, Iron Rose Ranch University Endowed Chair in Musculoskeletal Research, Colorado State University
David Frisbie	M.S., Ph.D.	1996, 1999	Professor, Orthopaedic Research Center, Colorado State University
Brigitte von Rechenberg	Ph.D.	1999	Dean, College of Veterinary Medicine, University of Zurich
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
Louise Southwood	M.S., Ph.D.	1998, 2002	Associate Professor, University of Pennsylvania School of Veterinary Medicine
Tara Ruttley	M.S.	2000	Engineer for NASA
Carson Shellenberger	M.S.	2000	Engineer for private industry

Student	Degree	Date Graduated	Current Position
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., Ph.D.	1999, 2003	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
Awad Al-Zaben	Ph.D.	2003	Faculty member, Electronics Engineering Department, Yarmouk University, Irbid, Jordan
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, NE
Chris Sorensen	Ph.D.	2004	Post-Doctoral, National Mass Spectrometry Facility, Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA
Brandon Santoni	Ph.D.	2006	Postdoctoral Research Fellow, OBRL, Colorado State University
Katja Duesterdieck	Ph.D.	2006	Assistant Professor, Oregon State University
Marti Shearin (Drum)	D.V.M., Ph.D.	2006	Assistant Doctoral Fellow, University of Tennessee
Valerie Perino	M.S., Ph.D.	2001, 2006	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, Texas
Erin Contino	M.S.	2009	Assistant Professor, Equine Sports Medicine and Rehabilitation, Colorado State University
Ryan Carpenter	M.S.	2009	Equine practice, Southern California
Jennifer Antonnici	Ph.D.	2010	University of California San Diego
Christina Lee	Post-Doc	2010	
Myra Barrett	M.S.	2011	Assistant Professor, ORC, CVMBS, Colorado State University
Melissa King	D.V.M., Ph.D.	1997, 2011	Assistant Professor, Equine Sports Medicine and Rehabilitation, Colorado State University

Student	Degree	Date Graduated	Current Position
Katrina Easton	D.V.M., Ph.D.	2011	University of Sydney
Carrie Adrian	Ph.D.	2011	Director of Rehabilitation Services, VCA Animal Hospitals
Katie Seabaugh	M.S.	2011	Assistant Professor, Equine Sports Medicine and Rehabilitation, ORC Veterinarian
Lacy Kamm	M.S.	2012	Equine surgeon, Veterinary Associates, Auckland, New Zealand
Brad Nelson	M.S., Ph.D.	2013, 2017	Assistant Professor of Surgery, Colorado State University
Valerie Moorman	Ph.D.	2013	Assistant Professor, Equine Medicine and Surgery, University of Georgia
Ali Daniel	M.S.	2014	Private referral practice, Florida
Josh Donnell	M.S.	2015	Partner, Equine Sports Medicine LLC, Pilot Point, TX
Aimee Colbath	M.S., Ph.D.	2015, 2019	Assistant Professor of Surgery, Michigan State University
Ellison Aldrich	M.S.	2016	Equine Surgeon, Faculty, Massey University, Palmerston North, New Zealand
Frances Peat	M.S.	2017	Ph.D. student, Colorado State University
Sherry Johnson	M.S.	2018	Ph.D. student, Colorado State University

Resident	Years of Residency	Date Achieved Board Certification in the American College of Veterinary Surgery
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, 1986-1987	
C. Kobluk	Phase III, 1987-1988	1990
K. T. Gibson	1986-1989	1990
S. A. Gustafson	1986-1989	1990
M. J. Reeves	1986-1989	1990
D. French	Phase III, 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
A. McCoy	2008-2010	2011
K. Seabaugh	2009-2011	2013
L. Kamm	2010-2012	2013
B. Nelson	2010-2013	2014
A. Daniel	2010-2014	2015

Resident	Years of Residency	Date Achieved Board Certification in the American College of Veterinary Sports Medicine and Rehabilitation
D. Ferris	2010-2013	2015
E. Contino	2011-2014	2015
J. Donnell	2012-2015	2016
P. Manchon	2013-2016	
F. Peat	2014-2017	
S. Johnson	2015-2018	2019
J. Daglish	2016-2019	

PROGRAM SYNOPSIS

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. The goals of our program are summarized in our research focuses. As we developed arthroscopic surgical techniques to treat these clinical conditions, we identified limitations in terms of secondary osteoarthritis (OA) and articular cartilage loss and this led into phase two of our program of finding solutions through scientific research.

A major goal of the program has always been to find solutions to musculoskeletal problems, especially joint injuries and arthritis. As clinicians and researchers, we 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 and methods of early detection, and develop better treatments to prevent permanent damage to injured joints and validate manual therapies and rehabilitation techniques.

The ORC now includes the Orthopaedic Bioengineering Research Laboratory (OBRL), and we function as a single unit. The ORC and OBRL, together with the Pre-clinical Surgical Research Laboratory (previously Small Ruminant Orthopaedic Research), and Orthopaedic Oncology make up the Musculoskeletal Research Program, which is a Program of Research and Scholarly Excellence at Colorado State University. This designation of PRSE to us was originally granted in 2004, and has been renewed in 2008, 2012 and 2016. The significant collaborations with the College of Engineering, School of Bioengineering, as well as the Department of Health and Exercise Sciences,

has added considerably to our research strengths. In recent years, considerable human-based funding (Orthopaedic Foundations, NIH, and corporate grants) has added to our support.

Another significant addition to the ORC has been the development of the equine ambulatory sports medicine service and an Equine Sports Medicine and Rehabilitation Residency Program. This followed the accreditation of the new American College of Veterinary Sports Medicine and Rehabilitation specialty and four of our faculty being made Charter Diplomates. Since that time, we have added four Diplomates (board certified in the American College of Veterinary Sports Medicine and Rehabilitation (equine specialty). As faculty, they support an ever expanding clinical and research program in equine sports medicine and rehabilitation. This has led to both considerable clinical and research advancements in the rapidly emerging field.

Most recently, we have achieved funding of $65 million to build the Translational Medicine Institute (initially called the Institute of Biologic Translational Therapies) that is going to take us to a new level in orthopaedic research in translational musculoskeletal research (as well as allied areas of biologic therapies and stem cell research), doing what we have always done for horses but greatly expanding our efforts in human musculoskeletal disease. This was made possible by a lead gift of $35 million from John and Leslie Malone, $10 million from CSU and the $20 million matching gift from Princess Abigail K. Kawananakoa of Hawaii.

Research Activities

The following are the research focuses of the ORC. Updates of recent and current projects of 2017-2018 can be found on pages 102-192.

1. Musculoskeletal Tissue Healing

Until a few years ago, we have principally addressed articular cartilage healing and continue to do so, but we have enlarged the focus to include bone tendons, ligaments, and menisci.

Projects published in 2017-2018 relevant to this focus include

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, and c) Monitoring of therapy, with the long-term goal of preventing severe

osteoarthritis or failure of joints, tendons, ligaments, and menisci. Work in biomarkers has progressed into imaging biomarkers with particular emphasis on the use of ultrasonography, MRI and computed tomography (CT) in diagnosing early disease change in the limb. Considerable work has also been accomplished using subject-specific finite element modeling of the equine metacarpal phalangeal joint which helps us better understand the stresses that play a role in injury of this critical joint.

There were a number of studies in 2017-2018 of importance in the area of early diagnosis of bone and joint disease. A study on the relationship between lesions and performance outcome in survey radiographs of yearlings placed in the repository at the National Cutting Horse Association futurity sale showed that despite many previous concerns with lesions in the femorotibial articulation, radiologic lesions of the medial femoral condyle of the stifle including minor defects through complete subchondral cystic lesions were not significantly associated with performance outcomes.

3. Improvement in the Understanding of the Pathogenesis of Musculoskeletal 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.

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 biological therapies 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. Recent projects summarized in

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, chiropractice 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 bio-mechanically 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 research in the area of rehabilitation to address critical questions at a more basic level. The rehabilitation research has been led by Dr. Melissa King. In a paper published in 2017, we showed overall improvements in thoracic limb function, joint range of motion and synovial membrane integrity and significant reduction in synovial membrane inflammation in experimental osteoarthritis with underwater treadmill exercise. The reduction in inflammation resulted in significant clinical improvement with regard to symmetric thoracic limb loading, uniform activation patterns, patterns of select thoracic limb muscles and return to baseline values for carpal joint flexion, compared with results for horses with simulated hand walking.

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 under-graduate 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 under-graduate 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 ORC's extensive collaboration with the Steadman Philippon Research Institute and biotechnology companies, as well as collaboration in five NIH research grants, 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 ORC's findings.

Potential Impact of Translational Medicine Institute

Program Trends

1. Faculty and Staff: Over the last 12 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 ORC research involves fourteen full-time faculty members (including three Bioengineering Faculty) in our Center. To support the work of the Faculty Researchers, we now have eight research associates. We had ten Ph.D. students in the program in 2018. Current funding is around $4 million annually.

2. Facilities: Thanks to generous private donors, the construction of the Gail Holmes Equine Orthopaedic Research Center and the remodeling of the orthopaedic research laboratories was completed 16 years ago. In addition, a state-of-the-art equine MRI facility has been in operation for 12 years, and this was also funded by private donations. More recently, a state-of-the-art gait analysis facility has been added and the roof of the ORC Laboratories has been replaced

as a gabled roof, and with additional renovations to accommodate expansion of Bioengineering. Last but not least, the new Translational Medicine Institute (TMI) is allowing us to play a much larger role in translational research to people in addition to our animals.

3. Endowed chairs: We have also received three $3 million University Endowed Chairs from Barbara Cox Anthony, Iron Rose Ranch, and Abigail K. Kawananakoa, a $1.5 million Chair in Musculoskeletal Imaging from the estate of Kenneth and Virginia Atkinson, and most recently, a $6 million Presidential Endowed Chair from John and Leslie Malone. We continue to pursue endowed funding to make all of our positions permanent.

4. Further development of the Equine Ambulatory Sports Medicine Service: An equine ambulatory sports medicine service was initiated in 2010, and has now grown to where Drs. Chris Kawcak and Melissa King have been joined by Dr. Mindy Story, Dr. Erin Contino and Dr. Katie Seabaugh. There are now three research associates, Lindsey McCormick, Whitney McMillan, and Meredith Park assisting in this service offering state-of-the-art expertise in equine musculoskeletal problems in athletic horses. Britt Mactavish is the Equine Operations Manager of the program. We have three equine sports medicine residents (one in each year) and have graduated our seventh resident from their three-year program in 2018. The service commenced in 2011 and has continued to exceed our expectations in demand.

5. Establishment of Equine Sports Medicine and Rehabilitation Residencies: A new American veterinary specialty, the American College of Veterinary Sports Medicine and Rehabilitation was accredited by the American Veterinary Medical Association in May 2009. There were 27 Charter Diplomates established 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 then established an equine sports medicine and rehabilitation residency program to train future specialists in 2010. Our first resident, Dr. Dora Ferris commenced in July 2010 followed by our second resident, Dr. Erin Contino starting in July 2011, and our third resident Dr. Josh Donnell started in July 2012. These first three residents had their credentials accepted and passed the examination to be-come board certified as Diplomates of the American College of Veterinary Sports Medicine and Rehabilitation. Drs. Ferris and Donnell have gone into private practice and Dr. Erin Contino is a faculty member in our Equine Sports Medicine Service.

6. Unrestricted Funding from Donors and Foundations: The period 2017-2018 has been one of continuing to function with good support and further increase in faculty and staff positions. Donor support is critical to our continued operation and growth. The funding and building of the TMI in this time period is a particular highlight.

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 orthopaedic 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 groundbreaking 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 a 96-well plate format, using Molecular Devices SpectraMax, 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. (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.
- **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.
- **Sircol Assay:** Assay to assess the amount of newly synthesized collagen in cartilage, tendon or cell culture media.

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.

Pre-Assay Sample Processing Including:

Papain, hyaluronidase, and collogenase 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.

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

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
- Flow cytometry analysis of MSC and other cellular cultures

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 Fresh Frozen Sectioning and Staining of Paraffin Embedded Samples

Live/Dead Cellular Tissue Staining and Fluorescent Imaging

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, cardiovascular, neuronal or skeletal injury and/or disease. The primary research foci 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 development and application 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 and resorbable biomaterials

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 biomechanical strength
c. Dynamic and Quasi-static analyses
d. Fatigue and life-cycle analyses

8. Histological structural analyses

a. Micro Computed Tomography (µCT) – High resolution imaging of bone and/or implants to determine bone growth and healing
b. Decalcified and non-decalcified tissue histology
c. Dynamic and Static Histomorphometric analysis

2017-2018
SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

2018 Textbook Chapters

1. **Contino E.** (2018) "Management and Rehabilitation of Joint Disease in Sport Horses" In Veterinary Clinics: Equine Practice – Equine Sports Medicine. Ed: Garcia-Lopez, J.
2. **Johnson SA, Frisbie DD.** Musculoskeletal System: Synovial Joint Biology and Pathobiology. In, Equine Surgery, JA Auer and JA Stick (eds), 5th edition. Philadelphia, Elsevier Saunders. August 2018.
3. **Johnson SA, Frisbie DD.** Musculoskeletal System: Medical Treatment of Joint Disease. In, Equine Surgery, JA Auer and JA Stick (eds), 5th edition. Philadelphia, Elsevier Saunders. August 2018.
4. **Johnson SA, Frisbie DD.** Musculoskeletal System: Surgical Treatment of Joint Disease. In, Equine Surgery, JA Auer and JA Stick (eds), 5th edition. Philadelphia, Elsevier Saunders. August 2018.

2017 Textbook Chapters

1. **McIlwraith C.W.** Joint injuries and disease in osteoarthritis. In, Adams and Stashak's Lameness in Horses 7th edition, Baxter GM (ed). Wiley 2017 In Press.
2. **McIlwraith C.W.** Osteochondrosis. In, Adams and Stashak's Lameness in Horses 7th edition, Baxter GM (ed). Wiley 2017 In Press.
3. **McIlwraith C.W.** Joint injuries and disease in osteoarthritis. In, Adams and Stashak's Lameness in Horses 7th edition, Baxter GM (ed). Wiley 2017 In Press.
4. **McIlwraith C.W.** Osteochondrosis. In, Adams and Stashak's Lameness in Horses 7th edition, Baxter GM (ed). Wiley 2017 In Press.
5. **Waldorff EI, Fang S, Zhang N, Visal L, Imbriani M, Magalinl E, Preve E, Robotti P, Raines A, Goldberg E, Jiang J, McGilvray KC, Easley JT, Seim HB, Puttlitz CM, Ryaby JT.** PEEK titanium composite (PTC) for spinal implants" in "Orthopaedic Biomaterials – Advances and Applications", 2017, Editors: Li B and Webster T, Published by Springer

2018 Refereed Publications

1. **Aldrich E, Goodrich L, Contino E, et al.** (2018) "Usefulness of caudomedial-craniolateral oblique radiographic views for diagnosis of injury of the origin of the cranial cruciate ligament in two horses." *J Amer Vet Med Assoc*; Accepted.
2. **Ball AN, Donahue SW, Wojda SJ, McIlwraith CW, Kawcak CE, Ehrhart N, Goodrich LR.** The challenges of promoting osteogenesis in segmental bone defects and osteoporosis. J Orthop Res. 2018; 36(6):1559-1572. doi:10.1002/jor.23845.
3. **Ball A.N., Phillips J.N., McIlwraith C.W., Kawcak C.E., Samulski R.J., Goodrich L.R.** Genetic modification of scAAV-equine-BMP-2 transduced bone-marrow-derived mesenchymal stem cells before and after cryopreservation: an "off-the-shelf" option for fracture repair. J Orthop Res 2018. Doi: 10.1002/jor.24209. [Epub ahead of print].
4. **Barrett M.F., Selberg K.T., Johnson S.A., Hersman J., Frisbie D.D.** High field magnetic resonance imaging contributes to diagnosis of equine distal tarsus and proximal metatarsus lesions: 103 horses. Vet Radiol Ultrasound 2018; 59:587-596. doi: 10.1111/vru.12659.
5. **Barrett M.F., McIlwraith C.W., Contino E.K., Park R.D., Kawcak C.E., Frisbie D.D., zumBrunnen J.R.** Relationship between repository radiographic findings and subsequent performance of Quarter Horses competing in cutting events. J Am Vet Med Assoc 2018; 252:108-115. doi: 10.2460/javma.252.1.108.
6. **Barrett MF, Manchon PT, Hersman J, Kawcak CE.** Magnetic resonance imaging findings of the proximal metacarpus in Quarter Horses used for cutting: Retrospective analysis of 32 horses 2009-2012. Equine Vet J. 2018; 50(2):172-178. doi:10.1111/evj.12746.
7. **Barrett M.F.** Recent advances in articular cartilage evaluation using computed tomography and magnetic resonance imaging. *Equine Veterinary Journal*. 2018 Feb 27; Published online.
8. **Barrett M.F.** Incomplete fracture of the talus secondary to maladaptive stress remodeling in a horse. *Journal of the American Veterinary Medical Association*. 2018 Mar 5; accepted for publication.

9. **Colbath A.C., Dow S.W., Hopkins L.S., Phillips J.N., McIlwraith C.W., Goodrich L.R.** Induction of synovitis using interleukin-1 beta: are there differences in the response of middle carpal joint compared to the tibiotarsal joint? Front Vet Sci 2018. doi: 10.3389/fvets.2018.00208.

10. **Coleman M.C., Belknap J.K., Eades S.C., Galantino-Homer H.L., Hunt R.J., Geor R.J., McCue M.E., McIlwraith C.W., Moore R.M., Peroni J.F., Townsend H.G., White N.A., Cummings K.J., Ivanek-Miojevic R., Cohen N.D.** Case-control study of risk factors for pasture- and Endocrinopathy-associated laminitis in North American Horses. J Am Vet Med Assoc. 2018 Aug 15; 253(4):470-478. doi: 10.2460/javma.253.4.470.

11. **Daglish J., Frisbie D.D., Selberg K.T., Barrett M.F.** High field magnetic resonance imaging is comparable with gross anatomy for description of the normal appearance of soft tissues in the equine stifle. Vet Radiol Ultrasound 2018; 59:721-736. doi: 10.1111/vru.12674.

12. **J Easley J, CM Puttlitz, H Seim, N Ramo, C Abjornson, FP Cammisa, KC McGilvray.** "Biomechanical and histological assessment of a novel screw retention technology in an ovine lumbar fusion model." *Spine Journal* doi: 10.1016/j.spinee.2018.07.021, 2018.

13. **Gadomski BC, McGilvray KC, Easley JT, Palmer RH, Qin YX, Puttlitz CM.** An Investigation of Shock Wave Therapy and Low-Intensity Pulsed Ultrasound on Fracture Healing Under Reduced Loading Conditions in an Ovine Model. J Orthop Res. 2018; DOI:10.1002/jor.23666.

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21. **McIlwraith C.W.** Current status informational treatments for traumatic joint disease. In *Proceedings, Florida Association of Equine Practitioners*, Promoting Excellence Symposium, 2017.
22. **McIlwraith C.W.** New biologic protein therapies in the treatment of equine traumatic joint disease. In *Proceedings, Florida Association of Equine Practitioners*, Promoting Excellence Symposium, 2017.
23. **McIlwraith C.W.** Mesenchymal stem cells – appropriate use in equine joint disease. In *Proceedings, Florida Association of Equine Practitioners*, Promoting Excellence Symposium, 2017.
24. **McIlwraith C.W.** Equine joint injury – the past, present and future. In *Proceedings, South Africa Equine Veterinary Association 50th Anniversary*, Kruger National Park, South Africa. February 13-16, 2017.
25. **McIlwraith C.W.** President's Community Lecture Series, Colorado State University, Fort Collins, CO, "Joint injury in arthritis: helping horses and humans." April 18, 2017
26. **Johnson S.A., Frisbie D.D., Valdez-Martinez A., Barrett M.F., Turk P., McIlwraith C.W.** Longitudinal tendon healing assessed by advanced multi-modality imaging and endpoint data correlation: a closer look. In Proceedings, Third Havemeyer Foundation Symposium on Tendon Injury and Tendonitis, Home Ranch, Clark, CO. October 15-19, 2017.

2018 Oral Presentations

1. **Contino E.K.** "Back Pain: How Do They Present and How Do You Image That Area?"; AAEP Focus on Integrative Imaging, Raleigh, NC; Invited speaker.

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2. **Contino E.K.** "How Does the Imaging Diagnosis Dictate a Rehabilitation Program?"; AAEP Focus on Integrative Imaging, Raleigh, NC; Invited speaker. 06/2018
3. **Contino E.K.** "Focus on the Pre-Purchase Exam and Imaging" panel discussion AAEP Focus on Integrative Imaging, Raleigh, NC; Invited speaker. 06/2018
4. **Contino E.K.** Wetlab instructor – ultrasound of the thoracolumbar back (4 hours); AAEP Focus on Integrative Imaging, Raleigh, NC; Invited instructor. 06/2018
5. **Contino E.K.** Wetlab instructor – proprioceptive techniques (4 hours), clinical lameness cases (6 hours); AAEP 360° 'Back Pain and Pelvic Dysfunction' course, Fort Collins, CO; Invited instructor. 06/2018
6. **Contino E.K.** Communications Junior Practicum Guest Speaker, Colorado State University, Fort Collins, CO. September 2018 – 7 hours lecture.
7. **Contino E.K.** Equine Lameness; Junior practicum, Colorado State University, Fort Collins, CO. September 2018 – 1 hour lecture, 3 hours lab.
8. **Contino E.K.** Treatment of Osteoarthritis; VM 763, Colorado State University, Fort Collins, CO. March 2018 – 1 hour lecture.
9. **Contino E.K.** Equine Sports Medicine and Rehabilitation; CSU Junior practicum, Colorado State University, Fort Collins, CO. March 2018 – 8 hours lab.
10. **Contino E.K.** Diagnostic Analgesia, VM 763, Colorado State University, Fort Collins, CO. February 2018 – 1 hour lecture.
11. **D.D. Frisbie.** American Association of Equine Practitioners Annual Convention – "Intra-articular therapies – what do we know?" – Facilitator, San Francisco, CA, December 3, 2018.
12. **D.D. Frisbie.** Florida Association of Equine Practitioners 14th Annual Promoting Excellence Symposium – "Working up stifle abnormalities, the clinical side," "Specific surgical treatments to be aware of in the stifle," "Diagnostic techniques: specificity and sensitivity to stifle disease including how standing stifle arthroscopy fits in," "Intra-articular biologics," Naples, FL, October 18-21, 2018.
13. **D.D. Frisbie.** Vail Scientific Summit – "Implications of providing a cover for microfractured defects (pre-clinical equine model)," Vail, CO, August 19-21, 2018.
14. **D.D. Frisbie.** Ultrasound and Standing Arthroscopy of the Equine Stifle Joint, Colorado State University, Fort Collins, CO, August 18, 2018 – Lecture, "Stifle: Anesthesia, normal anatomy, standing arthroscopy, case examples and medical treatments," Lab and demo – 2 hours of lecture, 4 hours of lab.
15. **D.D. Frisbie.** American Association of Equine Practitioners Annual Convention – "How to diagnose poor performance in the equine athlete" – Moderator, San Francisco, CA, December 3, 2018.
16. **D.D. Frisbie.** American Orthopaedic Society for Sports Medicine Biologics for Prevention and/or Treatment of Post-Traumatic Osteoarthritis in Sports Injuries, Think Tank – "Role of animal studies in PTOA," Washington DC, November 1-2, 2018.
17. **D.D. Frisbie.** Advanced Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, August 16-17, 2018 – Lecture, "Femoropatellar joint," "Femorotibial joints" Lab: carpal slab fracture, internal fixation, and carpal sheath, distal limb, proximal hindlimb and delegates choice – 1 hour of lecture, 6.5 hours of lab.
18. **D.D. Frisbie.** American Association of Equine Practitioners 360 Meeting – Back Pain and Pelvic Dysfunction – Lectures, 9 hours of lab, Fort Collins, CO, June 3-6, 2018.
19. **D.D. Frisbie.** Basic Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, August 15, 2018 – Arthroscopic surgery of the carpus and fetlock – 4 hours of lab.
20. **Kawcak C.E.** AAEP 360 – Back Pain and Pelvic Dysfunction. Colorado State University, Fort Collins, CO. June 3-6, 2018.
21. **Kawcak C.E.** The Injured Equine Athlete: Early Diagnosis through Imaging. University of Chicago, Department of Radiology, Committee on Medical Physics Seminar. Chicago, IL. March 14, 2018.
22. **Kawcak C.E.** Cervical Pain in the horse; Soreness vs Lameness; Diagnostic Blocks; Case Studies – 5 hours. Northwest Equine Practitioner Association. Bend, OR. February 2018.

23. **McIlwraith C.W.** American Association of Equine Practitioners 360 Meeting – Back Pain and Pelvic Dysfunction – Lectures, 9 hours of lab, Fort Collins, CO, June 3-6, 2018.
24. **McIlwraith C.W.** Ultrasound and Standing Arthroscopy of the Equine Stifle Joint, Colorado State University, Fort Collins, CO, August 18, 2018 – Lecture, "Stifle: Anesthesia, normal anatomy, standing arthroscopy, case examples and medical treatments," Lab and demo – 2 hours of lecture, 4 hours of lab.
25. **McIlwraith C.W.** Advanced Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, August 16-17, 2018 – Lecture, "Femoropatellar joint," "Femorotibial joints" Lab: carpal slab fracture, internal fixation, and carpal sheath, distal limb, proximal hindlimb and delegates choice – 1 hour of lecture, 6.5 hours of lab.
26. **McIlwraith C.W.** Basic Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, August 15, 2018 – Arthroscopic surgery of the carpus and fetlock. 4 hours of lab.
27. **McIlwraith C.W.** Vail Scientific Summit – "Implications of providing a cover for microfractured defects (pre-clinical equine model)," Vail, CO, August 19-21, 2018.
28. **McIlwraith C.W.** American Orthopaedic Society for Sports Medicine Biologics for Prevention and/or Treatment of Post-Traumatic Osteoarthritis in Sports Injuries, Think Tank – "Role of animal studies in PTOA," Washington DC, November 1-2, 2018.
29. **McIlwraith C.W.** American Association of Equine Practitioners Annual Convention – "Intra-articular therapies – what do we know?" – Facilitator, San Francisco, CA, December 3, 2018.
30. **McIlwraith C.W.** American Association of Equine Practitioners Annual Convention – "How to diagnose poor performance in the equine athlete" – Moderator, San Francisco, CA, December 3, 2018.
31. **McIlwraith C.W.** Florida Association of Equine Practitioners 14th Annual Promoting Excellence Symposium – "Working up stifle abnormalities, the clinical side," "Specific surgical treatments to be aware of in the stifle," Diagnostic techniques: specificity and sensitivity to stifle disease including how standing stifle arthroscopy fits in," "Intra-articular biologics," Naples, FL, October 18-21, 2018.
32. **McIlwraith C.W.** Karaka Sales, New Zealand Veterinarians Symposium, "What radiographic lesions on medial femoral condyles of Thoroughbred lesions lead to clinical disease?" January 28, 2018.
33. **McIlwraith C.W.** Mini-Symposium on novel equine and human therapeutics. Scripps Florida, Wellington, FL. "Latest equine/human advances in osteoarthritis and panel discussion." February 2, 2018.
34. **McIlwraith C.W.** International Cartilage Repair Society, Macau, China. Invited lecture. Early osteoarthritis and cartilage repair – Lessons from horses. April 9, 2018.
35. **McIlwraith C.W.** University of Notthingham, School of Pharmacy Seminar, "Experiences in articular cartilage resurfacing in the horse with possible translation" and a full day discussing research. June 11, 2018.
36. **McIlwraith C.W.** University of Notthingham, School of Veterinary Medicine Seminar, "Advances in treatment of joint disease" and day of discussion on collaborative research. June 12, 2018.
37. **McIlwraith C.W.** Baylor College of Medicine, Houston, TX, Translation Biology and Molecular Medicine Program Invited Lecture, "The use of equine models to evaluate articular cartilage repair and how it leads to translation." June 14, 2018.
38. **McIlwraith C.W.** Zoobiquity Colorado, Connecting Human and Animal Health Through Regenerative Medicine, Colorado State University and University of Colorado Anschutz Medical Campus Invited Lecture, "An equine to human journey in musculoskeletal translational medicine." October 5, 2018.
39. **McIlwraith C.W.** Kentucky Equine Research 30 Years, Lexington, KY Invited Lecture, "A review of past and upcoming advances regarding bone and joint disease in horses." October 29, 2018.

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40. **McIlwraith C.W.** Purdue University David van Sickle Musculoskeletal Days, three Keynote Lectures. Day 1: "Osteoarthritis (OA) equine-human translation, cartilage defect repair, the future of OA research." Day 2: "Orthopaedic health: focus on arthritis." Invited lecture Regenerative Medicine and Osteoarthritis and breakout session with Tim Lescun discussing early-mild osteoarthritis early, mild, moderate and late, severe osteoarthritis. November 9-10, 2018.
41. **McIlwraith C.W.** Ontario Veterinary College, University of Guelph, Guelph, Canada Invited to give Schofield Memorial Lecture, "Joint injury and arthritis. Helping horses and humans using translation of clinical and research science" (also received Schofield Memorial Medal). November 14, 2018.
42. **Puttlitz C.M.** Invited Lecturer, University of Eastern Finland, Kuopio, Finland, October 24-26, 2018. "Electromagnetic Coupling With and Without bioMEMs Sensors to Predict the Course of Bone Fracture Healing."

2017 Oral Presentations

1. **Contino E.K.** Equine Lameness; CSU Junior practicum. Lecture (1 hour) and lab (3 hours). September 2017.
2. **Contino E.K.** Diagnostic imaging of clinical cases; CSU Equine Sports Medicine Junior Practicum, Lab (4 hours). April 2017.
3. **D.D. Frisbie.** Standing stifle arthroscopy course, Malaren Hastklinik, Sigtuna, Sweden, August 10-12, 2017 – 2 hours of lecture, 6 hours of lab.
4. **D.D. Frisbie.** Ultrasound and Standing Arthroscopy of the Equine Stifle Joint, Colorado State University, Fort Collins, CO, July 29, 2017 – Lecture, "Stifle: Anesthesia, normal anatomy, standing arthroscopy, case examples and medical treatments," Lab and demo – 2 hours of lecture, 4 hours of lab.
5. **D.D. Frisbie.** Standing stifle arthroscopy course, Tierkarztliche Klinik fur Pferde, Grosswallstadt, Germany, June 16-19, 2017 – 2 hours of lecture, 6 hours of lab.
6. **D.D. Frisbie.** Advanced Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, July 27-28, 2017 – Lecture, "Femoropatellar joint," "Femorotibial joints" Lab: proximal forelimb, distal limb, proximal hindlimb and delegates choice – 1 hour of lecture, 6.5 hours of lab.
7. **D.D. Frisbie.** Basic Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO, July 26, 2017 – Arthroscopic surgery of the carpus and fetlock 4 hours of lab.
8. **D.D. Frisbie.** American Association of Equine Practitioners 360 meeting – Diagnosing, imaging and treating the hind suspensory and stifle: everything you need or want to know – Lectures, "Perineural and intrasynovial anesthesia, stifle and hind suspensory," "Arthroscopic normal anatomy of stifle," "Standing stifle arthroscopy," "Medical treatments" and 18 hours of lab, Fort Collins, CO July 9-12, 2017.
9. **Kawcak C.E.** Stifle Injuries in Western Performance Horses. American College of Veterinary Surgeons Surgery Summit, Indianapolis, IN, October 12-14, 2017.
10. **Kawcak C.E.** Panel Discussion: Stifle Injury in the horse. American College of Veterinary Surgeons Surgery Summit, Indianapolis, IN, October 12-14, 2017.
11. **Kawcak C.E.** Arthroscopy Laboratory Instruction. Colorado State University Arthroscopy Course. July 26-29, 2017.
12. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting – gross lab, 3 hours. Fort Collin, CO, July 9, 2017.
13. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting – Case presentation, 2 hours. Fort Collins, CO, July 10, 2017.
14. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting. 1-hour course – Treatment of proximal suspensory ligament injury. Fort Collins, CO, July 11, 2017.
15. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting – Case Discussions. Fort Collins, CO, July 11, 2017.

16. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting. Lameness lecture. 1.5 hours. Fort Collins, CO, July 12, 2017.
17. **Kawcak C.E.** American Association of Equine Practitioners 360 Meeting. Lameness lab. 7 hours. Fort Collins, CO, July 12, 2017.
18. **Kawcak C.E.** Practical Fracture Fixation Laboratories. AO Advanced Course. (8 hours per day) Columbus, OH, July 27-30, 2017.
19. **Kawcak C.E.** AO Course. 16 hours of laboratory instruction on equine fracture repair. Columbus, OH. April, 2017.
20. **Kawcak C.E.** Treatment of open and infected fractures. AO Advanced Course. Columbus, OH, April 30, 2017.
21. **Kawcak C.E.** Understanding bone disease. AO Advanced Course. Columbus, OH, April 27, 2017.
22. **Kawcak C.E.** Use of computed tomography in equine surgery. Virginia Maryland Equine Medical Center, Leesburg, VA. (2 hours). January 27, 2017.
23. **King M.R.** Rehabilitation of the Stifle and Hindlimb Proximal Suspensory Ligament. Proceedings AAEP 360 Focus Meeting: Stifle and Hindlimb Proximal Suspensory Ligament. Fort Collins, CO. 2017
24. **McIlwraith C.W.** Standing stifle arthroscopy course, Tierkarztliche Klinik fur Pferde, Grosswallstadt, 2 hours of lecture, 6 hours of lab. Germany, June 16-19, 2017.
25. **McIlwraith C.W.** American Association of Equine Practitioners 360 meeting – Diagnosing, imaging and treating the hind suspensory and stifle: everything you need or want to know – Lectures, “Perineural and intrasynovial anesthesia, stifle and hind suspensory,” “Arthroscopic normal anatomy of stifle,” “Standing stifle arthroscopy,” “Medical treatments” and 18 hours of lab, Fort Collins, CO July 9-12, 2017.
26. **McIlwraith C.W.** Ultrasound and Standing Arthroscopy of the Equine Stifle Joint, Colorado State University, Fort Collins, CO – Lecture, “Stifle: Anesthesia, normal anatomy, standing arthroscopy, case examples and medical treatments,” Lab and demo – 2 hours of lecture, 4 hours of lab. July 29, 2017.
27. **McIlwraith C.W.** Advanced Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO – Lecture, “Femoropatellar joint,” “Femorotibial joints” Lab: proximal forelimb, distal limb, proximal hindlimb and delegates choice – 1 hour of lecture, 6.5 hours of lab. July 27-28, 2017.
28. **McIlwraith C.W.** Basic Arthroscopy Surgery Course, Colorado State University, Fort Collins, CO – Arthroscopic surgery of the carpus and fetlock 4 hours of lab. July 26, 2017.
29. **McIlwraith C.W.** Standing stifle arthroscopy course, Malaren Hastklinik, Sigtuna, Sweden – 2 hours of lecture, 6 hours of lab. August 10-12, 2017.
30. **McIlwraith C.W.** South Africa Equine Veterinary Association 50th Anniversary, Skukusa, Kruger National Park, South Africa, Opening Keynote Lecturer, “Equine joint injury and disease – the past, present and future.” February 13-15, 2017.
31. **McIlwraith C.W.** Interventional Orthopaedic Foundation Annual Conference. “The basic science behind the use of mesenchymal stem cells in orthopaedic injuries and diseases.” Participation in panel discussion on tendon injury and PRP (with Drs. Centeno, Lutsc and Steinmetz). February 17, 2017.
32. **McIlwraith C.W.** European tenoscopy and bursoscopy course, New Market, UK. Three lectures, four 2 hour laboratories. April 21-22, 2017.
33. **McIlwraith C.W.** ICRS Heritage Summit, Gothenberg, Sweden. “Update on the treatment of cartilage lesions in the equine athlete.” Invited faculty lecture. June 29-July 1, 2017.
34. **McIlwraith C.W.** Colorado State University Basic Arthroscopic Surgery Course. 4 hours lecture and 4 hours laboratories. July 26, 2017.
35. **McIlwraith C.W.** Colorado State University Advanced Arthroscopic Surgery Course. 3 hours lecture and four 2 hour wet labs. July 27-28, 2017.
36. **McIlwraith C.W.** Specialty stifle arthroscopy and imaging, Cornell University, Ithaca, NY – 6 lectures and four 2 hour wet labs. August 4-5, 2017.
37. **McIlwraith C.W.** Mesenchymal stem cell 2017, Cleveland, OH. “Use of MSCs in equine sports medicine” – invited lecture. August 14-16, 2017.

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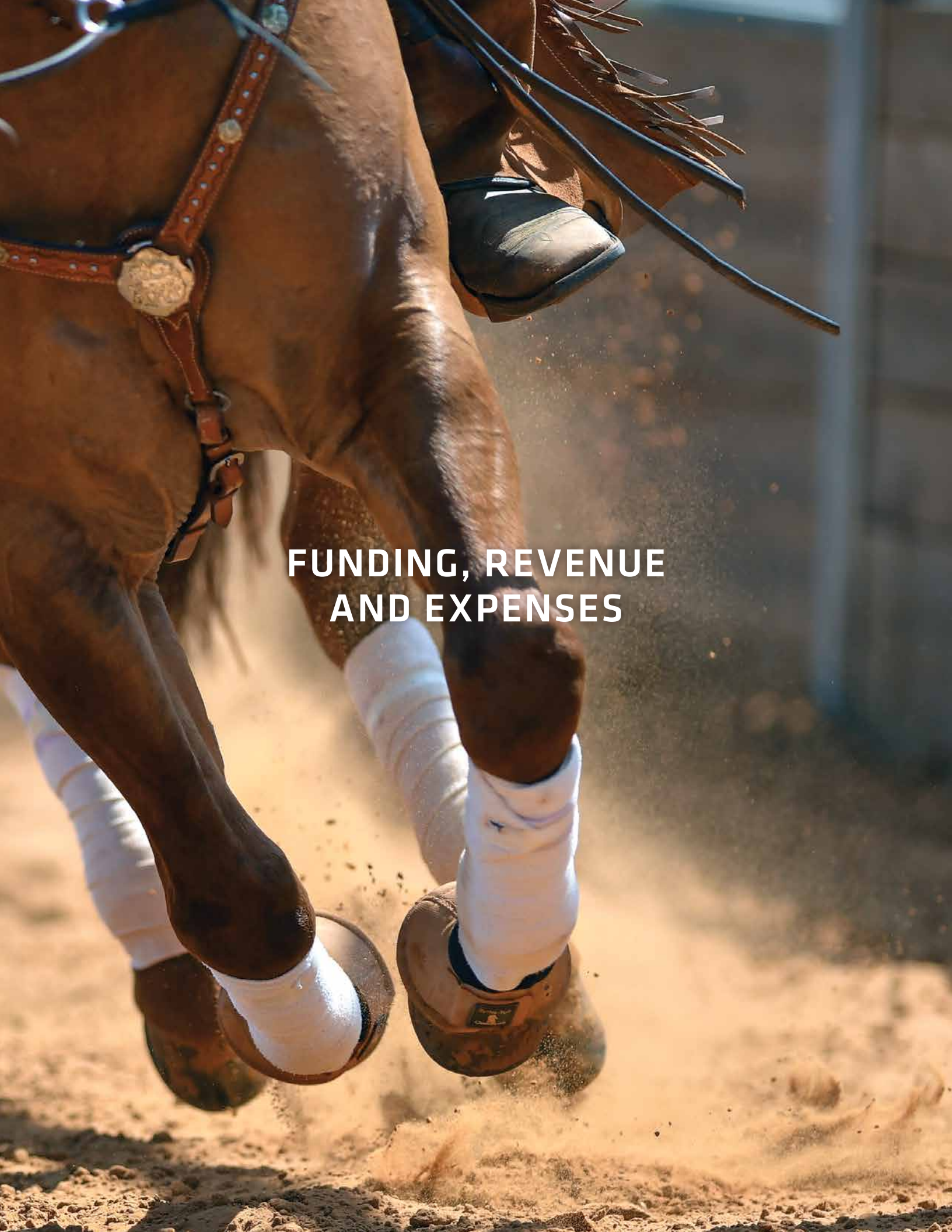
38. **McIlwraith C.W.** Regenerative Medicine Summit, Medical Center of the Rockies, Loveland, CO. Invited lecture, “Equine orthopaedic research: MSCs in equine sports medicine.” August 17, 2017.
39. **McIlwraith C.W.** Third Vail Scientific Summit, Vail, CO. Invited lecture, “Equine clinical and pre-clinical studies with bone marrow-derived MSCs translatable to human sports medicine.” August 23-26, 2017.
40. **McIlwraith C.W.** Third Havemeyer Meeting on tendon injury and disease. The Home Ranch, Clark, CO. Co-Organizer with Dr. Roger Smith and presentation, “Longitudinal tendon healing assessed by advanced multi-modality and endpoint data point correlation: a closer look. October 15-19, 2017.
41. **McIlwraith C.W.** Florida Association of Equine Practitioners 13th Annual Promoting Excellence Symposium. Three invited lectures, “Current status of conventional treatments for traumatic joint disease,” “New biologic protein therapies and the treatment of equine traumatic joint disease,” and “Mesenchymal stem cells – appropriate use in equine joint disease.” October 20-22, 2017.
42. **McIlwraith C.W.** American Association of Equine Practitioners Annual Meeting, San Antonio, TX. Post table topic, “Diagnosis and management of axial skeletal problems.” November 18-20, 2017.
43. **Puttlitz C.M.** Visiting Professor, Department of Orthopaedic Surgery, and the Florida Orthopaedic Institute, University of South Florida, Tampa, FL, March 31, 2017. Grand Rounds Lecture: “Sensor-based fracture healing prediction.”

2018 Honors

1. **Ross Palmer.** Mark Bloomberg Research Excellence Veterinary Orthopedic Society Snowmass, CO
2. **C. Wayne McIlwraith.** The American Association of Equine Practitioners (AAEP) Research Award

2017 Honors

1. **Laurie Goodrich.** Founding Fellow in Equine Minimally Invasive Surgery: Arthroscopy, Bursoscopy, Tenoscopy and Fracture Repair American College of Veterinary Surgeons Bethesda, MD
2. **Laurie Goodrich.** Cabaud Award in Research American Association of Orthopedic Sports Medicine Toronto, Canada
3. **Chris Kawcak.** Cabaud Award in Research American Association of Orthopedic Sports Medicine Toronto, Canada
4. **Dave Frisbie.** Cabaud Award in Research American Association of Orthopedic Sports Medicine Toronto, Canada
5. **C. Wayne McIlwraith.** ACVS Founding Fellow, Minimally Invasive Surgery (Large Animal Orthopedics)
6. **Kevin Haussler.** American College of Veterinary Sports Medicine and Rehabilitation, Scientific Abstract Award, Second place – Equine. Application of Low-Level Laser Therapy and Chiropractic Care for Acute Back Pain in Horses. Haussler KK, Manchon PT, Donnell JR, Frisbie DD.



FUNDING, REVENUE AND EXPENSES

Investigators	Sponsor	Title	Period	Amount
Frisbie,David D (Primary PI) 1678; Trella,Katie J (Co-PI) 1678	University of Colorado	Understanding the Role of Energy Metabolism in the Initiation of Tendinopathy Using a Murine Model	05/01/2017-04/30/2018	$37,175.02
Goodrich,Laurie R (Primary PI) 1678; Kawcak,Christopher E (Co-PI) 1678; Frisbie,David D (Key Person) 1678; Barrett Frisbie,Myra Frances (Key Person) 1681; McIlwraith,C Wayne (Key Person) 1678	Boston University	Polyacrylate Gel to Treat Equine OA in an Osteochondral Chip Fragment Model	10/15/2016-10/14/2017	$187,798.00
Johnson,Sherry Ann (Primary PI) 1678; Frisbie,David D (Co-PI) 1678; Santangelo,Kelly Susan (Key Person) 1682	American Quarter Horse Association	The Effect of Exercise-Based Rehabilitation in a Tendinopathy Model	10/01/2017-09/30/2018	$19,716.00
Moorman,Valerie Jean (Primary PI) 1678; Pezzanite,Lynn Marie (Co-PI) 1678; Kawcak,Christopher E (Key Person) 1678; Goodrich,Laurie R (Key Person) 1678	American Quarter Horse Association	The Relationship Between Sagittal Hoof Conformation and Hindlimb Lameness in the Horse	10/01/2017-09/30/2018	$19,864.00
McGilvray,Kirk (Primary PI) 1374; Puttlitz,Christian M (Co-PI) 1374; Easley,Jeremiah T (Co-PI) 1678; Palmer,Ross H (Key Person) 1678	HHS-NIH-National Institutes of Health	Sensor Development for Predicting Bone Allograft Incorporation	07/01/2017-06/30/2019	$378,729.00
McGilvray,Kirk (Primary PI) 1374; Puttlitz,Christian M (Co-PI) 1374	Aroa Biosurgery	Evaluation of Aroa Biosurgery's Ovine Extracellular Matrix Device in an Ovine Model of Acute Rotator Cuff Repair: Histological Sample Preparation Part 2	08/01/2017-01/31/2018	$21,912.00
McGilvray,Kirk (Primary PI) 1374; Puttlitz,Christian M (Co-PI) 1374	Aroa Biosurgery	Evaluation of Aroa Biosurgery's Endoform Collagen Matrix in an Ovine Model of Acute Rotator Cuff Repair	02/17/2017-09/18/2017	$10,296.00
McGilvray,Kirk (Primary PI) 1374	Vertera, Inc.	Evaluation of Ovine Lumbar Fusion and Bone Ingrowth with Porous PEEK Device	08/01/2016-07/31/2017	$79,298.00
James,Susan Patricia (Primary PI) 1374; Williams,John D (Co-PI) 1374	Plasma Controls, LLC	Task Order #18 Assembly of Plasma Diagnostic System	10/15/2017-07/15/2018	$6,999.41
James,Susan Patricia (Primary PI) 1374; Yalin,Azer P (Co-PI) 1374	mAirSure, LLC	Assembly and Testing of Open-Path Methane Sensors	09/15/2017-12/31/2017	$10,746.96
James,Susan Patricia (Primary PI) 1374; Williams,John D (Co-PI) 1374	Plasma Controls, LLC	TO#15: Iodine Hollow Cathode Testing on Argon	06/15/2017-06/14/2018	$20,999.99
James,Susan Patricia (Primary PI) 1374; Williams,John D (Co-PI) 1374	Plasma Controls, LLC	Task Order #14 Testing of Hall Thruster	04/01/2017-09/30/2017	$4,831.23
James,Susan P (Primary PI) 1374; Volckens,John (Co-PI) 1374	Access Sensor Technologies, LLC	Low Cost Personal Sampling Pump	10/01/2016-06/30/2017	$49,944.00

Investigators	Sponsor	Title	Period	Amount
Santangelo,Kelly Susan (Primary PI) 1682; Dean,Gregg Alan (Co-PI) 1682	Morris Animal Foundation	Novel Approaches to the Diagnosis and Prognostication of Feline Infectious Peritonitis	08/01/2017-07/31/2020	$139,924.77
Santangelo,Kelly Susan (Primary PI) 1682	HHS-NIH-Arthritis, Musculoskel, and Skin	Iron Accumulation as a Driver of Osteoarthritis During Aging	07/01/2017-06/30/2019	$418,000.00
Kawcak	CRC CVMBS CSU	Racing CRC Kawcak	07/01/2016-06/30/2017	$14,607.00
Kisiday	CRC CVMBS CSU	Chondrogenic preconditioning of mesenchymal stem cells to enhance meniscus repair	07/01/2016-06/30/2017	$12,298.00
McIlwraith	CRC CVMBS CSU	Effect of IL-1 beta in a Cervical Articular Facet Joints in Horses	07/01/2016-06/30/2017	$30,000.00
Frisbie	CRC CVMBS CSU	Safety evaluation of the intra-articular application of allogeneic freeze-dried platelet- rich plasma or conditioned serum in equine normal joints	07/01/2016-06/30/2017	$24,592.00
Kawcak	CRC CVMBS CSU	Racing CRC Kawcak	07/01/2017-06/30/2018	$14,273.00
Barrett	CRC CVMBS CSU	Barrett AHD FY18 CRC	07/01/2017-06/30/2018	$25,000.00
Contino	CRC CVMBS CSU	Prevalence of lameness and correlation with performance outcomes in the equine athlete	07/01/2017-06/30/2018	$16,880.00
Goodrich	CRC CVMBS CSU	Does extracorporeal shockwave cause enhanced osteogenesis of equine bone marrow derived mesenchymal stem cells?	07/01/2017-06/30/2018	$24,964.00
Frisbie	CRC CVMBS CSU	The development of a mechanically induced ex-vivo injury model in equine SDFTs using a custom loading device	07/01/2017-06/30/2018	$25,000.00
Total				**$1,593,848.38**

Interest on Endowments	FY 2017 Amount	FY 2018 Amount
McIlwraith Scholarship	7,995	6,736
Cox Anthony Chair	146,409	156,658
Iron Rose Ranch Chair	114,193	122,186
Atkinson Chair	50,863	54,423
Kawananakoa Chair	101,248	108,336
Malone Chair	237,889	259,764
Interest Total	658,597	708,103

Fee for Service Activity		
Fee for Service Total	**23,581**	**81,736**

Medical Center Clinical Services		
Clinical Services Total	**206,564**	**143,397**

Research Projects		
Research Accounts Total	**418,904**	**1,961,378**

State Funds				
	Kawcak CRC Grant	14,408	Kawcak CRC Grant	14,408
	Kisiday CRC Grant	12,298	Barrett CRC Grant	25,000
	McIlwraith CRC Grant	30,000	Contino CRC Grant	16,880
	Frisbie CRC Grant	24,592	Goodrich CRC Grant	24,964
			Frisbie CRC Grant	25,000
State Funds Total		**81,298**		**106,252**

Total Donations	**466,496**	**595,060**
Continuing Education Activities	**47,806**	**54,350**
Stallion Auction	**41,604**	**20,372**
TOTAL REVENUE	**$ 1,944,843**	**$ 63,675,648**

Expenses	FY 2017 Amount	FY 2018 Amount
Faculty Salaries	640,445	493,449
Research Associate Salaries	305,220	456,745
Administrative Salaries	237,944	386,370
Residents	277,951	178,713
Graduate Student Salaries	n/a	26,600
Hourly EORC students	110,393	86,458
Interest Total	**1,571,953**	**1,628,335**
Faculty Travel	55,708	103,324
Materials and Supplies	470,612	323,957
Other Direct	1,490,495	1,701,078
Building	20,471	531
Equipment	35,366	186,578
Expense Subtotal	**3,644,605**	**3,943,803**
Facility and Administrative Overhead Costs	268,511	315,761
Expense Total	**3,913,116**	**4,259,565**
ACCOUNT BALANCE	**($ 1,968,273)**	**($ 583,917)**

SUMMARIES OF RESEARCH PROJECTS

Influence of changing lateral recumbency and mode of ventilation on the alveolar-arterial oxygen tension gradient and selected laboratory analysis in adult isoflurane anesthetized horses

This is a summary of an article by Dr. Khursheed Mama published in Journal of Veterinary Medicine in 2018.

Take home message

Arterial oxygen decreases in both spontaneously breathing and ventilated horses after a change in lateral recumbency. In healthy horses, this improves over time.

Introduction

There was an opportunity to partner to assess arterial oxygenation amongst other variables during a year-long project in which horses were anesthetized repeatedly and where a change in position from one to the other lateral recumbency was necessary to facilitate data collection. Uniquely these horses were anesthetized at an elevation of approximately 1525 m above sea level.

Methods

The study was completed in 8 healthy horses. Horses underwent multiple anesthesias during which they were positioned in either right or left lateral recumbency for computed tomography. Physiological parameters (e.g., blood pressure) were assessed during anesthesia maintenance with isoflurane. Breathing was spontaneous or controlled. Blood was sampled from an arterial catheter prior to, within 5 min of changing lateral recumbency and prior to circuit disconnection for measurement of pH, blood gases and laboratory analytes.

Results

The oxygen tension decreased after turning and then increased to prior to disconnection regardless of the starting lateral recumbency. The oxygen prior to disconnection was however lower than pre turning. As anticipated, there were differences in carbon dioxide tensions and pH between spontaneously breathing and ventilated horses. Changes over time in electrolytes and blood glucose and lactate while evident were minor.

Conclusions

This study provides baseline blood gas and electrolyte values in horses anesthetized at an altitude of approximately 1525 m. Results confirm results obtained at sea-level that changing lateral recumbency during anesthesia decreases blood oxygen tension and hence caution is advised especially in animals with pre-existing hypoxemia.

Current and novel injectable hydrogels to treat focal chondral lesions: properties and applicability

This is a summary of an article by Drs. C. Pascual-Garrido, F. Rodriguez-Fontan, E.A. Aisenbrey, K.A. Payne, J. Chahla, L.R. Goodrich, and S.J. Bryant published in the Journal of Orthopedic Research.[1]

Take home message

This article summarizes the innovative approaches being used to engineer tissue through use of injectable hydrogels for cartilage repair. Currently, many young adults are commonly receiving cartilage restoration procedures but these procedures are built for relief rather than a cure. Further investigation into tissue engineering, specifically injectable hydrogels, shows they are an adequate substitute that restores, maintains, and improves tissue functions.

Introduction

Injectable hydrogels have received increased attention for the treatment of articular cartilage. It has a wide range of properties including retaining a large amount of water, allowing it to have an excellent permeability for nutrients and metabolites as well has being very compatible for the body. Cells and bioactive molecules are also easily added into the hydrogels. It further allows the ability to provide cues for cell differentiation. The procedure of injecting hydrogels is also minimally invasive.

The objective of this article is to present information on the use of bio-compatible hydrogels within cartilage regenerative procedures that should be considered as a potential solution to the limited effectiveness of current methods of treating cartilage degenerative diseases.

Methods

A comprehensive look at the literature on the physical and biochemical properties of hydrogels, the biological properties of the cellular components of the system, and the current best practices in treatment (both approved and experimental) which were used to provide a complete picture of the current and past research on this topic.

Results

This thorough review of the literature and the studies previously conducted on this mode of treatment shows there is conclusive research indicating that, while there is a limited sample size for clinical applications, the scientific community should continue to look into this method of treatment. Through *in situ* and *in vivo* studies, it has been shown that hydrogels provide an environment which promotes recruitment of cells and regrowth of cartilage. The literature currently shows that this can be done through either placement of stem-cell-recruiting cytokines (such as SDF-1, IL-8, PDGFs or TGF-β) or direct integration of stem cells (ACIs, ESCs, MSCs, or iPSCs) within the system. Additionally, physical tests have been done which show that hydrogels adequately mimic the physiological properties of healthy cartilage – something which is a beneficial attribute as it serves as a symptomatic relief as the cartilage is regenerating. Lastly, the literature indicates that the clinical studies which have been performed, while limited at time of publication, show that the hydrogel system is beneficial in improved regeneration when compared to current best practices.

Conclusion

By using injectable hydrogels, there is improved control over in situ gelation, new processing techniques for the hydrogels (e.g. like being able to make multiple layers), and better incorporation of biological signals. Multiple studies on animal models evaluating cartilage repair with hydrogels have shown promising results in osteochondral defects. There is limited experience with human patients, but blinded randomized controlled trials will further determine the effectiveness of hydrogels in cartilage repair.

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Genetic modification of scAAV-equine-BMP-2 transduced bone-marrow-derived mesenchymal stem cells before and after cryopreservation: an "off-the-shelf" option for fracture repair

This is a summary of an article by Drs. A.N. Ball, J.N. Phillips, C.W. McIlwraith, C.E. Kawcak, R.J. Samulski, and L.R. Goodrich published in the Journal of Orthopedic Research.[1]

Take home message

This is the first published study that demonstrates the utility of screening allogeneic cells for donor responsiveness to gene therapy aimed at inducing osteogenesis in vitro. We have provided evidence that an ex vivo transduction technique, employed with a screening process, produces detectable levels of BMP-2.

Introduction

Conventional clinical management of segmented bone defects in humans continues to result in 5-10% of fractures forming non-unions, meaning it is necessary to search for new innovative approaches to bone repair. In parallel, fracture repair in the horse and the difficult recovery is a major challenge because of chronic pain, the development of support limb laminitis, and association with ischemia and infection, which can result in euthanasia of the patient. Further, because of the challenges associated with fracture repair in the horse, and parallels between poor soft tissue coverage and blood supply to the distal limb of horses and the limbs of humans, a translational incentive exists such that success in the equine model may help heal the 5-10% of human fractures that do not heal despite clinical intervention.

Mesenchymal stem cells derived from bone marrow aspirates (BMDMSCs) are often combined with growth factors to induce bone formation and accelerate healing. The most prominently studied osteoinductive growth factor in bone healing is bone morphogenetic protein-2 (BMP-2). Recombinant BMP-2 (rhBMP-2) has met with varying clinical success and has well documented limitations. Gene therapy is considered an alternative to recombinant protein therapy. BMDMSCs can be genetically modified to produce therapeutic proteins in quantities that affect clinical outcomes in bone. Self-complementary adeno associated virus (scAAV) is a vector designed such that therapeutic protein production begins within hours of transduction. It has also been shown to selectively transduce target BMDMSCs and produce high levels of therapeutic proteins even in BMDMSCs with low proliferations rates.

The objectives of this study were to evaluate scAAV-BMP-2 osteogenic induction in equine BMDMSCs in vitro and to investigate if selective cryopreservation of scAAV-BMP-2 cells would not reduce the BMP-2 delivery capacity of the genetically modified cells following recovery in vitro.

Methods

Mesenchymal progenitor cells from five skeletally mature horses were isolated aseptically from the sternum. The cells were transduced in serum free Dulbecco's Modified Eagle Media containing 48,000 viral particles per cell (vpc) scAAV-equine-BMP-2 or 8,000 vpc scAAV-GMP for three hours. Cells in non-vector control groups were incubated in incomplete media during this time. BMP-2 protein expression was evaluated on days 7 and 14. Morphology was graded on days 7 and 14 as well. The two horses producing the most BMP-2 protein expression were selected to undergo cryopreservation studies. The cells were transduced and cryopreserved for 48 hours and later, the viability of the cells were assessed.

Results

The genetically modified scAAV-equine-BMP-2 cells produced significantly more protein than any other group. Cells treated with rhBMP-2 produced similar amounts of BMP-2 protein as scAAV-equine-BMP-2 genetically modified cells, as expected. However, following cryopreservation, scAAV-equine-BMP-2

cells produced less BMP-2 protein when compared to transduced cells that were not cryopreserved but the change in this small sample size was not significant. In addition, cryopreserved rhBMP-2 treated cells produced similar amounts of BMP-2 protein when compared to non-cryopreserved cells.

Conclusion

This is the first evidence that cryopreservation of genetically modified BMDMSCs would not alter osteoinductive potential or clinical use of allogeneic donor cells in cases of equine fracture repair. Further work remains to be done to establish the therapeutic effectiveness of BMP-2 genetically modified allogeneic cells in clinical cases of fracture repair, and use of an equine pre-clinical model, translational to humans, should be performed to establish therapeutic effectiveness in vivo.

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The development and validation of a numerical integration method for non-linear viscoelastic modeling

This is a summary of an article by Drs. Nicole L. Ramo, Christian M. Puttlitz, and Kevin L. Troyer published in PLOS One.

Take home message

The nonlinear viscoelastic characterization of biological soft tissues for computational studies is limited by methods of fitting a constitutive model to experimental data. We have developed a direct fitting method using a stress history variable which is unlimited in the loading profile and improves both fitting efficiency and computational tractability.

Introduction

Compelling evidence that many biological soft tissues display both strain- and time-dependent behavior has led to the development of fully non-linear viscoelastic modeling techniques to represent the tissue's mechanical response under dynamic conditions. Since the current stress state of a viscoelastic material is dependent on all previous loading events, numerical analyses are complicated by the requirement of computing and storing the stress at each step throughout the load history. This requirement quickly becomes computationally expensive, and in some cases intractable, for finite element models. Therefore, the objective of this study was to develop a strain-dependent numerical integration approach for capturing non-linear viscoelasticity that enables calculation of the current stress from a strain-dependent history state variable stored from the preceding time step only.

Methods

This work expanded upon a previously developed comprehensive viscoelastic characterization for modeling nonlinear viscoelasticity in which the time-dependent behavior is approximated by a discrete Prony series [1,2,3]. While this method requires a multi-step approach and a pre-defined strain history, the novel direct fit method developed in this study uses a strain-dependent history variable that is defined to recursively update the stress at each incremental time step. This allows a direct fit using any arbitrary strain history. This methodology was validated based on its ability to recover non-linear viscoelastic coefficients from simulated stress-relaxation (six strain levels) and dynamic cyclic (three frequencies) experimental stress-strain data.

Results

The simulated stress relaxation and cyclic data were fitted using the direct fit method to both a nonlinear and a linear viscoelastic model. The nonlinear model successfully fit each data set with average errors in recovered coefficients of 0.3% for stress-relaxation fits and 0.1% for cyclic (Figure 1). In contrast, the linear model was unable to capture the strain-dependent stress-relaxation data (Figure 2).

Conclusions

The results support the use of the presented direct fit methodology to develop linear or non-linear viscoelastic models from stress-relaxation or cyclic experimental data. The contrast between nonlinear and linear viscoelastic fits supports the use of nonlinear modeling for characterizing biological soft tissues.

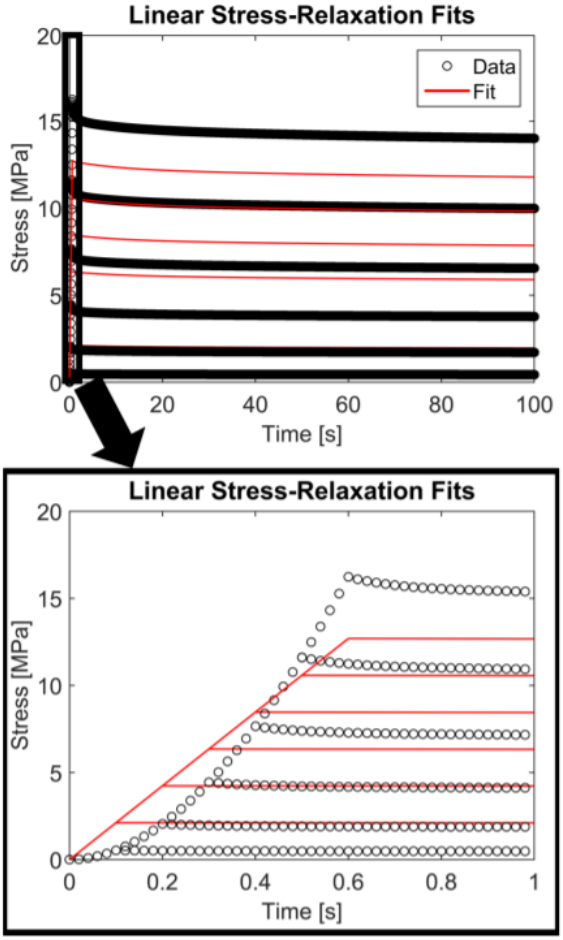

Figure 1: Non-linear stress-relaxation fits. The proposed numerical integration direct fit method for non-linear viscoelastic characterization was able to accurately fit the idealized stress-relaxation experimental data, including the non-linear stress-strain behavior during the ramping phase and the strain-dependent relaxation indicative of non-linear viscoelastic behavior.

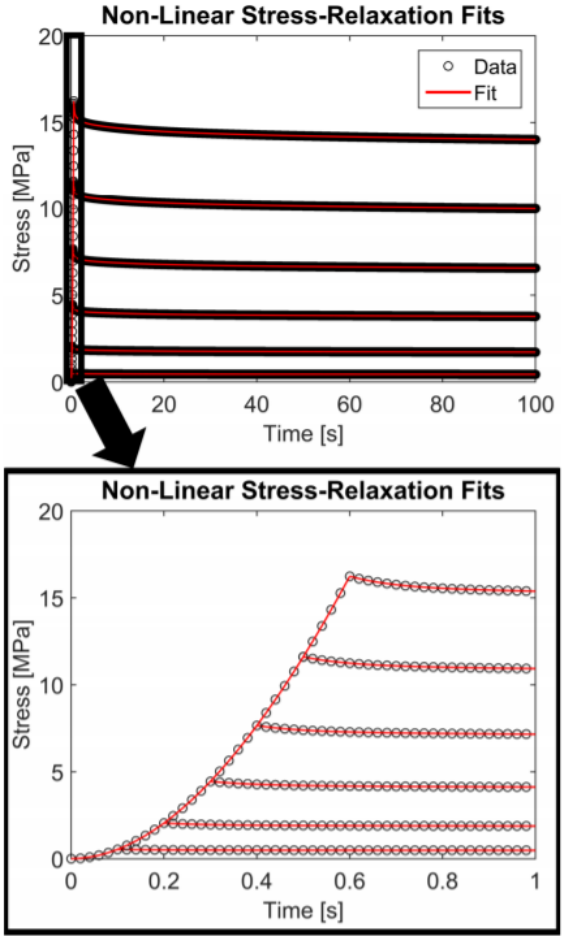

Figure 2: Linear stress-relaxation fits. The linear viscoelastic formulation was not able to capture the idealized strain-dependent stress-relaxation data, resulting in large RMSE values compared to those of the non-linear viscoelastic formulation.

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Biomarkers for equine joint injury and osteoarthritis

The article below represents the results of a symposium aimed at identifying valid data biomarkers that can be used to complement clinical observations for diagnosis and prognosis of joint injury leading to osteoarthritis (OA). It reviews the current state of knowledge and is reprinted here as the original review.

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Abstract

We report the results of a symposium aimed at identifying validated biomarkers that can be used to complement clinical observations for diagnosis and prognosis of joint injury leading to equine osteoarthritis (OA). Biomarkers might also predict pre-fracture change that could lead to catastrophic bone failure in equine athletes. The workshop was attended by leading scientists in the fields of equine and human musculoskeletal biomarkers to enable cross-disciplinary exchange and improve knowledge in both. Detailed proceedings with strategic planning was written, added to, edited and referenced to develop this manuscript. The most recent information from work in equine and human osteoarthritic biomarkers was accumulated, including the use of personalized healthcare to stratify OA phenotypes, transcriptome analysis of anterior cruciate ligament (ACL) and meniscal injuries in the human knee. The spectrum of "wet" biomarker assays that are antibody based that have achieved usefulness in both humans and horses, imaging biomarkers and the role they can play in equine and human OA was discussed. Prediction of musculoskeletal injury in the horse remains a challenge, and the potential usefulness of spectroscopy, metabolomics, proteomics, and development of biobanks to classify biomarkers in different stages of equine and human OA were reviewed. The participants concluded that new information and studies in equine musculoskeletal biomarkers have potential translational value for humans and vice versa. OA is equally important in humans and horses, and the welfare issues associated with catastrophic musculoskeletal injury in horses add further emphasis to the need for good validated biomarkers in the horse.

Osteoarthritis (OA) is the most common disease affecting the joints in humans and is an important cause of pain, disability, and economic loss.[1-3] Traumatic joint injury and OA are equally important in the equine athlete,[4] not only for joint disease but also for bone failure. In September 2014, the third Dorothy Russell Havemeyer Foundation Symposia on Equine Musculoskeletal Bio-markers was held (the second Havemeyer Foundation Symposium has been reported[5]). The aim was to identify validated biomarkers that could be used to complement clinical observations for diagnosis and prognosis of joint injury leading to OA, to predict pre-fracture subchondral bone disease which can lead to catastrophic bone failure in equine athletes, and to discuss development of a point of care diagnostic platform.

The definition of a biomarker varies but a recent consensus suggests it is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[6] Further, this definition stated that "biomarkers can be anatomic, physiologic, biochemical, or molecular parameters associated with the presence and severity of specific diseases and are detectable by a variety of methods including physical examination, laboratory assays, and imaging." Biomarkers have been differentiated into "dry" (e.g., imaging parameters) and "wet" biomarkers (genetic and biochemical entities that can be detected in blood, serum, urine, synovial fluid [SF], and tissues) in OA.[7]

There has been much work in biomarkers in OA in humans for over 25 years.[8,9] The quest is still ongoing to define a validated and qualified biomarker panel that could be used to complement clinical observations for diagnosis, prognosis and response to treatment, with the most recent data from the NIH Osteoarthritis Initiative reported.[10] The first report

demonstrating a relationship between biomarkers and osteochondral change in equine joints was published in 1999.[11] Panels of some biomarkers have been validated in experimental equine OA,[12,13] and the status of equine biomarkers was reviewed in 2005[14] and more recently in 2015.[15] The Dorothy Russell Havemeyer Foundation Symposia in 2005, 2009, and recently in 2014 have allowed exchange of updated information in human and equine musculoskeletal biomarkers as well as planning best paths for the future in both disciplines. The current narrative review represents the key findings from the presentations by the attendees, the issues and questions arising from their discussion and the formal break-out sessions held at the 2014 Symposia.

Equine musculoskeletal biomarkers: current knowledge and future needs

Previous studies have promoted development of targeted molecular diagnostics and predictive biomarkers as models for personalized equine orthopedic medicine.[5,14,15] Diagnostics are sought that are non-invasive, repeatable/reproducible and have specificity and sensitivity for early stages of OA.[16] Spontaneous joint disease is a common clinical problem in the horse and surveys estimate that up to 60% of lameness is related to OA.[17] There is therefore a need for diagnostics designed to predict risk of clinical injury and not just manage the extent of OA, bone disease, catastrophic fracture, and tendon/ligament injury, but to monitor the health and training of competition horses and prevent such injuries. This workshop focused on the current status of diagnostic and point of care platforms for predictive biomarkers.

Biomarkers in human OA: current state of the art in osteoarthritis biomarkers

There is an urgent need for qualified biomarkers to monitor OA development, predict the long-term clinical treatment response and outcome, and identify individuals with the highest risk of disease progression.[7,9,16,18] Osteoarthritis biomarkers could assist clinical trials by delivering essential early information of treatment response, speeding up compound evaluation, and thereby making OA a more manageable target for new drug development. Since a disease starts when detected by the best marker available to define it, herein lies the power of biomarkers. This is especially important for OA, a disease with a prolonged asymptomatic molecular and pre-radiographic phase. Biomarkers could provide an early warning of biochemical and structural alterations leading to earlier treatment prior to irreversible disease, which is likely recalcitrant to therapy.

An Osteoarthritis Research Society International (OARSI) White Paper[7] was produced in response to the Food and Drug Administration (FDA) call for a critical appraisal of fundamentals of the science related to biomarkers of OA, particularly relating to drug development. A subsequent OARSI White Paper reviewed FDA guidance on biomarkers and made recommendations for their use in preclinical development and phase I to IV clinical trials.[18] These documents catalyzed the OA Biomarker Consortium study managed by the Foundation for the National Institutes of Health (FNIH)[10] and highlight how advances in the field of OA research and treatments can be accelerated by a systematic paradigm that encompasses development, validation, qualification, and regulatory approval of OA-related biomarkers for clinical trial and clinical use (also see http://oarsi.org).

In addition to robust disease definitions, there is a recognized need for a consensus on a nomenclature defining the disease. According to the FDA[19] the “currently used disease classification systems define diseases primarily on the basis of their signs and symptoms.” Consequently, many disease subtypes with distinct molecular causes are still classified as one entity, with little ability to stratify or link distinct phenotypes. The National Academy of Sciences has called for a “New Taxonomy” of disease to advance our understanding of disease pathogenesis and improve health, that defines and describes diseases on the basis of their intrinsic biology in addition to traditional signs and symptoms.[20] Biomarkers are key to this new taxonomy for heterogeneous diseases such as OA. To aid in this, a standardized nomenclature has been proposed, describing disease (molecular, anatomic, and physiological aspects) and illness aspects of OA.[21]

Use of personalized health care (PHC) to stratify OA phenotypes

OA is a heterogeneous disorder, with numerous drivers of disease progression. However, up to 50% of OA patients in clinical studies and approximately 85% in the background population do not show both symptom and structural progression over 2 years.[22,23] It is therefore important to identify the individuals that progress and determine the drivers of progression. This would enable enriching of clinical trial populations, and when effective treatment is available to slow disease progression, to identify those in need of it. There is a need to pair the paramount risk factor for progression with personalized treatment approaches, in which "one size does not fit all." A number of drivers for PHC in OA have been identified[24]: (i) Identification of patients who respond optimally, with the highest efficacy and lowest safety concerns, to a given treatment; (ii) Specific development strategy for a selected subpopulation of patients; and (iii) Efficient use of healthcare resources. To date, three different OA subpopulations have been identified: (i) Inflammation mediated OA; (ii) Subchondral bone turnover driven OA; and (iii) Trauma driven OA. Biomarkers can identify different pathophysiological processes potentially leading to identification of these phenotypes (Figure 1 [from Lotz et al.[24,25]]).

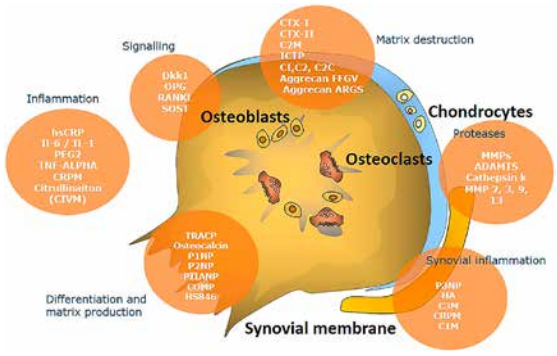

Figure 1. Overview of currently used markers in the rheumatology, divided into areas Inflammation, signaling, matrix destruction, matrix production and differentiation, proteases and synovial inflammation. Reproduced from Lotz M, Martel-Pelletier J, Christiansen C, et al. Value of biomarkers in osteoarthritis: current status and perspectives. Ann Rheum Dis 2013; 72:1756-1763.

Transcriptome analyses of meniscus and anterior cruciate ligament injuries may provide insights into early OA

These were novel discovery studies seeking to determine signaling pathways and specifically expressed transcripts that are different between samples. As with most transcriptomic profiling studies, these investigations are usually undertaken as "hypothesis-free" discovery studies, and do not rely on previous investigations to develop preliminary hypotheses. Clinical studies of athletes and revision anterior cruciate ligament (ACL) reconstruction patients indicate that having a partial meniscectomy, increasing age and elevated BMI are all associated with degenerative changes in knee articular cartilage. Englund et al. have suggested that weakening of the meniscus due to processes similar to OA may be sentinel for the disease.[26] However, little is known about the molecular signatures in injured meniscus. An extensive analysis of gene expression from meniscal fragments recovered from meniscal repair surgery was evaluated for association with the presence or absence of a concomitant ACL injury, age, BMI, and articular cartilage disease in the patient.[27-30] Transcripts associated with extracellular matrix (ECM) synthesis were down regulated in obese individuals (BMI >30) perhaps indicating a higher risk of developing meniscus degeneration. Transcripts upregulated in obese compared to lean or overweight patients were associated with increased apoptosis and suppression of ECM deposition. Patients >40 years of age demonstrated repression of genes for skeletal development, cartilage development, and cartilage ECM synthesis and elevation of genes involved in cell cycle and cell division, immune response, and inflammation pathways. Results such as these may provide a molecular rationale for the known clinical effects of partial meniscectomy, increasing age, and increasing obesity on the development of cartilage degeneration.[31-33]

Further investigation of the relative gene expression levels in the ACL at various times after injury from acute (<3 months) to chronic (>12 months) showed that processes representing angiogenesis were repressed in acute tears. In intermediate tears, processes representing stem cell proliferation con-

comitant with cellular component organization were elevated. In chronic tears, processes denoting myosin filament organization were elevated while those representing cellular component organization and ECM organization were repressed. An ACL tear appears to stimulate local repair processes early after rupture that recede over time. Further transcriptome analysis of injured and OA joint tissues may provide candidates for molecular biomarkers as well as targets for treatment that would reduce the risk of developing OA.[29,32,33]

Fluid ("wet") biomarker assays that are antibody based

Biomarker assessment by immunologic assay has been the standard for analysis in both humans and horses (reviewed recently).[14,15] Progress continues with development of biomarkers for human OA and their use in clinical trials[7,16,18] and knowledge has advanced in parallel in the horse (Table S1, *see page 120*).[5,15] Studies in the horse have shown significant exercise related changes in serum biomarkers of collagen metabolism in young horses.[33] Equine serum markers have also been shown to distinguish changes associated with exercise from pathologic change in exercising horses, and to correlate to clinical parameters of pain in an equine OA model.[12] A clinical study in 238 racehorses, employing monthly musculoskeletal examinations and blood samples, showed that it was possible to correctly predict horses that would sustain an injury 74% of the time.[34]

Recent work evaluating proteinases has shown that: (i) the presence of lumican and a [29] kD lumican catabolite increased with the onset and progression of OA;[35,36] (ii) a splice variant of one of the aggrecanases (ADAMTS4) was identified that appears to be specifically synthesized by human OA synovium and is associated with aggrecan degradation in the superficial zone of articular cartilage;[37] and (iii) synovial fluid ADAMTS4 activity is a marker of inflammation and effusion.[38] Such findings have biologic/disease rationale as confirmed by OA onset in a STR/ORT mouse model being significantly reduced using monoclonal antibodies directed against substrate recognition domains of ADAMTS5.[39]

An anti-cathepsin K antibody has demonstrated significant involvement of cathepsin K in naturally occurring equine and human OA.[40-43] In equine OA cartilage an alternate equine type II collagen specific cathepsin K cleavage site was identified in the N-terminal region of the C-terminal collagen fragment using proteomic and immunological techniques.[43] A novel ELISA assay (C2K77) has been developed to measure the activity of cathepsin K in culture media and is being validated in body fluids.[44]

While trauma is pivotal in the pathogenesis of human knee OA, seemingly equivalent injuries do not invariably result in post-traumatic (ptOA). For instance, only 50% of patients with ACL rupture develop ptOA 10-15 years later, and these numbers are not substantially affected by surgical reconstruction and "restoration" of joint biomechanics.[45-48] This suggests that factors other than joint instability may play a role in the risk, rate of onset, and progression of ptOA after injury. Differences between non-ptOA inducing (sham) and ptOA-inducing joint injury in mice showed differing phases of synovial inflammation with distinct cyclically increased macrophage, CD4 and CD8 T-cell infiltration into the synovium without associated systemic change. Data from Jaffa mice (protected from cartilage damage) suggest that proteolysis of aggrecan by ADAMTS plays a critical role in regulating the inflammatory response in the joint, particularly in macrophage activation and M1/M2 polarization. As has been done in inflammatory arthropathies, monitoring the pattern of cell influx into the joint after injury may be diagnostic and enable differentiation between OA-inducing and non-inducing joint trauma.[49-51]

Examination of proteins from harvested media in an interleukin-1 beta cartilage explant model analyzed by liquid chromatography mass tandem spectrometry (LC-MS/MS) identified cartilage oligomeric matrix protein (COMP) as a potential OA diagnostic in horses. The unique fragments of COMP include the amino acid sequences that form a new terminal (neo-epitope) sequence; polyclonal antibodies that react specifically with this new cleavage site have now been developed.[52] It was concluded that an increase in the COMP neo-epitope in synovial flu-

id from horses with acute lameness suggested that this has the potential to be a unique candidate biomarker for the early molecular changes in articular cartilage associated with OA.

Imaging biomarkers in the horse

Imaging lacks evidence as a biomarker technique for predicting and characterizing musculoskeletal injuries, especially to inform prognosis. Hurdles include limited ability to discern normal tissue adaptation from early disease, limited use of frontline volumetric imaging techniques (usually due to cost), lack of prospective data on imaging biomarkers in relation to disease presence and outcome in the horse, modest correlation between pain and imaging results, and limited follow-up/longitudinal imaging.[13,53] However, progress is being made and novel techniques including digital radiography, ultrasound, nuclear scintigraphy, computed tomography (CT) and MRI are developing. The use of digital radiography, nuclear scintigraphy, CT, and MRI to distinguish changes with exercise versus OA has been published.[13]

Digital radiography technology allows image manipulation to improve lesion detection but a 30-40% change in bone mineral density is still needed to detect lesions, allowing for significant tissue changes to occur prior to detection.[54] Radiological changes in OA are slow to develop, and thereby inhibit intervention in a timely fashion. Joint space width has been used for decades as a measure of joint disease severity, yet it lacks predictive ability for clinical outcomes in humans.[55] Joint space width measurements in equine femorotibial joints have recently been assessed for accuracy and standardization of positioning, as in humans, is essential for maximum accuracy.[56] Radiography, however, continues to be a useful outcome measure in a common model of OA.[13]

Nuclear scintigraphy has been useful in defining the presence of disease compared to increased uptake that occurs with exercise alone in horses.[57] Although nuclear scintigraphy appears helpful in early diagnosis of disease, it lacks the specificity to fully define the lesion, but may be useful for screening and monitoring OA onset or progression in both horses and humans.

Computed tomography has been used clinically to detect occult lesions in subchondral bone. Detection of altered patterns of subchondral bone density by computed tomographic osteoabsorptiometry (CTO) has been used to define joint disease in horses.[13] It appears that CTO density patterns can characterize insidious disease processes, such as palmar osteochondral disease. Intra-articular application of contrast has also been used and provides critical information concerning soft tissues of joints,[58] especially those such as the equine femorotibial joint that can rarely be imaged using MRI.[59] Dual energy CT has also been studied and appears to have value in characterization of soft tissues and detection of bone marrow edema.[60]

MRI has revolutionized the detection of subtle joint disease in all species, and in particular, the detection of soft tissue and articular lesions. However, its resolution is limited and subtle bone and joint lesions can sometimes be missed.[61] MRI has significant potential as a predictive marker of disease as shown by many studies including the MRI component of the OARSI/FNIH study.[61] A recent review has shown that measures of quantitative cartilage morphology, cartilage defect and bone marrow lesions, bone shape and attrition, and subchondral bone area were the most promising as imaging biomarkers.[62]

Quantitative MR imaging has improved characterization of articular cartilage matrix (GAG, collagen, and water) in humans and research animals, with limited use in the horse. dGEMRIC imaging uses intraarticular or IV administration of gadolinium based contrast medium measured in relation to the fixed-charged matrix components, giving an indication of GAG concentration in the cartilage matrix.[63] T1rho has been used in people but not horses, and can give information on GAG content, but can also be influenced by collagen content.[64] Therefore T2 mapping is often necessary for comparison. Sodium MR imaging is also correlated to GAG, but requires special equipment and high field strength for scanning.[65] T2 and T2 imaging can be used to charac-

terize collagen content within articular cartilage, but often require long scan times.[66] Diffusion weighted techniques measure water diffusion through the matrix and appear to have promise in best characterizing matrix integrity.[67]

Standing low-field MRI systems have been useful in the horse for identifying osseous pathology, which appears to carry various (but ill-defined) risks of sustaining catastrophic injury,[68,69] but their usefulness is limited to the distal limb; because of low quality resolution only rudimentary visualization of the articular cartilage is possible limiting early identification of cartilage pathology.

All imaging modalities to date focus on identifying tissue changes after the initiating insult. Much like genetic markers, using biomechanical modeling to identify those horses with joints that may be geometrically predisposed to disease has potential uses for identifying risk and modulating exercise to lower risk and/or severity of disease.[70]

The use of spectroscopy as a biomarker

In the case of naturally occurring equine traumatic OA, the Fourier transform infrared spectroscopy (FTIR) approach has been confirmed as highly accurate for synovial fluid when compared to arthroscopy.[71] The limitations of such studies are that they have been conducted on clinically apparent cases and have not been tested in a preclinical population of horses for which prospective synovial fluid analysis would be impractical.[35]

One of the significant advantages of FTIR as a biomarker tool is that the spectra generated from serum or any other body fluid, encompass not only known markers but also unknown markers.[71] Current work has used transmission FTIR that is expensive but more cost-effective clinical platforms are being developed.[72]

Metabolomics and proteomics

There has been increasing interest in profiling the metabolome, consisting of the low molecular weight end products of cell metabolic processes which indicate the cellular function of a given cell type or tissue under specific conditions.[73,74] The principal analytical techniques used in metabolomics are mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.[75] Compared to MS, NMR spectroscopy is non-destructive and requires little sample preparation, and can generate a comprehensive metabolomics profile from intact bio-fluids and tissues.[76] However, in certain instances this technique is insufficient to provide information that will fully characterize a metabolite and MS analysis has the advantage of higher sensitivity.

In OA, metabolomic fingerprinting has been performed on urine samples from Hartley guinea-pigs which spontaneously develop OA.[77,78] MS-based proteomics techniques have also been used to determine the underlying mechanisms of musculoskeletal aging, OA and tendon injury in equine SF from normal and OA racing Thoroughbreds as well as equine cartilage and tendon from normal or diseased young and old donors (Table S1, *see page 120*).

Proteomic analysis of the OA cartilage secretome identifies molecules with roles in the pathologic processes and allows the global study of secreted proteins while also potentially enabling biomarker discovery. In one study an equine degradome using a mass spectrometry-based absolute quantification method using a concatamer of selected quantotypic peptides representative of proteins (QconCAT) was designed to measure specific cleaved ECM proteins.[79] There was a significant decrease with age of the mean concentration of aggrecan G3 that is explained by loss of G3 soon after cartilage aggrecan synthesis and a steady decline in turnover producing a loss of G3 in the resident aggrecan molecules. The result is that the average size of aggrecan decreases with age, and a large proportion of aggrecan lacks a G3 domain.[80]

Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) was used to examine proteins in situ at high spatial resolution in an examination of full-thickness equine cartilage slices; identified ECM proteins included COMP, fibromodulin, biglycan, and type II collagen. In addition, a number of OA and age specific markers were identified.[81]

Proteomic profiling of equine synovial fluid from normal and OA metacarpophalangeal joints using label-free quantification approaches following protein equalization techniques identified 754 proteins in synovial fluid, 593 with a significant Mascot score. Proteins identified included those relating to matrix proteins, inflammatory factors, complement activation proteins and proteases. A subset of 10 proteins were identified which were differentially expressed in OA synovial fluid. This distinct set of proteins could provide potential biomarkers to stratify OA.[82] Although frequently used in clinical research, substantial challenges remain before this technology can be employed as a biomarker in a clinical setting.

Next-generation sequencing (NGS) and a computational strategy to support biomarker and therapeutic discovery

With NGS approaches, it is possible to identify subtle unique genomic variations encoded in each individual's genome and identify the transcriptionally active genes in individual tissues. This provides the ability to explore associated differences in coding or transcriptional activity with clinical observations, ultimately affording cause-effect relationships that impact aspects of the individual's health status. Knowledge of the extent of an individual's unique genomic variation, which genes are transcriptionally active and the pathway assignments of each gene provides information about the metabolically active processes and how the host's tissues metabolic activity differs after injury compared to a healthy state. Further, this global approach holds the promise to not only discern early pre-symptomatic disease, but also identify susceptible individuals.

In addition to global post-genomic experimental techniques, powerful analytical strategies are required to fully utilize the resulting large and complex data-sets. To address this need, iterative feature removal (IFR) analysis was developed to identify molecular features that can be used as classifiers for metabolic activity and as diagnostics.[83] The IFR process works by repeatedly building a predictive model on training data using a classifier that assigns non-zero weights to only a minimal subset of non-redundant features. IFR assists investigators with process discovery in a way that alternative feature selection approaches cannot. IFR analysis, when applied to global biological data-sets, allows for more comprehensive evaluation of linked metabolic processes. When applied to transcriptional data, IFR identified sets of genes that were highly predictive even when the sets were comprised of genes that, taken individually, appeared non-discriminatory. The efforts here not only identify biomarkers that are classifiers for disease, but also provide biomarkers that hold the potential to screen for disease susceptibility.

Due to the global analysis offered by NGS, this strategy can also be used to identify pathways associated with therapeutic intervention and healing. Based on observations that IGF-I could function as an anabolic factor for the treatment of OA, a gene therapy approach was taken to produce IGF-I and NGS was used to map the biological response associated with the observed healing effects in an equine study.[84] Analysis of the resulting transcriptional response to IGF-I therapy revealed that genes and metabolic pathways associated with specific extracellular matrix collagen types were differentially regulated, as in cartilage development and chondrocyte differentiation. NGS analysis afforded a differential expression fingerprint that could potentially be used to monitor treatments of OA.

Biobanks to classify biomarkers in different stages of equine OA

In order to validate existing and develop new wet biomarkers it is critical that sufficient well-documented equine samples are available to the research community. Potential biomarkers can be tested using standard samples from biobanks and classified according to: Burden of disease (B), Investigative (I), Prognosis (P), and Efficacy of treatment (E), Diagnostic (D), and Safety (S) (BIPEDS).[85] Safety was added in a second OARSI White Paper.[18] Four equine biobanks are actively archiving specimens or are proposed:

1. Young horses sampled every third month during a training program with speed training gradually increasing during the study period. This biobank can test potential biomarkers for D (acute lameness) and P (initiation and progression).

2. Joints, sampled at one abattoir or necropsy. The articular cartilage should be characterized as being macroscopically normal or with mild, moderate, or severe lesions. Radiographic examination of the dissected bones should be included categorizing the bone according to the extent of sclerosis. These structural OA joints can be used for testing biomarkers as B (degree of structural OA) and D (Structural OA).
3. Horses in conventional training/racing and undergoing arthroscopy of different joints. The SF is aspirated during arthroscopy, and material from synovial membrane, synovial capsule and osteochondral fragment, when appropriate, is immersed in buffered formalin.
4. Clinically lame horses examined by routine lameness examination sometimes including the lameness locator test,[86] evaluating acute and chronic lameness before and after local anesthesia. These fluids can test biomarkers for clinical OA as P (prognosis), E (efficacy), and D (diagnosis).

These biobanks will consist of serum and synovial fluid (SF), and where possible tissues from synovial membrane/capsule and articular cartilage (including subchondral bone). Samples of the SF would be analyzed for total protein (g/L) and total number of leucocytes, and the remainder centrifuged for 20 min, 16,000g and aliquots' (100 m/L) frozen at 80°C and stored until analyzed. Signed ethical approvals and consent of the owners is mandatory for all samples.

Conclusions

New information and studies in equine musculoskeletal biomarkers have potential translational value for humans and vice versa. Osteoarthritis is equally important in both humans and horses and the welfare issues associated with catastrophic musculoskeletal injury in horses add further emphasis to the need for good validated biomarkers in the horse. Further progress in identifying useful human and equine biomarkers requires exploratory studies to identify promising candidates combined with the development of reliable assays. To prove clinical utility and acquire regulatory approval for a biomarker is a demanding task, requiring retrospective hypothesis-generating and prospective hypothesis-testing studies in several study populations. The equine athlete offers a unique "at risk" population with a high incidence of naturally occurring clinically important musculoskeletal disease including OA, that is ideal for the discovery and validation of biomarkers across the BIPEDS spectrum. In addition, by having established inducible models in the same species, the biomarkers can be used in development of new therapeutics which simultaneously validates their utility in monitoring disease progression and response to treatment. To take advantage of this opportunity will require establishing standardized methods of sample collection, reproducible biomarker measurement, and well-documented biobanks akin to those in human medicine. Meeting these challenges will not be insubstantial, but the potential rewards for the equine industry and how this will inform human health, are enormous.

Authors' contributions

CWM provided the initial 10,000-word summary, which was reviewed by all the other authors. CWM also wrote the first draft. All authors have read and approved the final manuscript.

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Biomarkers for equine joint injury and osteoarthritis: supplemental table S1 and references cited

Table S1. List of synovial fluid, serum and urine biomarkers that have been reported and used in the horse.

Name of biomarker	Process measured and fluid evaluated (reference)
Carboxy propeptide of type II collagen (CPII)	Removal of CPII as the triple helix of type II collagen is formed reflecting anabolic process of type II collagen synthesis (also useful in osteochondrosis). Synovial fluid and serum.[1-4]
CS-846	CS epitope associated with newly synthesized proteoglycan but mainly reflects activated metabolic rate with synthesis and cleavage of CS. Synovial fluid and serum.[2-5]
C2C	Degradation of type II collagen. Synovial fluid and serum.[3,5-12]
C1, 2C	Type I and II collagen degradation (also useful in osteochondrosis). Synovial fluid and serum.[3,4,13]
Type II Collagen C-telopeptide fragments (CTX-II)	This ELISA measure cleavage in the C-terminal telopeptide associated with type II collagen degradation. Synovial fluid and serum.[13-16]
Coll II-1 NO2	Type II collagen degradation. Synovial fluid and serum (also equine osteochondrosis).[17,18]
CPII/C2C ratio C2C and CS846/GAG ratio	Measuring ratios of anabolic vs. catabolic markers for specific cartilage matrix components useful in osteochondrosis. Synovial fluid and serum.[11,19]
Cartilage oligomeric protein (COMP)	A biomarker of degradation and synthesis correlated with OA. Antibody recognizes intact and breakdown fragments and a positive association with osteoarthritis and osteochondral fractures. Synovial fluid, serum and urine.[20-23] COMP neoepitope in synovial fluid is a potential unique candidate biomarker for the early molecular changes in articular cartilage associated with OA.[24]
Glycosaminoglycans (GAG)	Increases of serum GAG levels (measured with DMMB assay). Elevated consistently with clinical and experimental equine osteoarthritis as well as in response to phenylbutazone use.[2,11,25]
5D4 (specific KS epitope)	Biomarker of GAG degradation but serum levels found not useful in cases of clinical osteochondral disease in horse.[26] Another KS epitope-based ELISA in synovial fluid was consistently elevated after intraarticular administration of triamcinolone acetonide.[27]
Osteocalcin (OC)	Small noncollagenous protein associated with bone assembly and turnover. Synovial fluid and serum OC showed significant increase with OA affected horses compared to non-OA affected horses.[2] Decreases with exercise in non-OA horses in studies of clinical cases[28] and spikes 4-6 months pre-injury for intraarticular fractures.[29]
Bone-specific alkaline phosphatase (BAP)	Isoform of alkaline phosphatase that is expressed at high levels on the cell surface of the bone forming osteoblasts and plays a role in bone formation. Correlation between synovial fluid levels and arthroscopically defined joint damage.[28]
Type I Collagen C-telopeptide fragments (CTX-1)	Suggested as potentially useful for bone resorption and destruction but both serum and synovial fluid CTX-1 was not useful in separating early experimental OA from exercise alone[2] but significantly increased in serum of foals with exercise (but then decreased after two months of training)[30] and serum levels significantly increased in horses with experimental OA treated with extracorporeal shockwave therapy.[31]
Serum Amyloid A (SAA)	The major acute-phase protein in horses and dramatic increases in synovial fluid and serum are seen during severe, acute as in LPS induced synovitis[32,33] and septic arthritis.[34] Useful in discriminating between infectious and non-infectious joint conditions but appears of little value in non-infected arthropathies including OA.[35]
Prostaglandin E2 (PGE_2)	An arachidonic acid derivative locally released into synovial fluid by inflamed synovial membrane and in lesser part by articular cartilage[36] and has been used routinely to gauge inflammation level as well as effect of therapeutic agents reducing inflammation.[37,38] PGE_2 is one of the most useful treatment efficacy markers when investigating medications. PGE_2 concentrations were significantly increased in synovial fluid in association with experimental equine OA[2] compared to controls (exercised horses).

Name of biomarker	Process measured and fluid evaluated (reference)
Interleukin-1 (IL-1)	Despite lack of a consistent association of synovial fluid IL-1beta levels with disease presence or severity, attenuation of IL-1 induced changes in response to exogenous therapy is generally perceived as positive.[39] IL-1beta increased progressively with clinical OA.[40]
Interleukin-6 (IL-6)	Dramatic increases in synovial fluid IL-6 in carpal joints with osteochondral lesions has been reported[41] and suggested as an excellent predictor of OA compared to control horses.[42] Synovial fluid IL-6 progressively increased in equine clinical arthritis.
High Mobility Group Box Protein-1 (HMGB-1)	This is a nuclear chaperoned protein, rapidly reduced in synovial fluid but not serum with onset of local joint inflammation[43] and significantly higher synovial fluid concentrations found in 45 Thoroughbred racehorses with osteochondral injury compared with 40 sound controls.[44] Preliminary value as a diagnostic or prognostic marker for OA.
Tumor Necrosis Factor-alpha (TNF-alpha)	Cytokine biomarker of inflammation increased progressively with progression of PTOA and correlated with radiographic progression of PTOA.[44]
General Matrix Metalloproteinase (MMP)	Activity assay. This assay detects only MMP-mediated substrate conversion and reflects the overall MMP activity in synovial fluid samples. There were significant increases in activity associated with moderate to severe cartilage damage (joints were examined postmortem).[45] General MMP activity relative to control joints at 8 hours after single injections of reIL-1beta or LPS.[46]
Stromal Cell-Derived Factor-1 (SDF-1)	Activates and enhances release of MMPs from chondrocytes and serum SDF-1 concentrations were more sensitive than plasma and synovial fluid concentrations for detection of osteochondral injury in equine joints.[47]
Proteomics	Comprehensive protein profiling of synovial fluid in equine osteoarthritis following protein equalization. Synovial fluid was used from 9 normal and 9 OA Thoroughbred horses and analyzed with LC-MS/MS using a Nano Acquity LC coupled to a Ltq Orthitrap® velos (protein equalization using ProteoMiner®). Looking at lower abundance protein fractions as well as immunohistochemistry, Western blocking and mRNA expression analysis.[48]
Characterization of neo-peptides in equine articular cartilage degradation	This study was done with equine cartilage explants and looked at proteolytic cleavage products following trypsin digestion and identification using tandem mass spectrometry.[49] The identification of novel peptide fragments provides a platform for antibodies that could assist in the identification of biomarkers for OA. OA, as well as identification of basic biochemical processes underlying OA.

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Incomplete fracture of the talus secondary to maladaptive stress remodeling in a horse.

This is a summary of an article by Drs. S. Katzman, M. Spriet, B. Beck, M. Barrett, and D. Hendrickson in the Journal of the American Veterinary Medical Association.

Take home message

Incomplete fracture of the talus is an important differential diagnosis for horses with acute hind limb lameness following strenuous exercise with abrupt changes in direction. Oblique radiographic projections of the tarsus may be useful for fracture identification, but definitive diagnosis may require advanced diagnostic imaging modalities such as CT and MRI. Horses with incomplete fracture of the talus can successfully return to performance level with conservative management and treatment.

Introduction

A 6-year-old Quarter Horse gelding was evaluated for acute onset of non-weight-bearing lameness of the left hind limb following strenuous exercise, specifically barrel-racing. The injury was characterized as acute development of non-weight bearing lameness (lameness score, 5/5) of the left hind limb 30 minutes after its fifth and final run during a competition. The lameness rapidly improved with complete resolution within 1 hour after onset. Approximately 72 hours following the initial presentation of lameness, the horse again presented for non-weight bearing on the left hind limb. The veterinarian who evaluated the horse was unable to localize the source of the lameness during the physical examination and did not perform a full lameness test due to the severity of the lameness. Due to unremarkable findings of manipulation and palpation of the limb, there was concern that the lameness originated from the pelvic or proximal femoral region. The horse was referred to UCD VMTH for further evaluation and received phenylbutazone (2g, IV) prior to transport to the teaching hospital. The horse was bright, alert, and responsive with appropriate vital parameters within reference limits upon physical examination. The application of a hood tested to the left hind hoof elicited no response. There was noted mild symmetric effusion of the tarsocrural region bilaterally. The horse allowed flexion, extension, and abduction of the left hind limb, but mildly resented adduction of the limb. Digital pulses of the right hind limb were mildly increased from normal, and a support boot was applied to aid in prevention of laminitis in the left hind limb.

Methods

Nuclear scintigraphy was performed the day following initial examination at the teaching hospital. The horse was administered technetium Tc 99m methylene diphosphonate (0.3 mCi/kg [0.14 mCi/lb], IV) 4 hours before bone-phase scintigraphy. The horse was sedated with detomidine hydrochloride (0.01 to 0.03 mg/kg [0.005 to 0.014 mg/lb], IV) and butorphanol tartrate (0.01 to 0.02 mg/kg [0.005 to 0.009 mg/lb], IV) and positioned squarely on all 4 limbs; Full weight bearing position could not be achieved for the affected left hind limb. Lateral and plantar images of the appendicular skeleton of both hind limbs, and dorsal and oblique images of the lumbar vertebrae and pelvis were obtained.

Standard radiographic examination of the left tarsus was performed twenty-four hours after the nuclear scintigraphy evaluation. Radiographic projections obtained included dorsoplantar, lateromedial, and dorsal 45 ° lateral-plantaromedial, dorsal 45° medial-plantarolateral oblique projections, flexed lateromedial and flexed dorsoplantar. Ultrasonography was also performed. Intra-articular anesthesia of the left tarsocrural joint was performed to further understand abnormalities found during radiographic and ultrasonographic examination that were not correlated with the nuclear scintigraphy results. Following aseptic preparation of the left tarsal region, a 21-gauge, 1.5-inch hypodermic needle was inserted into the dorsomedial pouch of the tarsocrural joint,

and 20 mL of 2% mepivacaine hydrochloride was injected into the joint.

A series of 3 intra-articular injections of ACS into the left tarsocrural joint with a 2-week interval between injections was prescribed. The dosing interval for the ACS was based the clinical experience of the attending clinician (SAK). It was recommended that the horse be stall-confined for 30 days with reevaluation at 2-week intervals with a follow-up radiographic study of the left tarsus obtained at the end of the 30 day period.

A physical examination and MRI were performed one week post-discharge to assess lameness and baseline movement. The horse was positioned in left lateral recumbency, and MRI of the left tarsus was performed with an intermediate-feld 1.0-T magnet.g T1 fast-spin echo, proton-density fast-spin echo, T1 gradient echo, and short tau inversion recovery sequences were obtained in the sagittal, dorsal, and transverse planes. Following the MRI, additional radiographs were obtained to further characterize the fracture.

Results

Nuclear scintigraphy revealed marked focal IRU in the proximal left talus. Mild diffuse IRU at the distal right tarsus was identified but considered an incidental finding (Figure 1).

Radiographs included 2 approximately 1.5-cm-long, smoothly marginated, ovoid osseous fragments with 1 adjacent to the dorsal medial malleolus and 1 distal to the dorsomedial aspect of the medial malleolus. Mild tarsocrural joint intracapsular swelling and a small focal concavity on the proximal aspect of the medial trochlear ridge of the talus were noted but were considered static compared to radiographs from 8 months prior. Ultrasonography revealed moderate desmitis of the short component of the medial collateral ligament origin and moderate tarsocrural joint effusion and synovitis. The 2 osseous bodies identified on radiographs were associated with medial malleolar avulsion fractures.

Left hind limb lameness (lameness score, 2/5) improved one week post- initial examination MRI images revealed an undulating fracture with irregular

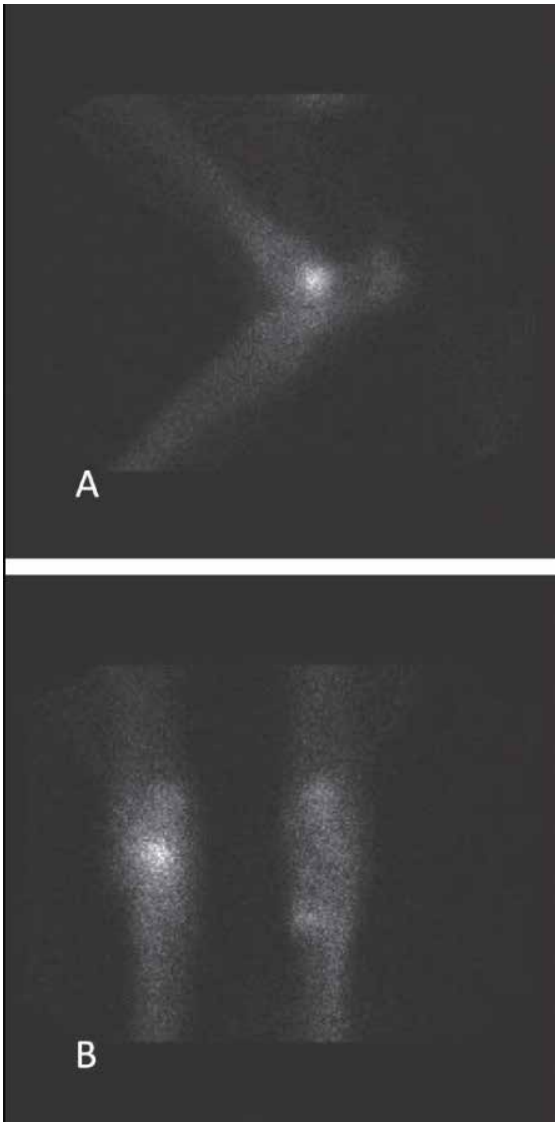

Figure 1. Lateral nuclear scintigraphic image of the left tarsus (A) and plantar nuclear scintigraphic image of the left and right tarsi (B) of a 6-year-old Quarter Horse gelding used for barrel racing that was evaluated for acute onset of non-weight-bearing lameness of the left hind limb following strenuous exercise. Notice the marked IRU within the tarsocrural region at the level of the talus.

margins that extended across the talus. The fracture originated at the trochlear groove and extended in a dorsomedial direction into the medial trochlear ridge of the talus (Figure 2). Marked osseous sclerosis coupled with an increase in the osseous fluid signal was present along the length of the fracture. A mild step defect associated with the fracture was

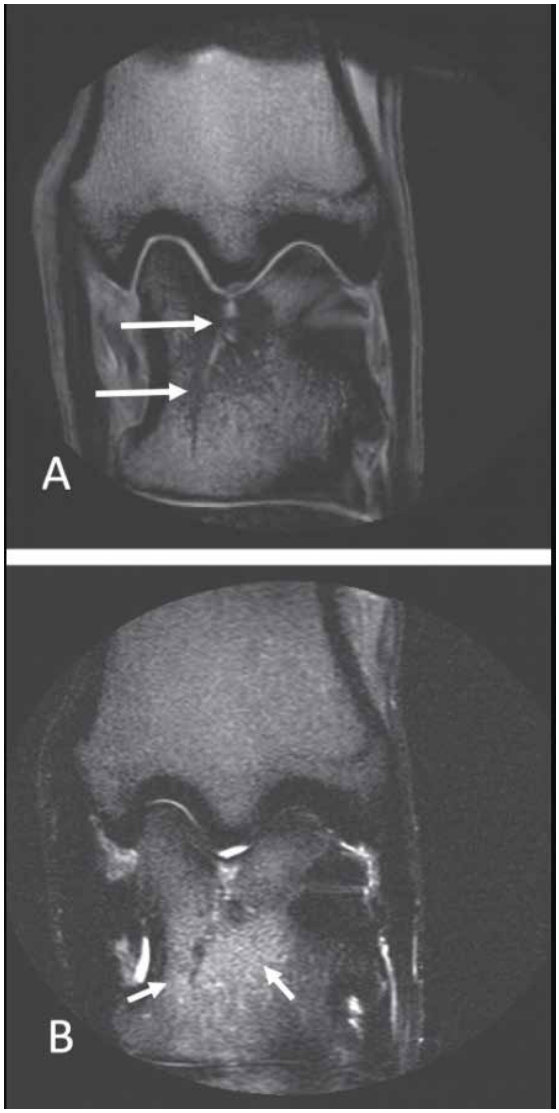

Figure 2. Dorsal plane T1 gradient echo (A) and short tau inversion recovery (B) MRI images of the left tarsus of the horse of Figure 1 that depict an incomplete fracture in the talus (long arrows; A). Notice the marked abnormal fluid signal (short arrows; B) that is characteristic of osseous contusion. These images were obtained approximately 2.5 weeks after the onset of lameness and the nuclear scintigraphic evaluation.

detected at the dorsoproximal aspect of the medial trochlear ridge. A small round osseous fragment was identified embedded in the origin of the short component of the medial collateral ligament and was associated with thickening of the ligament at its origin.

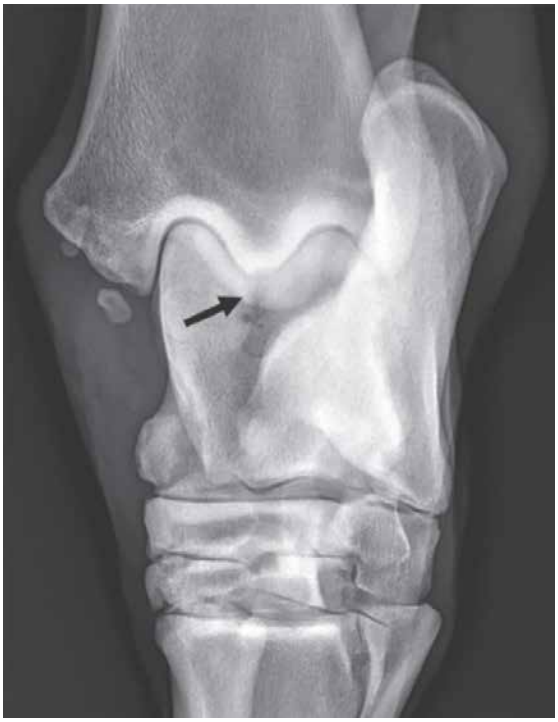

Figure 3. Dorsal 20° lateral-plantaromedial oblique radiographic image of the left tarsus of the horse of Figure 1 obtained following completion of the MRI examination. Notice that the talus fracture (arrow) is evident. The 2 approximately 1.5-cm-long, smoothly marginated, ovoid osseous fragments adjacent to the medial malleolus that were observed in the initial radiographic study performed 24 hours after the nuclear scintigraphic evaluation are also present. Medial is to the left.

Additional radiographs of the left tarsus further characterized the fracture. The fracture is best visualized on a dorsal 20° lateral-plantaromedial oblique projection, as depicted by the black arrow (Figure 3).

Conclusion

Incomplete fractures of the talus are an important differential diagnosis for severe acute hind limb lameness following strenuous activity in equine patients. Standard radiographic evaluation of the tarsus, consisting of the minimum number of projections, may be insufficient, thus it is essential to obtain additional oblique projections of the tarsus for proper diagnosis and to reduce the need for advanced diagnostic imaging modalities, such as CT and MRI. If properly identified, talus fractures in equine patients can be successfully managed with

conservative treatment to treat lameness and return to competing at previous levels within 15 months post-onset of lameness.

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The relationship between repository radiographic findings and subsequent performance of Quarter Horses competing in cutting events

This is a summary for a study performed by Drs. M.F. Barrett, C.W. McIlwraith, E.K. Contino, R.D. Park, C.E. Kawcak, D.D. Frisbie and JR zum Brunnen in the Journal of American Veterinary Medicine Association in 2018.[1]

Take home message

Most abnormalities identified at repository radiographs in cutting horses were not significantly associated with subsequent performance.

Introduction

Use of repository radiographs has not been included as standard practice as part of the sale process for Thoroughbred yearlings intended for racing. 2-3 common multiple studies 4-7 have been performed with the objective of examining the clinical importance of pre-sale radiographic findings in Thoroughbreds. Repository radiographs findings must be interpreted with consideration of the various breeds and athletic disciplines involved. 8 This paper was the second part of two studies initially evaluating the prevalence of radiographic lesions on repository radiographs 458 yearlings and 2-year-old quarter horses competing in cutting events. There was a follow up to see what the significance of these findings were. We hypothesize that many mild radiographic lesions would not be associated with reduced performance, whereas more severe lesions would be more likely to be clinically important.

Methods

Repository radiographs are obtained from the Western Bloodstock radiograph repository for horses offered at NCHA sales held between December 2005 and December 2006 and from a privately-owned cutting horse ranch as previously described. 9 Performance data was obtained through a review of performance record from the NCHA database and through the use of mail questionnaires and telephone calls to owners.

Lesions of the tarsal joint – The lateromedial, dorsoplantar, dorsolateral-plantaromedial oblique, and dorsomedial-plantarolateral oblique views of the tarsal joints were examined for radiographic evidence of osteophytes, subchondral lysis, and sclerosis of the distal portions of the tarsal joint and for malformation of the central and third tarsal bones. The presence of osteophytes was graded from 0 to 4, corresponding to none, very small, small, medium, and large. Subchondral lysis was also graded on a scale of 0 to 4, ranging from none to severe. The severity of lysis was evaluated on the basis of both the extensiveness of the lysis and the depth of the subchondral bone affected. Sclerosis was graded from 0 to 3, ranging from none to severe, with severe designated as increased bone density affecting > 50% of the bone. Malformation was defined as an abnormal wedge shape or crushing and graded on a scale from 0 to 2, with 0 representing no evidence of malformation, 1 representing mild dorsal wedging, and 2 representing unequivocal severe asymmetry in the proximal-distal diameter of the bone or central crushing.

Lesions of the proximal interphalangeal (pastern) joint – Evaluation of the pastern region was included in evaluation of the metacarpophalangeal and metatarsophalangeal joints. Thickening of the dorsoproximal cortex of the middle phalanx of the hind limb was graded as absent (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). If the dorsal border was irregular, a minimum of grade 2 was applied. Osteophytosis of the dorsoproximal aspect of the hind limb middle phalanx was graded as 1 (present) or 0.

Outcome parameters were as follows:

1. Did the horse compete in a cutting competition (yes or no)?
2. Did the horse earn money in a cutting competition (yes or no)?

3. If so, how much?
4. If the horse did not compete, why not?

Lesions of the medial femoral condyle were graded as follows:

Grade 0 = normal appearance of the medial femoral condyle

Grade 1 = flattened contour of the medial femoral condyle but no radiographic evidence of changes in the subchondral bone

Grade 2 = Subchondral bone sclerosis defects in the subchondral bone that did not extend all the way through the deep portions of the subchondral bone plate or both

Grade 3 = Defects that extended through the subchondral bone such as wide subchondral lucencies (SCL)

Grade 4 = Well defined round or oval radiolucent area in the middle of the medial femoral condyle that extended to and communicated with the femoral tibial joint.

Results

Of 458 eligible horses, 343 had complete radiographic studies available for review and were included in the study. For 27 of these horses, radiographs were obtained from the privately-owned cutting horse ranch. Earnings data were available from the NCHA database for 178 of the 343 (52%) horses included in the study. Horses for which earnings data were not avail-able either did not compete or competed but did not earn any money. Therefore, for all horses for which earnings data were not available, an attempt was made to determine whether the horse had or had not competed through the use of a mailed questionnaire or telephone calls to the owner.

Information on whether the horse did or did not compete was available for 103 of the 165 (62%) horses for which earnings data were not available. Of these 103 horses, 29 (28%) competed but did not earn money and 74 (72%) did not compete. For horses that did not compete, lameness diagnosed by a veterinarian was the most commonly reported reason (n = 16 [22%]). Lameness referable to the stifle region was reported in 2 of the 16 horses, 1 of which had radiographically normal stifle joints and 1 of which had bilateral grade 3 medial femoral condyle lesions. Lameness affecting both the tarsal and stifle joints was reported in 1 horse that radiographically had mild osteophytosis of the distal tarsal joints and radiographically normal stifle joints. Lameness affecting the tarsal joints only was reported in 2 horses, 1 of which had radiographically normal tarsal joints and 1 of which had mild osteophytosis of the distal tarsal joints. Four horses had lameness attributable to suspensory ligament injuries. Other reported reasons that horses did not compete included that they were used for other disciplines, had insufficient talent, had medical restrictions unrelated to lameness, and were still in training.

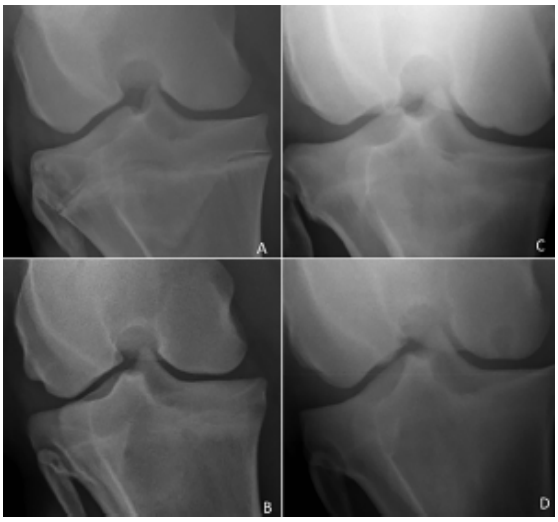

Figure 1. Representative caudocranial repository radiographic images of the stifle joint included in a study evaluating possible associations between repository radiographic findings and performance outcome for Quarter Horses (n = 343) competing in cutting events. These images illustrate the grading scheme for evaluation of the medial femoral condyle. A – A flattened contour of the medial femoral condyle is present but without any evidence of changes to the subchondral bone (grade 1). B – Notice the mild subchondral bone sclerosis and a subtle shallow defect in the subchondral bone that does not extend all the way through the deep portion of the subchondral bone plate (grade 2). C – A shallow concave defect that extends through the subchondral bone is evident (grade 3). D – A well-defined, round radiolucent cyst-like lesion is present in the trabecular subchondral bone of the medial femoral condyle (grade 4).

Analysis of the likelihood (yes vs no) that horses would compete included data only for the 281 horses that were definitively known to have (n = 207) or to have not (74) competed. Analyses of the likelihood (yes vs no) that horses earned money as a 3-year-old, as a 4-year-old, or as a 3- and 4-year-old combined were performed with data for all 343 horses included in the study Analyses of the amount of money earned as a 3-year-old, as a 4-year-old, and as a 3- and 4-year-old combined were performed with data for the 178 horses for which earnings data were available.

Only 2 of the 7 radiographic lesions included in the analyses were significantly associated with performance outcomes: presence of osteophytes involving the distal aspect of the tarsal joint, and presence of osteophytes on the dorsoproximal aspect of the hind limb middle phalanx. The presence of grade 2 osteophytosis involving the distal aspect of the tarsal joint was associated with significantly increased odds of not earning money as a 3-year-old (P = 0.01; OR, 3.19; 95% CI, 1.29 to 7.91; r2 = 0.05) and as a 4-year-old (P = 0.003; OR, 2.5; 95% CI, 1.38 to 4.56; r2 = 0.08). The presence of grade 1 osteophytosis of the distal aspect of the tarsal joint was also associated with a significantly increased odds of not earning money as a 4-year-old (P = 0.014; OR, 2.18; 95% CI, 1.17 to 4.06; r2 = 0.08). Conversely, the presence of an osteophyte on the dorsoproximal aspect of the hind limb middle phalanx was associated with significantly decreased odds of not earning money as a 4-year-old (P = 0.004; OR, 0.36; 95% CI, 0.18 to 0.71; r2 = 0.08).

The remaining 5 radiographic lesions included in the analyses (abnormalities of the medial femoral condyle, subchondral lysis of the distal aspect of the tarsal joint, sclerosis of the distal aspect of the tarsal joint, malformation of the central and third tarsal bones, and thickening of the dorsoproximal cortex of the middle phalanx of the hind limb) were not significantly associated with performance outcomes.

Table 1. Repository radiographic findings for 281 Quarter Horses that subsequently did (n = 207 [74%]) or did not (74 [26%]) compete in cutting events.

Radiographic finding and score	No. (%) of horses	
	Competed	Did not compete
Medial femoral condyle		
Grade 0	11 9 (75)	39 (25)
Grade I	44 (75)	1 5 (25)
Grade 2	22 (79)	6 (21)
Grade 3	1 3 (68)	6 (32)
Grade 4	9 (53)	8 (47)
Tarsal joint		
Osteophytes		
Grade 0	121 (79)	32 (21)
Grade I	35 (74)	12 (26)
Grade 2	33 (59)	23 (41)
Grade 3	12 (67)	6 (33)
Grade 4	6 (86)	I (14)
Subchondral lysis		
Grade 0	17 1 (75)	58 (25)
Grade I	1 3 (72)	S (28)
Grade 2	18 (64)	9 (36)
Grade 3	S (71)	2 (29)
Grade 4	0 (0)	0 (0)
Sclerosis		
Grade 0	197 (74)	68 (26)
Grade I	9 (64)	S (36)
Grade 2	0 (0)	I (10 0)
Grade 3	I (10 0)	0 (0)
Malformation		
Grade 0	195 (74)	67 (26)
Grade I	IO (5 9)	7 (41)
Grade 2	2 (10 0)	0 (0)
Middle phalanx of hind limb		
Thickening of dorsoproximal cortex		
Grade 0	17 4 (72)	67 (28)
Grade I	26 (84)	S (16)
Grade 2	6 (10 0)	0 (0)
Grade 3	I (33)	2 (67)
Osteophytes		
Grade 0	178 (74)	63 (26)
Grade I	29 (73)	1 1 (27)

Discussion

Results of the present study suggested that most abnormalities identified on repository radiographs of Quarter Horses competing in cutting events were not significantly associated with subsequent performance. However, the number of horses included in the study likely affected our results. In particular, some radiographic findings of interest affected a small number of horses, resulting in a low power to detect significant associations between the radiographic findings and subsequent performance.

Nonetheless, to our knowledge, the present study represented the largest study evaluating performance outcomes for horses competing in cutting events to date. The 2 radiographic findings that were significantly associated with performance outcome in the present study were osteophytosis of the dorsoproximal aspect of the middle phalanx of the hind limb and osteophytosis of the distal aspect of the tarsal joint. Interestingly, mild (grade 1 or 2) osteophytosis of the distal aspect of the tarsal joint was the only finding that was significantly associated with decreased performance (i.e., decreased likelihood of earning money as a 3-year-old and as a 4-year-old and decreased earnings as a 4-year-old). These findings were surprising, as we had hypothesized that mild changes would be unlikely to be clinically important, in contrast with severe lesions, which we expected to be more likely to be significantly associated with performance.

Whereas we, as other clinicians, have anecdotally considered small osteophytes of the distal aspect of the tarsal joint to be of questionable clinical importance, the results of the present study may lead us to question this assumption. The finding of the present study that the presence of medium and large osteophytes (grades 3 and 4) was not significantly associated with performance outcome could have been a result, in part, of the low number of horses with these lesions (31/343 [9%] horses) and the unequal numbers of horses in the osteophytosis categories, resulting in a low power to detect differences among groups. It is not uncommon in cutting horses for intra-articular medication of the tarsal joint to be administered without prior diagnostic analgesia to con-firm the joint as the source of lameness. Therefore, we suggest that it would also not be surprising to find that horses with moderate to marked radiographic changes were more likely to be treated empirically with intra-articular joint medications, which may have helped improve their performance. Conversely, milder osteophytosis may have been more likely to be dismissed as unimportant, such that these horses would then be less likely to receive intra-articular treatments.

Interestingly, although lameness referable to the stifle joint, particularly the medial femoral condyle, is a major concern for veterinarians treating horses competing in cutting events, radiographic abnormalities of the medial femoral condyle were not significantly associated with performance outcome in the present study. This result was in contrast to our hypothesis that moderate to severe lesions of the medial femoral condyle would be associated with a poorer performance outcome. Our results suggested that lesions of the medial femoral condyle may not restrict a cutting horse's ability to perform successfully, at least for the limited period of time early in its career that, in the cutting horse industry, is the time of greatest earning potential. As for other lesions, it is possible that the lack of significant findings could have been a result of a low power in some analyses, particularly analyses of earnings. However, the power for detecting a 15% difference in odds of earning money was 80%. Thus, we suggest that it should have been possible to detect a clinically relevant difference if one were present. However, it must be kept in mind when interpreting the results of the present study that lameness was not an outcome variable. Whereas an attempt was made to contact the owners of horses that did not earn money to find out why, further follow-up information was not obtained for horses that earned money. Thus, it is possible that there were horses with stifle joint lesions that were able to compete and earn money, yet still developed manageable lameness.

As in any study evaluating radiographs submit-ted to a repository, there was a risk that horses with more severe lesions may not have been included in the sale, decreasing the number of radiographs evaluated in the present study from horses with moderate to severe radiographic lesions. As such, we may have underestimated both the prevalence and potential effect that more severe lesions could have on performance outcome in the overall population of horses competing in cutting events. Nonetheless, we suggest that despite these limitations, the radiographic findings of the present study are a useful representation of the lesions found in horses sold for cutting events at major sales, and it is the performance outcome of these horses that is of particular concern in the cutting horse industry.

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A coaxial dipole antenna for passively sensing object displacement and deflection for orthopaedic applications

This is a summary of an article by Drs. Kevin M. Labus, Branislav M. Notaroš, Milan M. Ilić, Conor J. Sutherland, Amy Holcomb, and Christian M. Puttlitz published in IEEE Access.

Take home message

A non-invasive electromagnetic coupling measurement system for monitoring the progress of bone fracture healing was optimized and tested for feasibility. The study resulted in an antenna design with improved measurement sensitivity, and the feasibility was demonstrated for measuring deflections of a fracture fixation plate under an applied load. This technology has the potential to improve the treatment of fractures on the path to non-union by providing an early diagnosis of aberrant healing.

Introduction

Approximately 10% of bone fractures do not heal properly [1], and the deleterious effects and resultant costs of fracture non-union can be reduced by an estimated 50% if addressed in the early time period of healing when therapies can be most effective [2]. However, radiographic evidence which comprises the current standard of care, fails to provide an early diagnosis of non-union bone healing. A promising approach for monitoring and predicting the course of bone fracture healing is by measuring the mechanical load-sharing between the healing callus and the implanted fixation hardware. Previous technologies have used implantable sensors which require modification to the fixation hardware and may carry long term biocompatibility risks. The objective of this study was to optimize and evaluate a method of externally sensing hardware load-sharing based on the electromagnetic near field effects of a radio-frequency antenna.

Methods

Previous work by our group used a custom antenna, consisting of two parallel coaxial cables, to probe a passive strain sensor [3]. Because this antenna type was observed to be highly sensitive to a fracture fixation plate deflecting under an applied load, it was chosen for optimization as an object displacement sensor for the intended application and current study. The antenna works by detecting movement of a target object (metal fracture plate) within the electromagnetic near field via shifts in the antenna's apparent resonant frequency (ARF). Over time as a fracture heals, the callus stiffens, resulting in lower plate deflections (and thus lower ARF shifts) relative to the applied load. Therefore, the changes in consecutive measurements over time may inform on the progress of healing.

This study included a series of two parametric tests intended to optimize the sensitivity of the antenna to the displacement of a metal plate. The parameters tested were (1) the placement of the plate along the length of the antenna and (2) the spacing between the two coaxial cables. The results of these parametric tests formed the basis for a new antenna design. The resulting antenna underwent two tests to determine its efficacy for sensing the displacement of a metal plate and the deflection of a metal orthopaedic plate in a modeled fracture condition. The physical experiments were augmented with mechanical finite element and electromagnetic computational simulations.

Results

The parametric test on cable spacing indicated that as the distance between the two antenna cables increased, the sensitivity if the antenna ARF to plate movement also increased. The parametric test on plate location along the antenna length demonstrated multiple locations on the antenna that were most sensitive to movement of the plate, which depended on the harmonic frequency range tested. Specifically, for the third resonant frequency harmonic, there were three locations of greatest sensitivity along the length of the antenna. Therefore, an op-

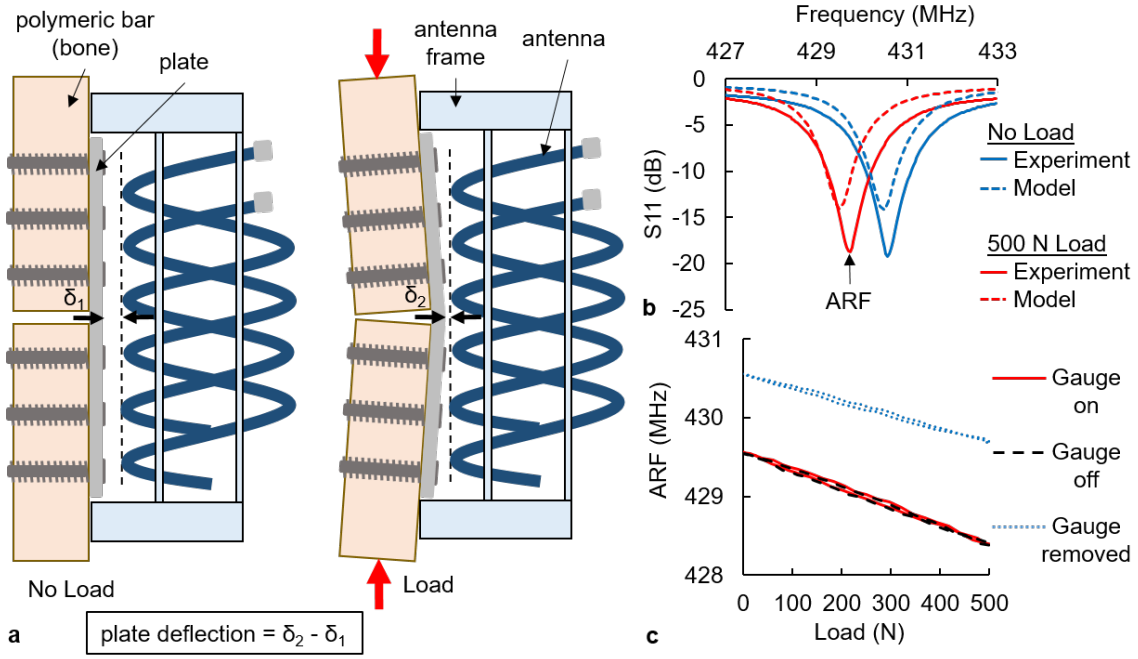

Figure 1. (a) Diagram demonstrating bending of a fracture fixation plate due to a compressive load and the resulting deflection of the plate relative to the antenna. This displacement produces a shift in the measured ARF. (b) |S11| frequency sweeps of the construct in (A) under 0 N and 500 N of load for the plate compression test and corresponding electromagnetic modeling results. ARF was obtained at the minima of the frequency sweep (as indicated by the black arrow for the 500 N experimental curve. (c) ARF versus load curves from the plate compression test comparing the effects of the strain gauge.

timized antenna was built using a three coil design such that all three sensitive locations were aligned on one face of the antenna. Also, the cable spacing was maximized to increase sensitivity, although limited by size constraints for the intended application. The plate displacement test on the optimized antenna showed a highly sensitive, nonlinear relationship between ARF and plate displacement. A test modeling a fracture condition under a compressive load showed a clear shift in the ARF due to the deflection of the fracture plate, and the ARF had a highly linear response to applied load (Figure 1). Electromagnetic computational predictions matched the experimental results within 0.05% error.

Conclusions

Parametric tests on the antenna's geometry resulted in an antenna design that was optimized for high sensitivity to displacements of a metal plate. Testing on that plate confirmed that this measurement system is feasible for measuring the bending deflection of a fracture fixation plate under an applied load. This system has the potential to be used to detect stiffness changes in a fracture that are related to healing to monitor the early progress of healing.

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A comparison of arthroscopy to ultrasonography for identification of pathology of the equine stifle.

This is a summary of a study performed by Drs. A.M. Adrian, M.F. Barrett, N.M. Werpy, C.E. Kawcak, P.L. Chapman, and L.R. Goodrich published in the Equine Veterinary Journal.[1]

Take home message

Ultrasonography and arthroscopy should be combined to best evaluate pathology of the stifle, since each modality has its own limitations depending on the location and type of lesion.

Introduction

Typically, the use of radiography and arthroscopy together with a physical examination and intra-articular anesthesia are used to diagnose equine stifle disease. However, radiography is unable to provide adequate information on the soft tissue structures. There is very minimal association between the radiographic severity grade of the surgery with cases specific to stifle lameness. Radiography also has a difficult time diagnosing mild to severe soft tissue lesions. Arthroscopy allows certain meniscal tears and their morphology and severity to be diagnosed but it also has its limitations. The narrow joint space limits the arthroscopic examination and by only using arthroscopy some injuries in certain locations can remain undiagnosed.

Ultrasonography is a more sensitive diagnostic instrument for intra-articular soft tissue evaluation in the equine stifle. It has a sensitivity and specificity in the diagnosis of meniscal injury and some meniscal lesions may only be diagnosed with ultrasonography. Ultrasonography is also the most adequate diagnostic technique for evaluating patellar ligament injuries.

The hypotheses of the study were:

1) Ultrasonography will detect more lesions of the menisci compared with arthroscopy, arthroscopy is more likely than ultrasonography to detect cartilage lesions of the femoral condyles and cranial meniscotibial ligament injuries.
2) Ultrasonography is less likely to detect more subtle intra-articular lesions in the stifle compared with arthroscopy.
3) Ultrasonography and arthroscopy alone are not sufficient to fully assess damaged tissues within the stifle.

Methods

The structures of the stifle joint were evaluated and graded for pathological change by scoring arthroscopic and ultrasonographic examinations. The presence and severity of the lesions were then compared between each instrument.

Results

It was then observed that medial meniscal lesions were detected more often with ultrasonography. Conversely, arthroscopy was better for the detection of cranial medial meniscotibial ligament tearing. Articular cartilage defects were best detected with arthroscopy and periarticular osteophytes of the medial femoral condyle with ultrasonography. Four cases had defects within one of the patellar ligaments, all of which were only characterized with ultrasonography.

Conclusion

The study found that some structures in the equine stifle are best evaluated by ultrasonography and others by arthroscopy. More meniscal lesions were diagnosed with ultrasonography than arthroscopy, however, articular cartilage lesions within the femoral condyles and tearing of the cranial meniscotibial ligament were better detected with arthroscopy. Subtle lesions in the lateral meniscus and articular cartilage defects of the medial femoral condyle are less likely to be detected with ultrasonography compared with arthroscopy. In conclusion, by us-

ing both ultrasonography and arthroscopy, there is an increased likelihood of detecting lesions in the equine stifle.

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A comparison of arthroscopy to ultrasonography for identification of pathology of the equine stifle

This is a summary of an article by Drs. A. Adrian, M. Barrett, N. Werpy, C. Kawcak, P. Chapman, and L. Goodrich published in the Equine Veterinary Journal.

Take home message

Lesions in equine stifle can be best characterized through thorough ultrasonographic and arthroscopic examination in addition to physical examination, radiography, and intra-articular anesthesia to fully appreciate pathologic changes associated with equine stifle disease.

Introduction

Physical examination, radiography, arthroscopy and intra-articular anesthesia have traditionally been utilized to diagnose and monitor lesions associated with equine stifle disease. Radiographic examination allows proper evaluation of osseous abnormalities in the stifle but provide minimal information regarding soft tissue structures. Despite normal radiographic findings, there can be mild to severe soft tissue lesions present that go undetected.

To properly diagnose and appreciate pathologic changes associated with equine stifle disease, it is ideal to utilize various imaging modalities, such as arthroscopy and ultrasonography, in addition to traditional diagnostic practices, depending on the location of the lesion. Arthroscopic examination is advantageous in diagnosing certain meniscal tears and aids in characterizing morphology and severity. However, examination of the cranial and caudal aspects of the medial and lateral femorotibial joints as well as the axial and abaxial menisci borders are limited with arthroscopy. Ultrasonography is a highly sensitive and specific diagnostic modality that aids in diagnosing and evaluating intra-articular soft tissues lesions; there are specific meniscal lesions that are only identifiable with ultrasonography. Magnetic resonance imaging (MRI) is diagnostically effective at providing additional anatomic detail, including cruciate ligaments and articular cartilage defects. Depending on the location and severity of the lesion, there are diagnostic modalities that better characterize equine stifle lesions in comparison. This retrospective study investigated and compared the ability of diagnostic imaging modalities, such as arthroscopy and ultrasonography, to properly identify presence, severity, and location of pathologic changes within the equine stifle by investigating medial and lateral meniscus, medial and lateral cranial meniscotibial ligament, medial and lateral femoral condyle, and patellar ligaments.

Methods

Ultrasonographic still and arthroscopic images and videos of 47 stifles were retrospectively evaluated from 37 horses that were admitted to the Colorado State University equine hospital for stifle lameness between 2007 and 2011. All ultrasonographic and arthroscopic examination and imaging protocols were similar between cases and performed by board-certified veterinary radiologists or a supervised resident. For the ultrasonographic images, 2 ACVR board certified radiologists and a radiology resident graded each unaware of the arthroscopic findings. For arthroscopy, each image was graded by 2 ACVR board certified surgeons that did not review the ultrasonographic findings prior to review. Lesion evaluation was based on grading systems designed for the medial and lateral meniscus, medial and lateral cranial meniscotibial ligament, medial and lateral femoral condyle, and patellar ligaments.

Medial and lateral meniscus ultrasonographic (transverse images) and arthroscopic images were graded with a total of 47 medial menisci evaluated with 25 identified lesions. For each modality, the grading criteria involved presence of a defect (yes, no), defect severity (slight, mild, moderate, severe), orientation (horizontal, vertical, oblique, round, surface defect), location (abaxial, axial, femoral, tibial,

intrasubstance), and degree of defect extension into the meniscus (axial, abaxial, full thickness, intrasubstance).

The medial and lateral cranial meniscotibial ligaments were assessed by evaluating the insertion of the ligaments (normal, resorptive, proliferative) with each modality. For ultrasonography, the echogenicity (homogenous/heterogeneous) of the ligaments in the long axis was assessed and compared to arthroscopic evidence of fibrillation/tearing (slight, mild, moderate, severe).The medial and lateral femoral condyles were graded for both ultrasonography (long and/or short axis views) based on the presence of an articular cartilage defect, the size of the articular cartilage defect, osteophytosis, and the presence of subchondral bone defect.

Ultrasonography (long and/or short axis views was the only diagnostic modality used to grade medial, lateral, and lateral patellar ligaments. Patellar ligaments were graded on echogenicity (homogenous/heterogeneous) of ligaments, presence of a defect, location, length, diameter, and thickening (slight, mild, moderate, severe) of defects associated with ligaments.

Results

Ultrasonography exhibited a significant difference (P = .02, number with differing findings = 16 (N=16), Supplementary Item 1) in medial meniscus lesion detection compared to arthroscopy. Although 9 lesions were identified by both modalities with 4 similar grades (2 slight and 2 severe), there were 13 lesions (4 mild, 7 moderate, and 2 severe lesions) detected by ultrasonography that were not detected by arthroscopy. For the lesions detected by both modalities, 2 lesions were located in the cranial horn, 4 within the middle aspect, and 2 extending from the cranial horn to the middle aspect. There were 3 lesions in the cranial horn only detected via arthroscopy. Arthroscopic examination did not detect 4 lesions in the cranial horn, 3 lesions in the body, 2 lesions in the caudal horn, 2 lesions that extended from the cranial horn to the body, and one lesion that extended through the entire medial meniscus. For the lesions only detected by ultrasonography, one extended along the axial surface, 7 extended along the abaxial surface, and 2 were full thickness lesions. One lesion was arthroscopically classified as a full thickness tear, however, the lesion was classified as an abaxial defect with ultrasonography. Two lesions detected on the axial margin of meniscus by arthroscopy were diagnosed as intrasubstance lesion and a lesion extending from the axial to abaxial aspects. Ultrasonographic evaluation yielded significantly higher severity grades compared to arthroscopy (16 vs. 6 times, P = 0.052, number with differing findings = 22 (N = 22), Supplementary Item 1). If the grading of the ultrasonography and arthroscopy images differed by more than one grade, the score of the ultrasonography images were higher significantly more often, 14 times, than the score of the arthroscopy examination, 4 times (P = 0.031, Supplementary Item 1).

There was no significant difference (P = 1.0, number with differing findings = 5 (N = 5), Supplementary Item 1) between ultrasonography and arthroscopy for lateral meniscus lesion detection. A total of 34 lateral menisci were evaluated with 6 detected lesions. There are 3 lesions (2 slight, one mild) located on the cranial horn only detected by arthroscopic examination; Ultrasonography revealed one lesion considered to be within the cranial horn extended throughout the entire lateral meniscus into the caudal horn. Arthroscopic examination revealed three lesions on the axial aspect that were not detected via ultrasonography; Ultrasonography revealed that one lesion found on the axial aspect of the lateral meniscus via arthroscopy was shown to extend from the axial to the abaxial aspect of the lateral meniscus via ultrasonography. Two lesions (one moderate, one severe) were only identified using ultrasonography with one lesion within the abaxial aspect of the meniscus and one extended from axial to abaxial aspect.

The osseous insertion of the medial cranial meniscotibial ligament was assessed in 25 equine stifles. Both arthroscopy and ultrasonography graded 15 osseous insertions as normal and one as proliferative. Arthroscopy graded 2 insertions as proliferative that were graded as normal via ultrasonography. Ultrasonography graded one insertion as resorptive and 6 as proliferative that were graded as normal with arthroscopy. Arthroscopic and ultrasonographic images of the medial cranial meniscotibial ligament from

29 stifles were evaluated for tearing. Arthroscopy exhibited a significant difference (P = 0.02, number with differing findings = 11 (N = 11), Supplementary Item 1) for detecting medial cranial meniscotibial ligament lesions compared to ultrasonography (Figure 3). There were 18 stifles with normal echogenicity and no arthroscopic evidence of tearing. There were 9 tears detected by arthroscopy with only one lesion exhibiting slight ultrasonographic echogenicity change. There were two ligaments with slight, 3 with mild, and 3 with severe tears that had normal ultrasonographic echogenicity.

Arthroscopy exhibited a significant difference (P<0.0001, number with differing findings = 21 (N = 21), Supplementary Item 1) in articular cartilage defect detection compared to ultrasonography. A total of 27 medial femoral condyles were evaluated with 21(2 slight, 9 mild, 9 moderate, and one severe, Figure 4) articular cartilage defects detected by arthroscopy that were not detected by ultrasonography. Arthroscopy also exhibited a significantly (23 times vs. 3 times, P<0.001, number with differing findings = 26 (N = 26), Supplementary Item 1) higher severity gradings associated with femoral condyle articular cartilage defects compared to ultrasonography. Two defects graded as severe with arthroscopy were graded as mild and moderate, respectively, with ultrasonography. If there was agreement within one grade

There was no significant difference (P>0.9, number with differing findings = 15 (N =15), Figure 5, Supplementary Item 1) in lesion detection regarding subchondral bone defects between arthroscopy and ultrasonography. Both modalities graded 8 as normal and 11 with subchondral bone defects. Only 4 defects were diagnosed using only one modality. Ultrasonographic gradings were lower than arthroscopic gradings for 5 defects with no significant difference when comparing absolute grades to arthroscopy. Two lateral femoral condyles were evaluated and both had subchondral bone defects only diagnosed with arthroscopy.

There was a significant difference (P<0.001, number with differing findings = 14 (N = 14), Supplementary Item 1) between the osteophyte detection with ultrasonography detecting more lesions than arthroscopy. From the total 18 medial femoral condyles that were evaluated for periarticular osteophytosis, there were 3 graded as normal by both modalities and one stifle with osteophytosis along the medial femoral condyle. Ultrasonography graded 14 stifles

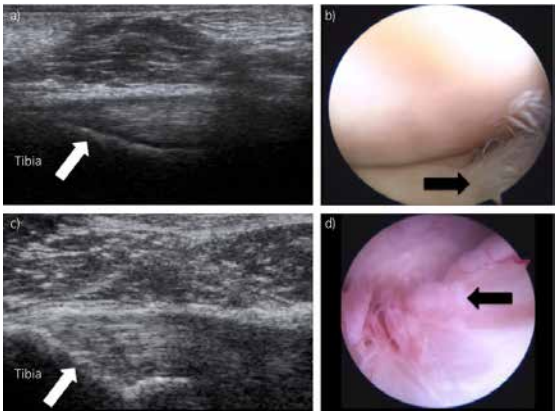

Figure 3. Ultrasound and arthroscopy images of the medial cranial meniscotibial ligaments from 2 different horses. The ultrasound images are longitudinal images of the ligament, obtained with the probe oriented perpendicular to the long axis of limb. Axial is to the left. Ultrasound (a) and arthroscopy (b) images of Horse 1. (a) Note the smooth tibial insertion of the cranial meniscotibial ligament to the left of the image (white arrow). No fibre damage is noted. (b) There is fraying of the medial cranial meniscotibial ligament (black arrow). Ultrasound (c) and arthroscopy (d) images of Horse 2. (c) There is irregular osseous proliferation of the insertion of the cranial meniscotibial ligament on the tibia (white arrow). This corresponds with the osseous proliferation (black arrow) diagnosed via arthroscopic evaluation shown in (d).

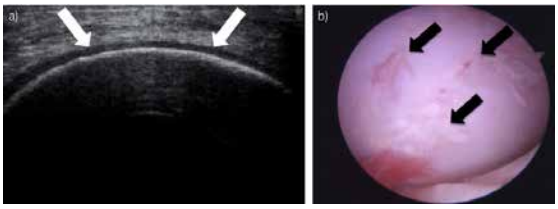

Figure 4: Ultrasound and arthroscopy images at the same area of the medial femoral condyle from one horse. The ultrasound image was obtained in the transverse plane relative to the long axis of the limb with axial to the left. (a) The articular cartilage (white arrows) of the medial femoral condyle appears normal. (b) Severe cartilage defects (black arrows) are noted on the weightbearing aspect of the medial condyle.

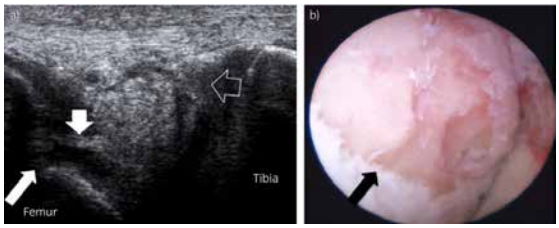

Figure 5: Ultrasound and arthroscopy images of the medial femoral condyle from one horse. The ultrasound image was obtained in the longitudinal plane relative to the long axis of the limb with proximal to the left. (a) On the ultrasound image a moderate subchondral bone defect is present in the distal aspect of the medial femoral condyle (white arrow). The echogenic region (white arrow head) overlying the subchondral bone defect indicates associated articular cartilage loss; however, the nontangential orientation of this image does not allow adequate direct assessment of the cartilage. The medial meniscus is moderately heterogeneous and has a moderate oblique defect at its tibial surface (open arrowhead) (b) On the arthroscopy image, articular cartilage and subchondral bone loss is apparent on the weightbearing surface of the medial femoral condyle (black arrow). No debridement had taken place at that point.

with osteophytosis along the abaxial periarticular margin of the medial femoral condyle. Four lateral femoral condyles were evaluated and no osteophytes found.

A total of 35 medial patellar, 41 middle patellar, and 34 lateral patellar ligaments were evaluated using ultrasonography, only. Two medial patellar ligaments had thickening and altered echogenicity (one moderate and one severe) while 3 medial patellar ligaments had a discrete defect (one mild, 2 severe) and one of these 3 was also one of the 2 with diffuse echogenic change (Figure 6). One middle patellar ligament had mild echogenic changes, while another middle patellar ligament had severe echogenic changes, thickening and a severe distinct defect in the middle to distal aspect. All lateral patellar ligaments had normal echogenicity; however, one was slightly thickened.

Conclusion

The study reveals that specific structures of the equine stifle are better evaluated with ultrasonography than arthroscopy and vice versa. Ultrasonography allowed enhanced evaluation of periarticular soft tissues and lesions associated with patellar ligaments. To properly evaluate pathologic changes associated with stifle disease, a thorough ultrasonography and arthroscopic examination should be performed.

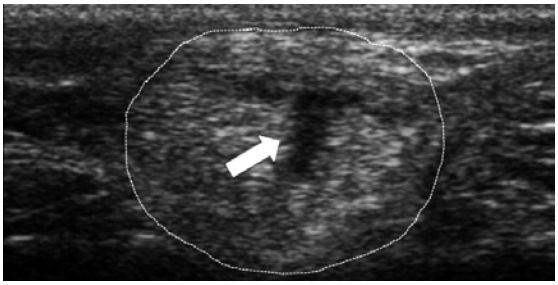

Figure 6: Transverse ultrasound image of the medial patellar ligament (outlined by dotted line). Lateral is to the left. The hypoechoic T shape area within the ligament (white arrow) represents focal moderate to severe fibre tearing, which extended from the proximal to mid-aspect of the ligament.

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Additional palmaroproximal-palmarodistal oblique radiographic projections improve accuracy of detection and characterization of equine flexor cortical lysis

This is a summary of an article by Drs. S. Johnson, M. Barrett, D. Frisbie published in Veterinary Radiology & Ultrasound[1]

Take home message

Skyline radiographs using a flatter angle of incidence improve radiographic detection and characterization of flexor cortical lysis severity, may allow the beam to be tangential to the more distal portion of the navicular bone, and are recommended when flexor cortical lysis is suspected.

Introduction

Radiographic flexor cortical lysis indicates advanced degenerative change and its earlier recognition may improve case outcome. Aims of this prospective, diagnostic accuracy study were to determine effects of radiographic beam angle and observer on accuracy of lesion detection.

Methods

The sample included 36 limbs from 31 horses. Palmaroproximal-palmarodistal oblique (skyline) radiographs were acquired at standard (n=38) and more shallow (alternate) angles (n=58). Images were independently reviewed by four experience levels of five observers each (n=20) for the presence and severity of flexor cortical lysis. Observers also reported their confidence in these answers. Responses were compared based on seeing a standard skyline or multiple projections. The definitive presence (or absence) and severity of lysis was based upon radiologist consensus agreement.

Results

When assessed by observer, the identification of lysis and the assessment of its severity was most similar to that of radiologists when observers of all levels of experience were able to view multiple skyline projections (P = 0.399 and P= 0.174). Using multiple views to detect lysis resulted in improved sensitivity (85.3% vs 97.2%, P<0.001), decreased specificity (82.8% vs. 74.5%, P = 0.03), and improved interobserver agreement (86.0% vs. 90.2%, P = 0.21). On average, observers of all levels of experience became more confident viewing multiple projections (P< 0.001).

Conclusions

Findings from this study supported acquiring an additional skyline view at an alternate angle of incidence. This view resulted in improved sensitivity, decreased specificity, and overall improved interobserver agreement among observers of various experience levels. Additionally, radiographic detection and characterization of flexor cortical lysis severity in observers of various experience levels was similar to that of radiologists when an additional, shallow view was provided. When one to two alternate angle skyline views were available in addition to the standard skyline view, interpreter confidence increased on average 30% of the time. The standard recommendation to obtain an optimally positioned navicular skyline radiograph by positioning the x-ray beam tangential to the flexor surface of the navicular bone13 may therefore not identify all lesions of the flexor cortex. The curvature of the flexor cortex may limit a single beam angle from highlighting the proximal to distal surface entirely within one radiographic projection; therefore, an additional skyline radiograph made with a more shallow beam angle is recommended when flexor cortical lysis is suspected.

Acknowledgements

We thank Dr. Ann Hess for statistical consulting.

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High field magnetic resonance imaging is comparable with gross anatomy for description of the normal appearance of soft tissues in the equine stifle

This is a summary of an article by Drs. Jodie Daglish, Dave Frisbie, Kurt Selberg and Myra Barrett, published in Veterinary Radiology and Ultrasound in 2018.[1]

Take home message

High field magnetic resonance imaging (MRI) of the equine stifle provides high spatial and contrast resolution of the soft tissue structures. This allows visualization of detailed structural characteristics, such as striations and variations in signal intensity that are not well documented on low field MRI. Findings were comparable between gross evaluation and MRI, and this study provides detailed descriptions of the normal appearance of the soft tissue structures of the equine stifle on high field MRI, including how to optimize evaluation of specific structures via selection of specific sequences or planes of acquisition.

Introduction

The equine stifle is a large and complex joint, with numerous intra- and periarticular structures that may be injured via trauma or overuse, resulting in the stifle joint being a frequent source of lameness or poor performance.[2,3] The deep location of multiple of the soft tissue structures and the restricted ability to distract the joint are factors that inhibit the diagnostic value of standard imaging modalities such as radiography or ultrasound.[4,5] In particular, complete assessment of the cranial and caudal meniscotibial ligaments, the cranial and caudal cruciate ligaments, the meniscofemoral ligament, articular cartilage, and subchondral bone is virtually impossible with any one modality, including diagnostic arthroscopy[6,7] but may be more thoroughly evaluated with three-dimensional advanced imaging.

Magnetic resonance imaging is considered to be the gold standard for non-invasive imaging diagnosis of soft tissue or cartilage abnormalities.[8,9] Currently, utility of high field equine stifle MRI is limited by there being only a small number of locations able to provide this service. Additionally, at present, patient selection for equine stifle in high field MRI systems is restricted by bore configuration and patient size.[10,11]

Continued pursuit of advanced imaging in the equine athlete has led to specific MRI techniques for regions of interest in the distal limb;[9,12] however, stifle MRI is still a new and developing field.[13,14] Accurate interpretation of clinical MRI studies of the equine stifle requires a good understanding of the normal anatomical appearance of the soft tissue structures of the stifle and clinical application of MRI physics. Development of an optimized protocol specific to the equine stifle is indicated.

The primary aims of this study were to develop an optimized high field MRI protocol for evaluation of the equine stifle, and to provide detailed descriptions of the normal high field MRI appearance of the soft tissues for use as a reference in future studies of clinically affected horses. A secondary aim was to specifically emphasize sequence selection and choice of slice orientation to optimize evaluation of structures that are poorly visualized with other non-invasive imaging modalities, such as the cranial and caudal cruciate ligaments.

Methods

The study was a prospective, anatomic design; it involved the use of no live animals. Materials were gathered postmortem and owner permission for postmortem examination and use of tissue for research was given. Nine cadaver stifles were collected from clinical cases euthanized for reasons unrelated to hind limb lameness. Each case history was reviewed to establish no record of stifle related lameness and each limb was confirmed within normal limits on palpation before inclusion in the study.

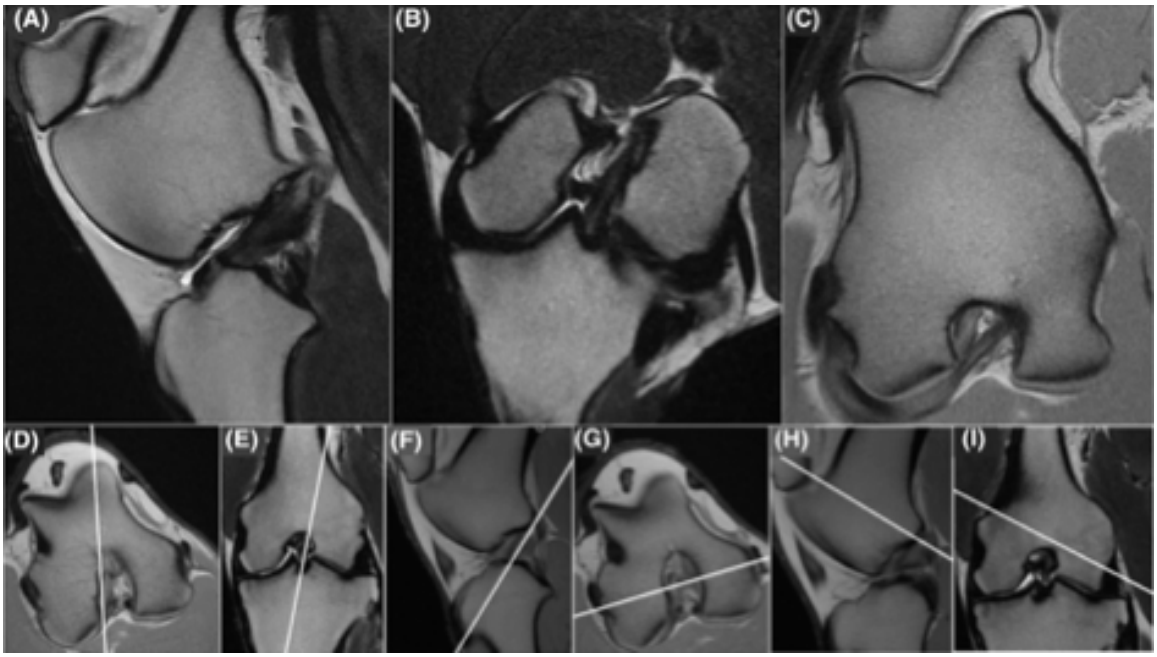

Figure 1. Oblique sequences specific to the cranial cruciate ligament orientation. A, Sagittal Oblique plane sequence. B, Dorsal Oblique plane sequence. C, Transverse oblique plane sequence. Positioning of the oblique plane relative to the standard dorsal, transverse and sagittal plane sequences is highlighted on images below each oblique. D, Standard transverse plane. E, Standard dorsal plane, white lines delineate the relative orientation of Sagittal oblique plane. F, Standard sagittal plane. G, Standard transverse plane, white lines delineate the relative orientation of Dorsal oblique plane. H, Standard sagittal plane. I, Standard dorsal plane, white lines delineate the relative orientation of transverse oblique plane. Image from Daglish et al., 2018.[1]

Limbs were each imaged 1-2 hours post mortem. Ultrasound evaluation of the stifle was performed as described by Barrett et al.,[4] to establish normality of the soft tissue structures of the joint. Each limb was then imaged in a 1.5 T MRI scanner, utilizing an extensive protocol designed to produce multiple imaging sequences suitable for comparison of the appearance of each soft tissue structure between sequences, and to best determine which sequences would be useful implemented in a clinical protocol. Specialized oblique plane images were acquired to capture the anatomical extent and appearance of the cranial cruciate ligament (Figure 1).

Each MRI sequence was evaluated to determine which best delineated the anatomical boundaries of each structure, including the patellar ligaments, joint capsules, collateral ligaments, articular cartilage of the femoral condyles, trochlear ridges, trochlear groove and patellar, menisci and associated ligaments, and the cruciate ligaments. Each structure was then described with respect to shape, position, signal intensity on each sequence and finally, relationship with associated structures. Areas previously reported as common sites of structural abnormality[13,14] were specifically evaluated for relative signal intensity, particularly for differences between proton density fast spin echo (FSE) and T2 FSE weighted sequence appearance, for subsequent comparison with gross findings.

Lastly, each stifle was grossly evaluated. The structures named above were evaluated in detail following dissection. Specifically, for each structure, signs of inflammatory change, alteration in structural integrity, and congruity of bone-soft tissue interfaces were assessed and recorded. The appearance of each structure was compared with the optimized MRI images for conformation of anatomical characteristics.

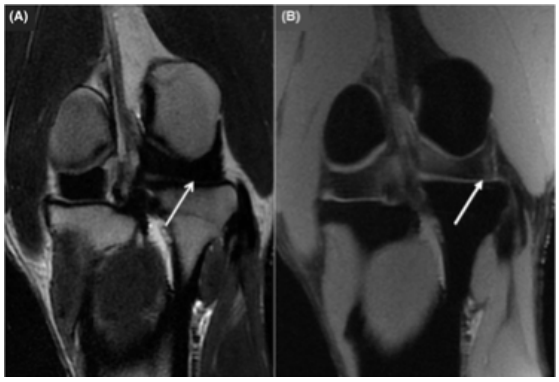

Figure 2. A, T2 fast spin echo Dorsal plane image demonstrating the normal, uniform low signal intensity lateral meniscus (white open arrow). B, Proton density fat saturation dorsal plane image at the same site, demonstrating the intermediate signal within the abaxial margin of the lateral meniscus (white closed arrow) created by magic angle artifact due to relative fiber orientation. The T2 sequence can be used to confirm normality of the tissue at this location. Image from Daglish et al., 2018.[1]

Results

Ultrasound evaluation of the 9 stifles identified no abnormalities of the soft tissue structures where evaluation was possible.[6] Similarly, no gross abnormalities were observed within the soft tissue structures of any of the stifles evaluated.

Preferred sequences found to be most useful for evaluation of the soft tissue structures of the stifle on MRI were presented and MRI details of specific structures are documented below.

Menisci

The curving fibers of the lateral and medial margins of the medial meniscus are subject to magic angle artifact that is observed as intermediate weighted signal compared to the low signal intensity of the mid body of the structure. Magic angle effect at this location is confirmed by comparing the region of increased signal on proton density FSE sequences to the homogenous, low signal of the meniscus on T2 FSE sequences. T2 FSE images are less susceptible to magic angle artifact (Figure 2).[15] and confirm this as the MRI appearance of a normal meniscus.

Meniscotibial ligaments

The medial and lateral cranial and (medial) caudal meniscotibial ligaments have low intensity signal with intermediate weighted signal striations best observed on transverse and transverse oblique planes. On sagittal plane images, the striations appear as punctate intermediate signal within the low signal intensity ligament. Grossly, all stifles had broadly separated and prominent striations within each of these ligaments, with no overlying fascial tissue, (Figure 3). High signal intensity striations within the ligament on short tau inversion recovery sequences were not associated with any gross findings that would be indicative of tearing at these

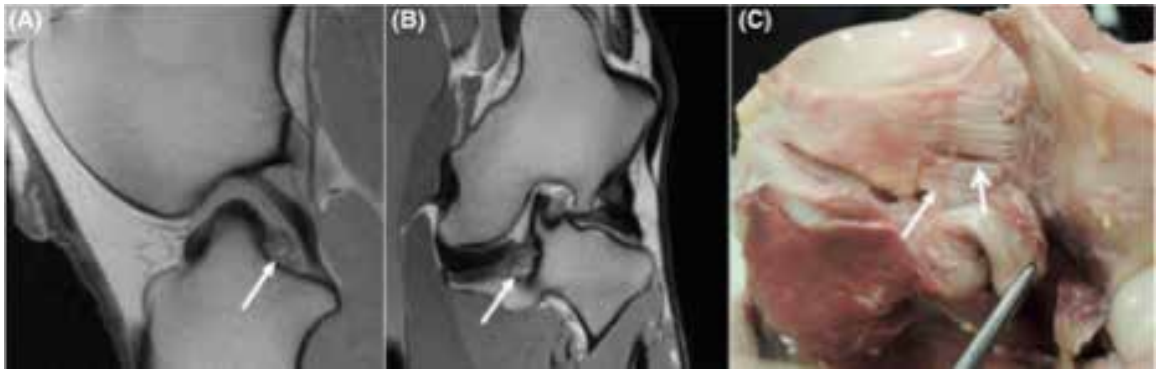

Figure 3. A, Sagittal proton density image demonstrating the loose fiber pattern of the medial caudal meniscotibial ligament in cross section. B, Transverse oblique proton density image of the longitudinal appearance of the medial caudal meniscotibial ligament at the insertion on the tibia. C, Corresponding gross specimen to demonstrate the appearance of the ligament fibers at the insertion onto the tibia. The large space within the ligament fibers (open white arrow) was a common finding to all specimens. Image from Daglish et al., 20181.

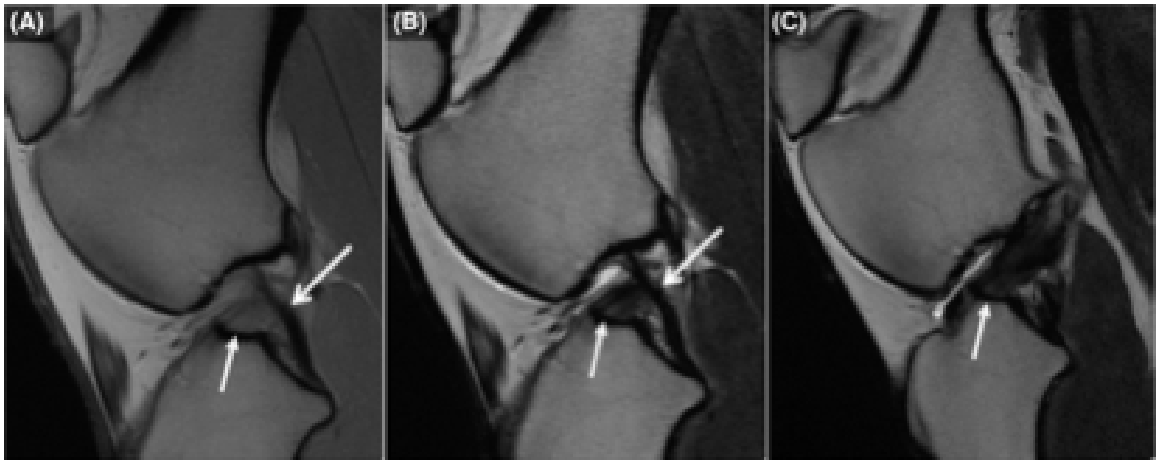

Figure 4. A, Sagittal proton density image demonstrating the tibial insertion of the cranial cruciate ligament (closed arrow) and the relationship to the caudal cruciate ligament (open arrow). B, The ligaments are better defined from the surrounding fascial tissue on T2 fast spin echo sequences, as shown here. This allows better assessment of the ligament margins and demonstrates the intermediate signal intensity throughout the cranial cruciate ligament well. C, Sagittal oblique T2 fast spin echo image, demonstrating how the oblique plane can improve the overall assessment of the cranial cruciate ligament and its relative bone interfaces. Image from Daglish et al., 20181.

sites, and therefore may be indicative of synovial fluid infiltration of the ligament striations

Popliteus

The tendon of popliteus originates at the lateral epicondyle of the femur, lies deep to the lateral collateral ligament and superficial to the lateral meniscus. The tendon of popliteus has a similar signal intensity as the lateral meniscus in all sequences, and causes the caudal margin of the lateral meniscus to appear irregular or undulating, with an inconsistent region of intermediate signal intensity observed at the junction between the two structures. The origin of the tendon of popliteus is also closely associated with the proximal third of the lateral collateral ligament, making differentiation of the two structures difficult on standard plane sequences. Careful consideration of these structures on tangential imaging planes is required to accurately assess the margins of each.

Cruciate ligaments

The cranial cruciate ligament is intermediate weighted on proton density FSE sequences and diffusely low signal intensity on T2 FSE sequences. On both proton density and T2 FSE sequences there is diffusely increased signal (high on proton density FSE and intermediate on T2 FSE sequences) associated with the proximal and distal surfaces of the ligament, and within the distal third of the ligament. This corresponds to twisting of the ligament fibers from proximal to distal and the presence of shallow striations and fanning of the ligament as it broadens to attach at the medial aspect of the cranial intercondylar eminence.

Due to the oblique orientation of the ligament from proximal, caudal, and lateral to distal, cranial, and medial, it is not seen in its entirety on any standard plane images. Specifically developed, obliquely oriented sequences (Figure 4) improve the assessment of the origin, body and insertion of the cranial cruciate ligament, as has been found in the human patient.[16] The ligament structure is best assessed with sagittal oblique plane images. Transverse oblique plane images allow complete evaluation of the bone attachment at origin and insertion and are useful for assessment of resorption or change in bone density associated with potential pathology at these sites.

Conclusions

In conclusion, high field MRI provides highly detailed assessment of equine stifle soft tissues. The structures of the stifle are numerous, are uniquely orientated relative to each other, and have specific anatomic or structural variations individual to each

structure. Interpretation of studies acquired is therefore challenging and requires good knowledge of anatomic detail, a clear understanding of the physics of MRI and exposure to multiple studies for familiarization before diagnoses can be made with confidence. Future studies are needed to determine the clinical application of high field MRI of the equine stifle. The MRI appearance of the soft tissues of the stifle compared with arthroscopic appearance of those visible intra-articularly will provide useful information. Similarly, performing cadaver studies on horses with known stifle disease can provide a greater understanding of how progressive disease within the joint may influence the health of the structures not commonly evaluated with routine radiographs and ultrasound.

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High field magnetic resonance imaging contributes to diagnosis of equine distal tarsus and proximal metatarsus lesions: 103 horses

This is a summary of an article by Drs. M Barrett, K Selberg, S Johnson, J Hersman, and D Frisbie published in Veterinary Radiology and Ultrasound.

Take home message

This study reveals that a range of lesions in the distal tarsus and proximal metatarsus are common in lame horses with a positive response to diagnostic analgesia of the proximal suspensory ligament. It is recommended that standardized MRI is utilized to include both the tarsus and proximal metatarsus for complete evaluation of both anatomic regions.

Introduction

Although radiography is often utilized to diagnose hind limb lameness due to lesions in the distal tarsus, there are many lesions in the distal tarsus and proximal metatarsus that are undetected by traditional radiographic examination. Magnetic resonance imaging (MRI) and localization of lameness via local and intra-articular analgesia possess diagnostic advantages that enhance evaluation of these lesions. Magnetic resonance imaging (MRI) can identify bone marrow lesions within distal tarsal bones, commonly affecting the central tarsal bones. The diagnostic modality has been reported to be useful in detecting osteoarthritis and subchondral cystic lesions of the distal intertarsal and tarsometatarsal joints that could not be characterized radiographically. MRI has also been proven to characterize fractures of the distal tarsus more effectively than radiographic examination. The goal of the study is to further characterize and describe MRI lesions found in the equine distal tarsus in a larger sample group than previous research. The study aims to compare these findings to the horses' clinical history, including degree of lameness and results of diagnostic analgesia to MRI findings.

Methods

The retrospective, observational study consisted of evaluation of MRI exams of the distal tarsus and proximal metatarsus between 2012 and 2014. There were 125 limbs from 103 horses evaluated by two board-certified veterinary radiologists, who were blinded to the clinical presentation of the patient. The MRIs were evaluated for soft tissue and osseous changes that were characterized and graded by degree of severity. Patient signalment, lameness severity, and results of diagnostic analgesia were recorded.

The following MRI lesions were recorded for each scan: osteoarthritic changes of proximal intertarsal (talocalcaneocentral), distal intertarsal, and tarsometatarsal joints (including articular cartilage damage, subchondral lysis, and osteophytosis); sclerosis and bone marrow lesions of the cuboidal bones; and degenerative changes of the second or fourth tarsal bones and their articulations with the central and third tarsal bones. Subchondral cystic lesions were also recorded and were defined as well-defined, smooth lesions that extended into the trabecular bone of the cuboidal bones and had internal signal characteristics consistent with fluid. Degenerative changes of the interosseous ligaments (intertarsal desmopathy) and their respective fossae of the distal intertarsal and tarsometatarsal joints (intertarsal enthesopathy) were defined as thickening and abnormal signal intensity of the ligament and altered shape and sclerosis of the ligament fossa. The proximal suspensory ligament was evaluated for evidence of desmopathy and the proximal third metatarsal bone for enthesopathy and bone marrow lesions associated with the origin of the suspensory ligament. All findings were graded on a scale of 0-3, for normal, mild, moderate, and severe change. The radiologists recorded scores independently and consensus was reached on discordant scores.

Results

The most common finding that affected 100 limbs was sclerosis of the central tarsal bone with 48 moderate and four severe cases. Sclerosis of the third tarsal bone affected 70 limbs with 27 moderate and four severe cases. Osteophytosis of the distal intertarsal joint affected 87 limbs, with 21 moderate and 10 severe, and affected the tarsometatarsal joint in 64 limbs with nine moderate and five severe. Subchondral bone lysis and articular cartilage damage was found in the distal intertarsal joint of 52 limbs and in the tarsometatarsal in 64 limbs.

Fifteen limbs had moderate distal intertarsal subchondral lysis and eight had severe subchondral lysis. Of the limbs with moderate or severe distal intertarsal lysis, the most change was appreciated dorsally in 10 limbs, while it was diffuse in nine and confined to the plantar third of the joint in four limbs. Articular cartilage damage in the distal intertarsal or tarsometatarsal joint without concurrent mild lysis of the subchondral bone was rare, affecting only eight limbs. While the articular cartilage was generally best assessed on the proton density-fat saturated images, often the more subtle concurrent subchondral bone lesions were best appreciated with the volumetric interpolated breath-hold examination images (Figure 1).

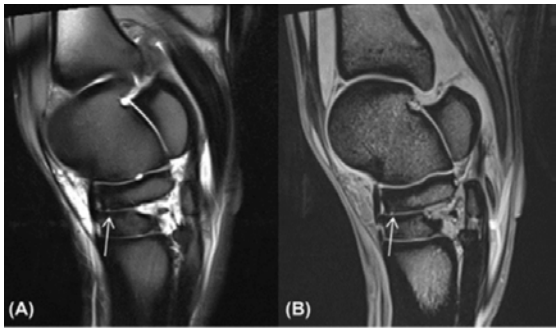

Figure 1. Sagittal plane proton density fat-saturated (A) and volumetric interpolated breath-hold examination (B) images. There is focal subchondral bone lysis of the distal intertarsal joint with alteration of the signal of the articular cartilage and sclerosis of the central tarsal bone

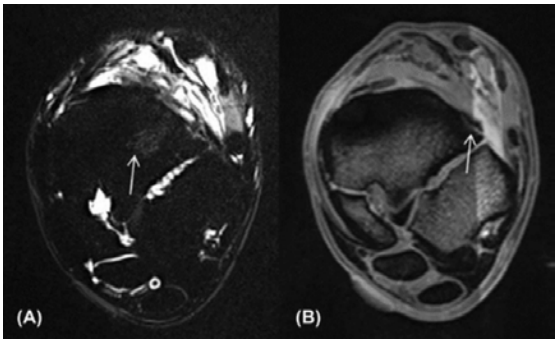

Figure 2. Transverse plane short tau inversion recovery (A) and volumetric interpolated breath-hold examination (B) images at the level of the central tarsal bone. There is a moderate bone marrow lesion (white arrow, image A) and a dorsolateral incomplete cortical fracture (white arrow, image B) of the central tarsal bone

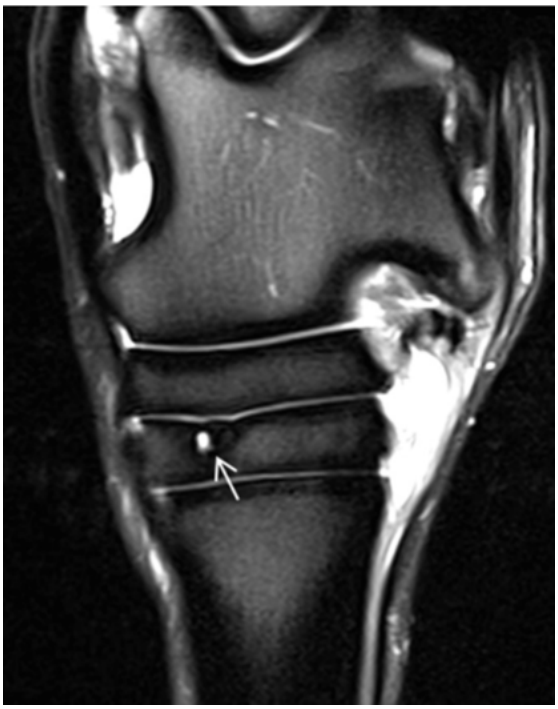

Figure 3. Dorsal plane proton density fat-saturated image. There is a focal subchondral cystic lesion of the third tarsal bone with surrounding bone sclerosis. There is subchondral lysis and mild alteration of the signal intensity of the articular cartilage in the distal intertarsal joint

Conclusions

Magnetic resonance imaging (MRI) should be utilized for complete evaluation of the distal tarsus and proximal metatarsus in lame horses with a positive response to diagnostic analgesia of the proximal metatarsus. The severity of MRI findings is not correlated with the degree of lameness. While radiographic examination is useful, it may be insufficient for complete evaluation of the distal tarsus and proximal metatarsus.

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Magnetic resonance imaging findings of the proximal metacarpus in Quarter Horses used for cutting: retrospective analysis of 32 horses 2009-2012

This is a summary of an article by Drs. M. Barrett, P. Manchon, J. Hersman and C. Kawcak published in Equine Veterinary Journal.

Take home message

Injury to the proximal metacarpal area is a common cause of lameness in cutting horses. Radiographic and ultrasonographic imaging of lesions responsible for lameness can provide ambiguous results, but MRI provides the most comprehensive diagnostic imaging evaluation of lesions specifically affecting cutting horses. Cutting horses with proximal metacarpal pain have significant pathological change within both the proximal suspensory ligament and its enthesis on the palmar cortex of McIII. However, severity of lesions and degree of lameness at the time of diagnosis does not influence return to performance. Accurate diagnosis of proximal metacarpal lesions based on MRI offers clinicians the capacity to select treatment protocols targeted to each disease process.

Introduction

Proximal metacarpal pain is a common source of lameness in performance horses.[1-5] The Quarter Horse is the primary breed used in the western discipline of cutting[1-2], and these horses commence training as 2-year-olds and compete in Limited Age Events from 3 to 6 years of age.[1-3] Cutting horses are of consistent phenotype, are selectively bred for athleticism and trainability, and undergo rigorous training prior to reaching skeletal maturity.[1-3] Lameness associated with proximal metacarpal pain can range from mild to severe.[6-8] Although frequently used for evaluation of the proximal metacarpus, ultrasonographic and radiographic studies have inherent limitations.[9-11] Nuclear scintigraphy (NS) of the proximal metacarpus and metatarsus has been described in normal and lame horses diagnosed with proximal suspensory desmitis, however, it lacks specificity.[12,13] Computed tomography (CT) is capable of identifying pathological bone change; however, CT cannot identify bone edema-like changes and provides limited soft tissue detail, even with contrast enhancement.[14] For these reasons, magnetic resonance imaging (MRI) is considered the gold-standard imaging modality capable of providing best characterization of changes in both hard and soft tissues.[15-18] Pathological changes within the proximal metacarpal area detected by MRI have shown good correlation with histological morphology of the PSL.[16-18] The purpose of this investigation is to describe clinical lesions present in a group of horses with limited physiological variation, and document lesions associated with clinical lameness specific to the discipline of cutting. Secondly, this study aims to establish prognostic indicators for return to performance by evaluating a 24-month follow-up period on each case.

Methods

Retrospective analysis of 32 cutting horses referred for MRI of the proximal metacarpus between 2009 and 2012 with a 2-year follow-up period. Cases were included in retrospective analysis if they were referred for an MRI of the proximal metacarpus following lameness examination that localized a component of lameness to the proximal metacarpus, and were registered Quarter Horses currently in training for, or competing in, cutting events. MRI studies were evaluated by a board-certified veterinary radiologist; the severity of lesions was graded from 0 (absent) to 3 (severe).

Results

A total of 20 right and 24 left forelimbs (12 bilateral studies) of 32 horses were evaluated. The most common findings were: third metacarpal (McIII) sclerosis at the proximal suspensory ligament (PSL) origin (42/44), McIII resorption at the PSL origin (32/44), PSL dorsal margin fiber irregularity (30/44) and McIII bone contusion (22/39). Of the 30 horses, 22 horses successfully returned to competition, irrespective of

severity of injury. Strong correlation exists between the degree of resorption in the palmar cortex of proximal McIII, degree of McIII sclerosis and severity of dorsal margin fiber irregularity.

Conclusions

It may be concluded that pathological change in both the PSL and proximal McIII are responsible for the clinical manifestation of PSD. Enlargement of the PSL can be subjectively assessed and confirmed with CSA measurement; an increase in this parameter supports the presence of concurrent osseous resorption and sclerosis in palmar McIII. The severity of these lesions does not appear to influence long-term athletic performance in the discipline of cutting. Future characterization of the disease process responsible for clinical lameness is essential to implement appropriate treatment and rehabilitation of PSD.

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Magnetic resonance imaging findings of the proximal metacarpus in Quarter Horses used for cutting: retrospective analysis of 32 horses

This is a summary of an article by Drs. M. Barrett, P. Manchon, J. Hersman, and C. Kawcak published in the Equine Veterinary Journal.

Take home message

Injury to the proximal suspensory ligament in cutting horses is a common cause of lameness. MRI imaging providing the most comprehensive diagnostic imaging modality to evaluate these lesions. Pathological changes within the proximal suspensory ligament and its enthesis on the palmar cortex of McIII are associated with proximal metacarpal pain in cutting horses. Based on the study, there was no significant association between the degree of lameness and severity of lesions at the time of diagnosis and time to successful return to performance.

Introduction

Proximal metacarpal pain is a common source of lameness in performance horses, specifically in the western discipline of cutting. Lameness resulting from proximal metacarpal pain ranges from mild to severe. Although lameness can be localized via anesthesia of the lateral palmar nerve at accessory carpal bone, anesthesia of the medial and lateral branches of the palmar metacarpal nerves, and direct infiltration of the proximal suspensory ligament, none of these techniques have proven specificity for diagnosing proximal suspensory disease (PSD).

Ultrasonographic and radiographic examinations are frequently used for evaluation of the proximal metacarpus but possess limitations due to their lack of specificity and sensitivity. Radiographic evaluation of the proximal metacarpus can reveal the structural integrity of the third metacarpal bone (McIII), osseous irregularity of the second and fourth metacarpal bones (McII and McIV), and concurrent abnormalities in the carpus. Ultrasound techniques to evaluate the proximal suspensory ligament (PSL) and its attachment to the third metacarpal bone (McIII) has been described but can be complicated by acoustic shadowing and by the presence of fat and muscle fibers within the ligament, resulting in a heterogeneous appearance. Nuclear scintigraphy of the proximal metacarpus and metatarsus has demonstrated sensitivity for identifying bone injury yet shows limited correlation with ultrasound in the forelimb. Computed tomography can identify pathologic bone changes, but cannot identify bone oedema-like changes and provides limited soft tissue detail.

Magnetic resonance imaging (MRI) is considered the gold-standard imaging modality to characterize changes in both hard and soft tissues. MRI detected pathological changes within the proximal metacarpus show strong correlation with histological morphology of the proximal suspensory ligament (PSL). MRI is commonly used to diagnose thickening of the suspensory ligament, discrete tearing, fibre disruption, osseous contusion of the palmar cortex of McIII at the PSL origin, and exostoses between McII and McIV.

The retrospective study aims to describe clinical lesions present in a group of horses with limited physiological variation and document lesions associated with clinical lameness specific to the discipline of cutting. Additionally, the study establishes prognostic indicators for return to performance by evaluating a 24-month follow-up period on each case.

Methods

A retrospective study investigated 32 cutting horses referred for MRI of the proximal metacarpus following a lameness examination that localized the lesion between 2009 and 2012 with a 2-year follow-up period. Cases included individuals that were registered Quarter Horses that were currently in training for, or competing in, cutting events. Records from the National Cutting Horse Association for each

horse were obtained to investigate the dollar value earned and date of competition. In absence of the record, owners were contacted to establish the horse's participation in competition.

MRI grading analysis was performed on a minimum of transverse proton density (PD) and sagittal fat-saturated proton density (PDFS) sequences, with frequent use of short TI inversion recovery (STIR) and T1 gradient echo (T1GRE) or T1 volumetric interpolated breath hold exam (T1 VIBE) sequences when available. A single board-certified radiologist graded severity of pathological change from 0 (absent) to 3 (severe) based on previously described pathologic lesions: PSL enlargement, dorsal margin, fibre damage, disruption of fat/muscle/ligament tissue architecture, periligamentous thickening, PD and STIR signal intensity of lesions, osseous proliferation at the enthesis on McIII, endosteal/trabecular fluid, sclerosis, and resorption within McIII, exostoses between McIII and McII/IV, and the presence or absence of osteoarthritis in the carpometacarpal and middle carpal joints. PSL cross-sectional area was measured on transverse PD sequences at four equidistant locations between carpometacarpal joint and the nutrient foramen of McIII.

Descriptive statistical analysis of type and distribution of lesions was performed using Microsoft Excel; mean values were calculated and reported with 95% confidence interval. Severity of lameness, overall severity of soft tissue lesions, overall severity of bone lesions, $LTE post diagnosis and time until return to performance were used for regression analysis, with both age and $LTE at the time of diagnosis as covariants in the model. An overall grade for bone and soft tissue pathologic change was assigned to each horse based on the most severe lesion in each tissue type in the predominantly lame limb (Table 1). These grades were incorporated into a logistic re-

TABLE 1: The subjective grading criteria used for each of the bone and soft tissue categories assessed by a single board-certified veterinary radiologist

Grade	0	1 (Mild)	2 (Moderate)	3 (Severe)
SL size	Normal	<10% increase	10-30% increase	>30% increase
Fat/muscle/ligament tissue architecture	Normal	<10% disruption	10-30% disruption	>30% disruption
Dorsal margin irregularity	Normal	Mild irregularity-one lobe	Moderate irregularity-one lobe or mild irregularity-both lobes	Significant irregularity affecting the entire dorsal margin of the SL
PSL PD signal	Normal	Mild increased signal-focal regions	Moderate signal-focal regions or mild diffuse signal	Diffuse signal increase affecting >50% of the PSL
PSL STIR signal	Normal	Mild increased signal-focal regions	Moderate signal-focal regions or mild diffuse signal	Diffuse signal increase affecting >50% of the PSL
McIII STIR signal	None	Mild signal-focal regions	Moderate signal-focal regions or mild diffuse signal	Diffuse signal increase-large region of the palmar aspect of McIII
McIII sclerosis	None	Mild sclerosis – focal regions affecting less than 10% of the bone	Moderate sclerosis – focal regions or mild diffuse sclerosis affecting 10-30% of the bone	Diffuse sclerosis – large region of the palmar aspect of McIII affecting >30% of the bone
McIII resorption	None	Mild resorption – focal regions affecting less than 10% of the palmar cortex of McIII	Moderate resorption – focal regions or mild diffuse resorption affecting 10-30% of the palmar cortex of McIII	Diffuse resorption of the palmar aspect of the McIII affecting >30% of the bone
McIII proliferation at PSL origin	Normal	Mild bone proliferation affecting <10% of the palmar cortex	Mild bone proliferation affecting 10-30% of the palmar cortex	Mild bone proliferation affecting >30% of the palmar cortex
Osseous reaction with McII and McIV	None	Mild osseous proliferation of axial aspect of splint bones or mild change in signal of the interosseous ligaments	Moderate osseous reaction between McIII/MtIII and splint bones or moderate change in signal of the interosseous ligaments	Severe osseous reaction with suspect adhesion formation to the SL and severe change in signal of the interosseous ligaments
Carpometacarpal joint osteoarthritis	None	Mild osteophyte formation, enthosopathy, sclerosis or subchondral bone damage	Moderate osteophyte formation, enthosopathy, sclerosis or resorption	Severe osteophyte formation, enthosopathy, sclerosis or resorption
Middle carpal joint osteoarthritis	None	Mild osteophyte formation, enthosopathy, sclerosis or resorption	Moderate osteophyte formation, enthosopathy, sclerosis or resorption	Severe osteophyte formation, enthosopathy, sclerosis or resorption

gression model to assess significance of lameness, overall severity of bone lesions and overall severity of soft tissue lesions on return to performance. In a similar fashion, a linear regression model was used to assess significance of lameness, overall severity of bone lesions and overall severity of soft tissue lesions on time to return to performance. Regression models and Pearson correlations were performed using Statistical Analyses Software (SAS).

Results

There were 32 total horses evaluated consisting of 20 right forelimbs and 24 left forelimbs (12 bilateral studies). Four horses had an MRI performed for bilateral forelimb lameness, while the remaining eight bilateral studies were performed for comparison purposes. The most frequent lameness score on presentation was a Grade 2/5, range 1-3. The mean age for all horses include 3.9 ± 0.3 years, range 3-7 years, and the mean duration of lameness until MRI diagnosis was 7 ± 2 weeks, range 1-22 weeks. The left forelimb was the primary lame limb in 17 horses, and the right forelimb was considered the primary lame limb for 15 horses. Follow-up data was available for 30 horses out of the total 32 horses included in the study. A total of 22 out of 30 horses successfully returned to competition with 14 horses returning within 6 months and 8 additional horses returning within 12 months. No additional horses returned to competition between 12 and 24 month post-injury; five out of the eight horses that did not return to competition were retired following injury and three never successfully competed in the cutting discipline. The mean time to return to competition was 157 ± 38.2 days, range 22-345 days.

Conclusion

A variety of pathologic processes in the tarsus and proximal metatarsus can contribute to lameness. Horses with lameness that resolves with diagnostic analgesia of the proximal metatarsus may have pathologic changes in the tarsus greater than that of the metatarsus.

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Quantitative evaluation of equine articular cartilage using cationic contrast-enhanced computed tomography

This is a summary of an article by Dr. Nelson, Stewart, Freedman, Patwa, Snyder, Goodrich and Grinstaff published in the journal 'Cartilage' Published online 12/2018 doi:10.1177/1947603518812562.[1]

Take home message

Time for the CA4+ cationic contrast agent to reach equilibrium in equine articular cartilage is affected by tissue volume. Quantitative cationic contrast-enhanced computed tomography (CECT) estimates the biochemical, biomechanical and histological state of normal and degradative articular cartilage.

Introduction

Early articular cartilage injury occurs with depletion of glycosaminoglycans in the extracellular matrix and is challenging to detect using currently available imaging technologies.[2] Contrast-enhanced computed tomography is a technique where contrast media is administered to highlight articular cartilage. Conventional agents are anionic or non-ionic and have limited diffusion into articular cartilage. A cationic contrast agent (CA4+) was developed that has a higher affinity for articular cartilage.[3] However, due to the different biochemical constituent proportions across species, it is unknown how the diffusion of CA4+ courses through and equilibrates in equine articular cartilage. Also, the ability for cationic contrast-enhanced CT to predict the biochemical and biomechanical properties of articular cartilage is unknown. We hypothesized that articular cartilage volume will influence CA4+ diffusion characteristics and that cationic CECT attenuation will correlate with GAG content and compressive modulus across a range of articular cartilage degradation.

Methods

Diffusion trajectory of CA4+

Osteochondral specimens from the femoral condyles of a 3-year-old horse were collected and visually inspected to ensure no surface erosion was present. The plugs were placed into a custom fixture and submerged in CA4+ (8 mg I/mL). Sequential micro CT scans were performed at multiple time points ranging from baseline to 24 hours of submersion. CT images were analyzed by segmenting the articular cartilage from the subchondral bone and the CT attenuation quantified. Comparisons over time were made and fit to a nonlinear least square regression equation to determine equilibration time.[4]

Cationic CECT characteristics of normal and degenerative equine articular cartilage

Under approval of the animal care and use committee at CSU, a 4 year old horse had three articular cartilage defects created on the femoral trochlea (defect joint) and the contralateral femoropatellar joint surface was examined arthroscopically to ensure macroscopically normal articular cartilage (control joint). Forty-seven days after surgery the horse was euthanized and osteochondral biopsies were collected along the surface, excluding the defect areas. After each plug was removed, it was assigned a macroscopic (Outerbridge) score.[5] Plugs with sufficient geometry were put in a mechanical testing system (BOSE, Eden Prairie, MN) and tested in unconfined compression to determine equilibrium compressive modulus. After mechanical testing, the plug was equilibrated in CA4+ for 24 hours and imaged with micro CT. The plug was washed to remove residual CA4+ and a portion of articular cartilage removed and analyzed with a dimethylmethylene blue assay to quantify GAG content.[6] The remainder of articular cartilage attached to bone was processed for histology and stained using safranin-O fast green to depict GAG distributions within the tissue. Comparisons between outcome parameters and statistical significance was defined as $P<0.05$.

Results

Diffusion trajectory of CA4+

The diffusion course progressed radially from the edges of the plugs (Figure 1). The medial femoral

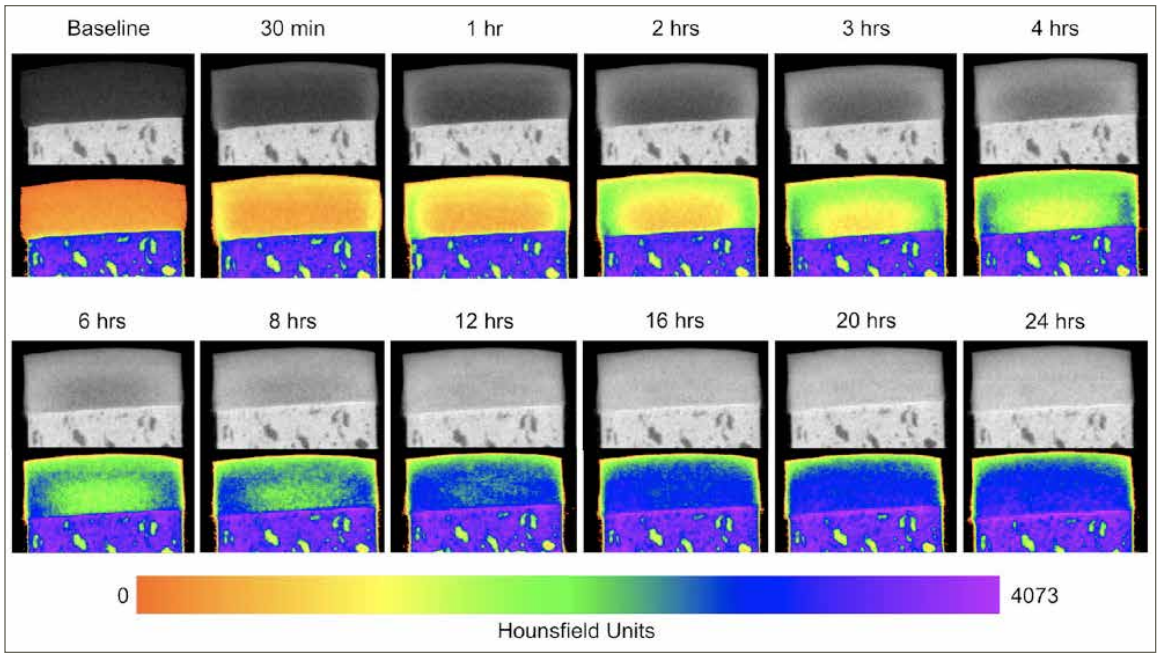

Figure 1. Temporal micro CT scans showing the diffusion of cationic contrast media in equine articular cartilage.[1]

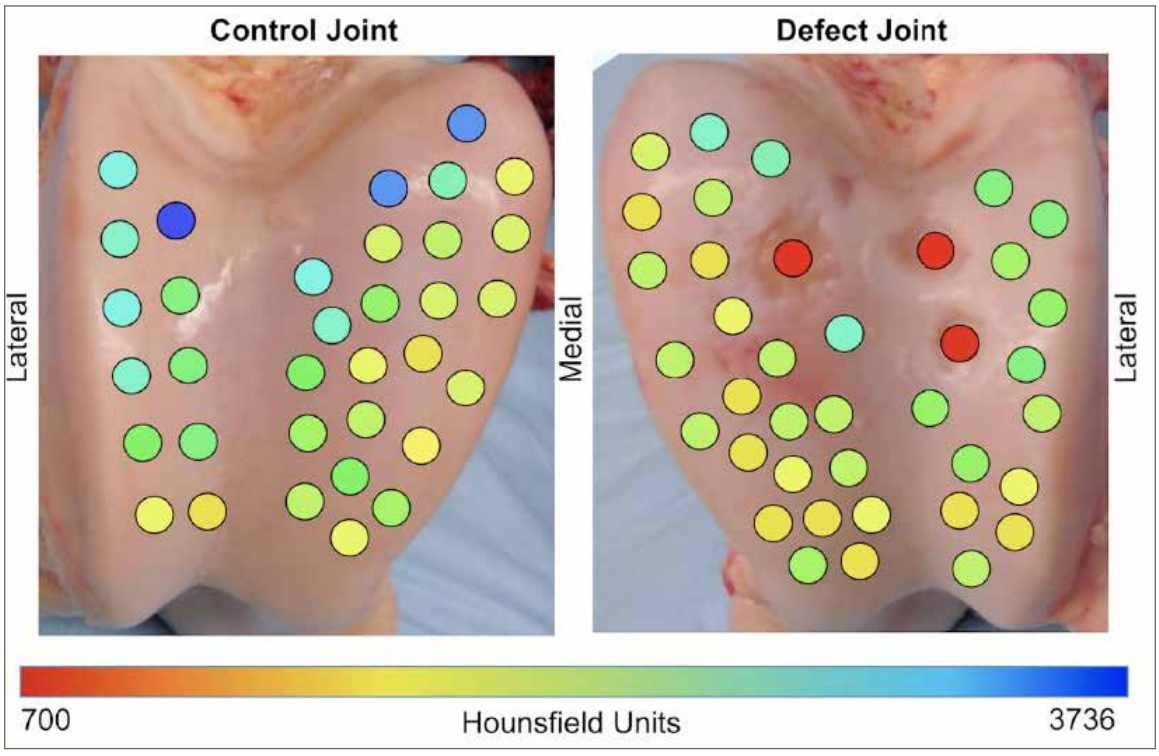

Figure 2. Topography of cationic CECT attenuation captured from the collected osteochondral plug biopsies. Red samples indicate the site of the arthroscopically created chondral defects. Note the higher attenuation values in the control joint and the lower attenuation values present adjacent to the cartilage defects.[1]

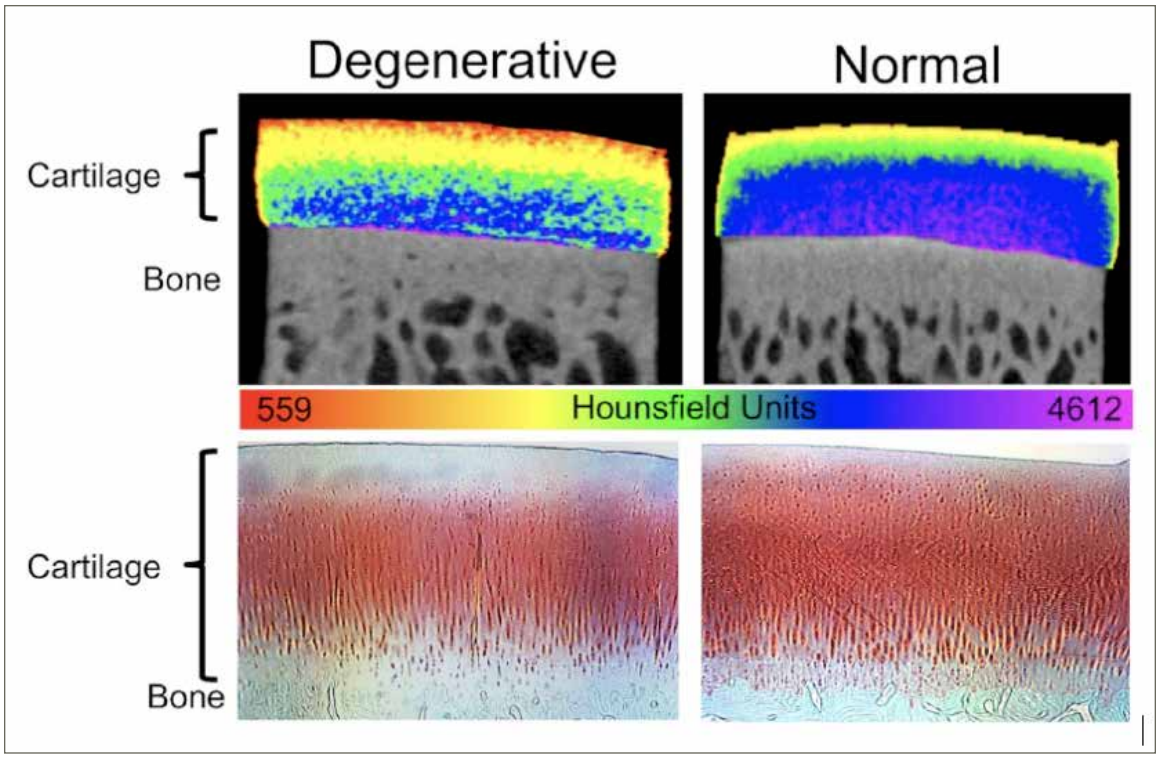

Figure 3: Cationic CECT images of articular cartilage samples from the degenerative (defect) joint and normal (control) joint. High amounts of safranin-O (red stain) uptake indicate high levels of GAGs in the tissue.

condyle cartilage was thicker and had a higher diffusion time constant (3.05 +/- 0.1 hours) compared with lateral femoral condyle cartilage (1.54 +/- 0.3 hours, P=0.04).

Cationic CECT characteristics of normal and degenerative equine articular cartilage

The distributions of cationic CECT attenuation varied between defect and control joints and was significantly lower in the defect joint than the control (P=0.005) (Figure 2). Cationic CECT attenuation strongly correlated with GAG (P<0.0001) and equilibrium compressive modulus (P<0.0001). The distribution of CA4+ diffusion in micro CT samples was similar to histologic stain uptake (safranin-O) (Figure 3).

Conclusions

Equine articular cartilage volume and anatomic location influence the CA4+ diffusion profile and time to reach equilibrium. Cationic CECT imaging is predictive of GAG concentrations and biomechanical properties and also reflects GAG distributions observed on histologic analysis. Cationic CECT has potential as a non-destructive imaging strategy to provide information on the biochemical, mechanical and histological properties of normal and degenerative articular cartilage.

Acknowledgements

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Radiographic localization of the entheses of the equine stifle

This is a summary of an article by Drs. E.D. Aldrich, L.R. Goodrich, M.K. Monahan, J.D. Conway, and A. Valdes-Martinez published in the Equine Veterinary Journal.[1]

Take home message

Injury to the tendons and ligaments of the equine stifle is a common cause of lameness in horses. Individual radiographic localization of each tendon and ligament of the stifle has not been previously reported or demonstrated in multiple radiographic projections.

Introduction

Radiographs, traditionally, are the most logical technique in evaluating and assessing the bony structures of the stifle. Other techniques can be used, but might be costly, or might be limited due to the anatomy of the equine stifle. Although radiographs cannot reveal lesions in the soft tissues, many of these injuries involve the origins and insertions of the soft tissues and may be accompanied by radiographic changes at these sites.

Increased knowledge of entheses will allow clinicians to extract vital information about soft tissue injuries from radiographs. The objective of this study was to produce a series of radiographs with each enthesis identified separately in 4 different projections. The optimal projections were identified for each structure and the radiographic landmarks were marked.

Methods

The location of all entheses were determined by gross dissection. The proximal tibia and fibula, distal femur, patella and menisci were isolated from one horse and used as a template. A series of 4 radiographs was obtained with each enthesis identified with barium paste. The radiographic landmarks for each enthesis were described and the best projection(s) for evaluation of each structure of interest identified.

Results

A complete series of radiographs outlining the entheses was created to serve as a guide for radiograph interpretation. Based on the evaluation of all images, the oblique was found to be the most useful in evaluating the cranial cruciate origin and it is also of value in the assessment of injuries to the medial patellar ligament.

Conclusion

Unlike a previous study done which highlights the close overlap of some of the entheses within the stifle, the current study reports the structures individually to avoid overlap. This information will valuable for evaluating radiographs of the equine stifle, particularly in cases where avulsion and enthesopathy exists, although its use is also limited by several factors.

This study is meant to provide clinicians with a descriptive reference of the soft tissue structures in the stifle. It includes the extensor and popliteal tendons and the patellar, meniscotibial, collateral, and cruciate ligaments.

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Recent advances in articular cartilage evaluation using computed tomography and magnetic resonance imaging

This is a summary of an article by Drs. Nelson, Kawcak, Barrett, McIlwraith, Grinstaff and Goodrich published in the 'Equine Veterinary Journal' 2018; 50:564-579.[1]

Take home message

This review article summarizes current CT and MRI applications capable of assessing equine articular cartilage, a critical joint tissue degraded in osteoarthritis. With a limited ability to heal, early detection of cartilage injury is important to prevent progressive deterioration; however, current strategies are unable to provide early detection. Quantitative evaluation using CT and MRI will provide an opportunity to improve diagnostic evaluation of articular cartilage in horses.

Introduction

Osteoarthritis is defined as a progressive deterioration of articular cartilage.[2] The progressive nature of the disease is due to the inability for articular cartilage to regenerate once the degradation process is initiated. Thus, early detection of articular cartilage injury is important for improving patient outcomes. Despite the need, early detection of articular cartilage with CT and MRI is limited. This article summarizes the compositional and physiological attributes of articular cartilage in horses and connects how those attributes can be imaged using CT and MRI, both morphologically and quantitatively.

Results

Articular cartilage is composed of an extracellular matrix made up of mostly water, followed by collagen and proteoglycans (glycosaminoglycans). The heterogeneous distribution of these constituents varies throughout tissue depth. Glycosaminoglycans (GAGs) have a negative charge, which attracts water affording compressive stiffness during loading, while collagen counteracts tensile forces at the articular surface.

Computed tomography

Articular cartilage is not visible on CT but once contrast media is administered, it diffuses into the tissue and becomes apparent. Morphological assessments including surface topography and articular cartilage thickness can be characterized. Based on the amount of contrast media that diffuses and equilibrates in articular cartilage, the resultant CT attenuation can be measured using commercial software. The characteristics of the contrast media used will dictate its affinity for articular cartilage.[3] Most contrast media are neutral or negatively charged. As such, the contrast media is repelled from the negatively charged GAGs. Cationic CT based contrast media has been developed by our collaboration with Dr. Mark Grinstaff's laboratory at Boston University. The cationic contrast media is electrostatically attracted to GAGs improving the diffusion into the tissue, the amount of which is quantified (Figure 1).[4-8]

Magnetic resonance imaging

Through a different process, MRI is valuable to assess articular cartilage. MRI capitalizes on the properties of hydrogen and since articular cartilage has a high water content, it is a tissue that is readily imaged with MRI. Similar to CT, surface morphometry and articular cartilage thickness can be measured thereby being a technique capable of detecting partial cartilage erosion.[9] However, this is a late stage manifestation of articular cartilage degradation. Because of the lack of early detection with morphologic sequences, quantitative strategies for MRI have been developed to detect early biochemical alterations in the tissue. Delayed gadolinium enhanced MRI of cartilage (dGEMRIC) is a quantitative technique that uses a gadolinium-based contrast media that diffuses into articular cartilage (Table 1).[10] Sim-

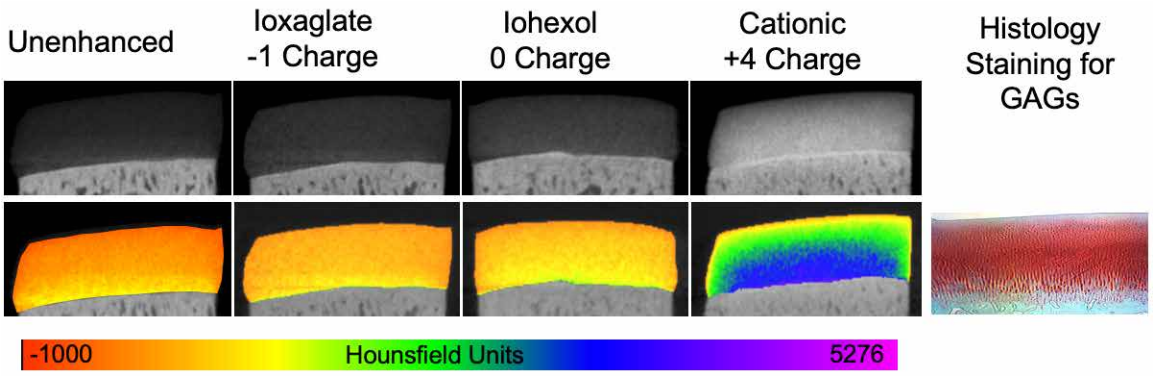

Figure 1. Appearance of articular cartilage after administration of variably charged iodinated contrast media. The top row of images show equine osteochondral samples without (unenhanced) and with iodinated contrast media. The bottom row of images shows the same plugs with an applied color map. Note the higher attenuation of articular cartilage with cationic contrast medium compared with ioxaglate (anionic) and iohexol (nonionic). A histologic sample of articular cartilage stained with safranin-O for GAGs shows the similar distribution between cationic CT imaging and histology.[1]

Table 1. Quantitative MRI techniques used to evaluate articular cartilage.[1]

Technique	Outcome parameter	Effect of imaging parameter in OA	ECM component highlighted	Advantages / disadvantages
dGEMRIC	T1 relaxation time	Decrease	Proteoglycans	*Advantages:* Well-validated; usable in current field strength scanners; largest database available; variety of sequences are used to generate T1 maps *Disadvantages:* risk of contrast media reaction (rare in horses); long scan times; delay after injectin required
T1rho	T1p time	Increase	Proteoglycans	*Advantages:* Sensitive to early proteoglycan degeneration; no contrast media needed *Disadvantages:* High RF power (potential tissue heating); SAR limitations
Sodium imaging	Na-23 signal	Decrease	Proteoglycans	*Advantages:* High specificity to GAG; high contrast resolution *Disadvantages:* Specialised hardware required to capture Na-23 signal; high-field strength (7T) needed; limited spatial resolution; long scan times; low signal-to-noise
gagCEST	Magnetisation transfer (CEST) asymmetry	Decrease	Proteoglycans	*Advantages:* High specificity to GAG; no contrast media needed *Disadvantages:* Complicated technique (advanced post-processing methods); low clinical feasibility; high-field (≥3T) needed
T2 (T2*) mapping	T2 * relaxation time	Increase	Collagen content and anisotropy	*Advantages:* No contrast media needed; easily adaptable to most MRI systems *Disadvantages:* Susceptible to magic angle artefact; dependent on MRI field strength; may detect early cartilage degeneration later than other qMRI techniques
Ultrashort echo time mapping	T2* relaxation time	Increase	Collagen content and anisotropy	*Advantages:* Examine deep articular cartilage and osteochondral junction *Disadvantages:* Long scan times; specialised pulse sequences; technical challenges
Diffusion imaging	Apparent diffusion coefficient	Increase	Water diffusion	*Advantages:* Widely available; simultaneous information on proteoglycan and collagen information (tissue integrity) *Disadvantages:* High strength MRI needed; low spatial resolution; technically complex; long scan times; motion sensitivity; limited deep articular cartilage evaluation

dGEMRIC, delayed gadolinium enhanced magnetic resonance imaging of cartilage; gagCEST, glycosaminoglycan chemical exchange saturation transfer; RF, radiofrequency; SAR, specific absorption rate; GAG, glycosaminoglycan; T, Tesla; MRI, magnetic resonance imaging; qMRI, quantitative MRI.

ilar to commercial CT contrast media, commercial gadolinium contrast media are negatively charged and diffuses into the tissue in inverse proportion to GAGs. The quantification of this imaging signal then can indirectly estimate GAG concentrations, nondestructively. T1rho is a quantitative method that also estimates GAG content, but without the requirement of contrast media.[11] T2 mapping is another quantitative MRI technique that correlates with collagen content.[10,11] Diffusion imaging techniques can estimate water diffusion. Most of these quantitative techniques are rarely explored in horses.

Conclusions

CT and MRI are widely used diagnostic techniques in horses. Improvements in technology, contrast media and access to developed quantitative imaging techniques used in humans are becoming increasingly available for horses and translational research. An understanding of these methods will increase awareness and offer substantial opportunities to improve our detection of early articular cartilage injury.

Acknowledgements

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The relationship between sagittal hoof conformation and hindlimb lameness in the horse

This is a summary of an article published by Drs. Lynn Pezzanite, Luke Bass, Chris Kawcak, Laurie Goodrich, and Valerie Moorman published in Equine Vet J.[1]

Take home message

Horses with hindlimb lameness localized to the distal tarsus and proximal metatarsus, but not the stifle, were more likely to have negative/neutral plantar angle of the distal phalanx (PADPs).

Introduction

The identification of factors associated with lameness could be one method to decrease lameness incidence and prolong the competitive life of the equine athlete. The objectives were to determine if there is an association between sagittal plane hoof balance and hindlimb lameness.

Materials and Methods

Eighty client-owned horses with hindlimb lameness localized with regional anesthesia (cases) and eighty horses with no detectable hindlimb lameness (controls) were prospectively enrolled. Lameness cases were categorized by location (stifle, tarsus, proximal metatarsus, and other sites). Lateromedial radiographs were performed of hind hooves and (PADP) determined. Mean PADPs were calculated. Logistic and linear regression were used to analyze PADPs. Odds ratios were calculated. Significance set at $P<0.05$.

Results

Mean PADP was significantly less in cases than controls. Mean PADP was significantly less in horses with lameness localized to tarsus and proximal suspensory, but not the stifle. Lame horses were 3.8 times more likely to have a negative/neutral PADP. Lameness localized to the tarsus and proximal suspensory were 5 and 5.2 times more likely to have a negative/neutral PADP.

Discussion

It is unknown whether the negative/neutral PADP contribute to lameness or lameness resulted in lower PADP. Corrective farriery to improve PADP may be investigated as one component in the prevention or treatment of hindlimb lameness localized to regions proximal to the foot.

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Use of contrast media in computed tomography and magnetic resonance imaging in horses: techniques, adverse events and opportunities

This is a summary of an article by Drs. Nelson, Goodrich, Barrett, Grinstaff and Kawcak published in the 'Equine Veterinary Journal' 2017; 49: 410-424.[1]

Take home message

This review article summarizes the current literature centered on the use of contrast media and adverse events that occur in horses, with comparisons to other veterinary species and humans. It also summarizes the current state-of-the-art uses and highlights potential applications of contrast-enhanced CT and MRI for assessment of diseased or injured equine tissues.

Introduction

Conventional CT and MRI techniques are often limited in their ability to distinguish one tissue from an adjacent one. Thus, contrast media are administered to improve identification and evaluation of tissues.[2,3] After contrast media administration, these techniques are denoted as contrast-enhanced CT and MRI. While the contrast media used in horses are manufactured for human use and generally considered safe, they are not devoid of risk and there are few detailed descriptions in horses. Since there is a paucity of data reporting in horses, this article drew from experiences in other species, in order to raise awareness of potential risks that may occur. This review describes the different contrast media available, reported techniques, adverse events and opportunities for their use in horses and then highlights the potential advantages and possibilities that are enabled by use of contrast-enhanced CT or MRI.

Results

Computed tomography contrast media

Iodinated contrast media are most commonly used in CT because of the high attenuation that occurs in the presence of x-rays. There are numerous commercially available solutions that exhibit different chemical structures, ionicity, osmolality and viscosity. Though usually safe, consideration of potential adverse events should be made: (1) Assessment of risk vs benefit, (2) consideration of alternative imaging strategies that would achieve the same clinical goals, and (3) valid clinical indication for administration.[4]

CT-based contrast media can be administered intravenously, intra-arterially, intrasynovially, and intrathecally. Each route of administration has applicable details regarding imaging acquisition settings, contrast medium concentration and delivery properties that must be understood to maximize image quality, while avoiding unnecessary risk to the patient. Intrasynovial deposition highlights the articular cartilage surface, thereby enabling surface characterization. Intrathecal administration can highlight spinal cord compression and the volumetric nature of the technology permits evaluation in multiple planes (Figure 1).

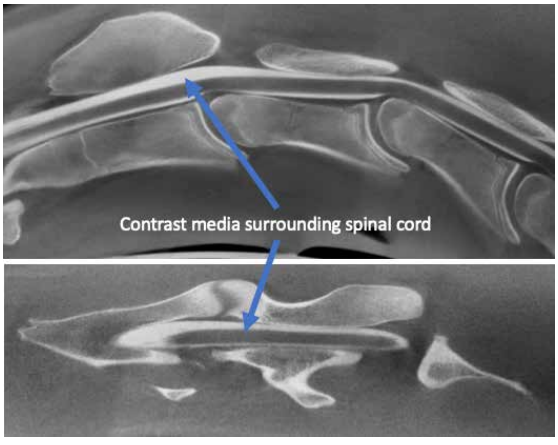

Figure 1. Contrast-enhanced CT myelography. The arrow points to the contrast medium surrounding the spinal cord. Compression of the spinal cord will not be able to be determined. The top image is a sagittal plane reconstruction and the bottom image is an oblique dorsal plane. The ability to assess the tissue volume in multiple planes improves diagnostic assessment over radiography.[1]

Adverse events are unintentional occurrences during or after contrast media administration and may occur acutely or be delayed. They range from minor reactions requiring no medical intervention to severe and life-threatening.[5,6] Some potential administration complications include air embolization and extravasation. While air embolization has the potential to be fatal, local inflammation secondary to extravasation is more common and quickly resolves.

Acute adverse events are allergic-like or physiological. In general, acute adverse events are reduced by ~80% in humans when using low osmolar instead of high osmolar iodinated contrast media. Severe reactions in humans are unpredictable and seem to occur with similar frequency using ionic and non-ionic formulations. Delayed adverse events in horses most commonly appear to manifest has hyperthermia and most self-resolve without treatment after intrathecal administration. Adverse events during and after myelography can include seizures, worsening neurological signs and prolonged anesthetic recovery. High osmolar contrast media are contra-indicated in horses because of severe adverse events and high fatality.[7]

Magnetic resonance imaging contrast media

Most contrast media used in clinical practice are complexed with gadolinium. Free gadolinium is toxic to tissue, but when chelated is considered to have a much higher safety profile. Gadolinium shortens T1 and T2 relaxation times on MRI and contrast media are classified as to how they concentrate in tissues – pool in the extracellular fluid, blood, or in the liver. As opposed to CT contrast media, the chelate structure is more important than ionicity, osmolality and viscosity. Administration routes used in horses consist of intravenous and intrasynovial;[8] however, intra-arterial and intrathecal administration is reported in humans.

Articular cartilage is a tissue that permits gadolinium contrast diffusion and provides improved discrimination from the synovial space and subchondral bone (Figure 2). It is denoted as gadolinium enhanced MRI of cartilage (dGEMRIC).[9]

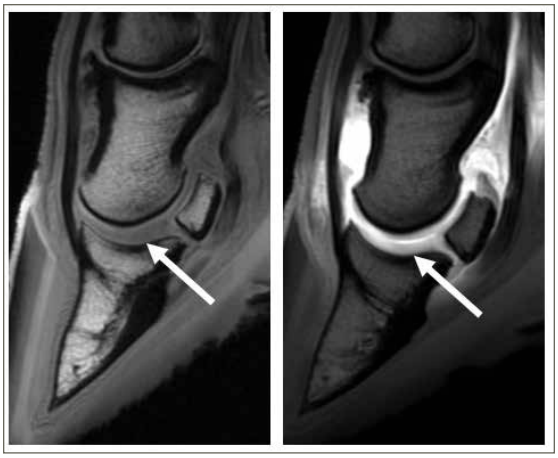

Figure 2. T1-weighted image of the distal interphalangeal joint in the horse before (left image) and after (right image) intrasynovial deposition of gadolinium-based contrast media. The arrow points to the articular cartilage. Note the increased imaging signal in the cartilage.[1]

Adverse events are minimally reported in horses and consist of mild and transient hypotension.10 However, in humans, nephrogenic systemic fibrosis is a rare and potentially fatal complication and even so, gadolinium deposits have been found in tissues decades after administration in humans. Horses with renal insufficiency or dehydration should be identified prior to systemic administration of gadolinium.

Conclusions

Contrast media use in CT and MRI is relatively in its infancy compared to the databases built in human medicine. The diversity of applications of its use enable equine clinicians to more comprehensively evaluate orthopedic tissues, while also ensuring minimal chances for adverse events to occur. Recognizing these limitations will expand our diagnostic capabilities and advancement of characterizing disease states in horses.

Acknowledgements

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Epidemiology of musculoskeletal injury during racing on New Zealand racetracks 2005-2011

This is a summary of a paper authored by Drs. Charlotte Bolwell, Chris Rogers, Erica Gee and Wayne McIlwraith published in animals in 2017[1]

Take home message

The rate of musculoskeletal injuries during a race, (0.72/1,000) starts in New Zealand was lower than the rates reported for other racing jurisdictions. The condition of the track and the distance of the race was associated with the rate of musculoskeletal injury during a race.

Introduction

Over the last few decades, numerous epidemiologic studies have focused on quantifying the incidence rate and risk factors for non-fatal and fatal race-day injuries in Thoroughbred racehorses worldwide. Internationally the incidence of fatalities ranges from 0.4/1000 starts[2] to 1-1.9/1000 starts[3] and the incidence of musculoskeletal injuries (MSI) reported on race day ranges per thousand starts[4] 4.5/1000 starts.[5]

Previous studies have reported the incident rates and risk factors for MSI occurring during training in New Zealand;[6,7] however, no studies have investigated the instances of musculoskeletal events occurring on race day during flat races in New Zealand. A previous study reported a failure of a finish rate of 2.8/1000 starts within flat racing in New Zealand.[8] Failure to finish represented a broad category of events (pulled up/fall/lost rider) that included MSI, which prevented a horse from completing the race.

Methods

A retrospective study was conducted from using data from all Thoroughbred flat race starts in New Zealand between August 1st, 2005 and July 31st, 2011 (6 racing seasons). The study utilized data from a previous study describing the broad failure to finish events (pulled up/fall/lost rider).[8] Briefly Stipendiary Steward's reports and race data were provided by New Zealand Thoroughbred Racing (NZTR) Race data was provided in a Microsoft excel file and included, race date, race track, age, horse gender, finishing position in race (used to identify horses that failed to finish), barrier draw, (positions in the starting gates), carded weight (weight allocated by race handicapper) carried weight (any apprentice weight allowances) in domestic rating (analogous to the official rating system).

Results

During the six racing seasons, there were 188,616 race starts for 16,646 individual horses. There were 544 failed to finish events, of which 177 (33%) were classed as veterinary events and 48% (85/177) of these were fatalities. Of the veterinary events reported, 136 (77%) were due to MSI and 41 (23%) were cardiac and respiratory events. The incidence of MSI on race day was 0.72 per 1000 starts, whilst the incidence of cardiac and respiratory events was 0.21 per 1000 starts.

Of the MSI events, 91 (67%) were reported as fractures, 10 (7%) were reported tendon or ligament injuries, 10 (7%) were reported as lameness, and 7 (5%) were reported as soft tissue injuries; 18 (13%) MSI events were undefined. Only one horse had multiple veterinary events (n = 2), of which one was lameness and one was tendon and ligament. The incidence of fractures, tendon or ligament injuries, and lameness, and soft tissue injuries was 0.48, 0.05, and 0.04 per 1000 starts, respectively. Of the MSI events 75 (55%) were forelimb, 33 (22%) were hindlimb, 1 (1%) was both fore and hindlimb, 9 (7%) were pelvic and 19 (14%) were non-defined. A total of 57% (78/136) of MSI were fatalities, whilst 81% (72/89) of fractures were fatalities. The incidence rate for race-day MSI fatalities was 0.41 per 1000 starts.

Table 1. Categories and definitions used to describe failure to finish veterinary events reported by Stipendiary Stewards occurring during Thoroughbred flat races in New Zealand (2005-2011).

Category	Definition
Musculoskeletal injury	A failure to finish event due to an injury to the muscular or skeletal system. Included terms such as: fracture, injury, broke down, sore, lameness, ruptured, tom, lacerations, damaged, broke.
Fracture	A failure to finish event due to a fracture. Included any event where fracture was specifically stated.
Tendon and ligament	A failure to finish event due to a tendon or ligament injury. Included terms such as tendon severed, ruptured suspensory, injury tendon, broke down right tendon.
Lameness	A failure to finish event due to lameness or soreness. Included terms such as sore behind, unsound, sore, lame
Soft Tissue	A failure to finish event due to a muscle or skin injury. Included terms such as pulled muscle, muscle soreness, torn muscle, cut leg.
Undefined	A failure to finish event due to an undefined MSI. Included terms such as injury hind quarters, injury left leg, injuries lower limb, broke down, fell and euthanized.
Cardiac and respiratory	A failure to finish event that was a result of a respiratory or cardiovascular event that was not influenced by injury. Such as epistaxis, atrial fibrillation, elevated heart rate, heart fibrillation, ruptured aorta, heart fibrillation, bled, ruptured, haemorrhage, respiratory distress.

Over half of the cardiac and respiratory events were recorded as epistaxis (23/41; 56%), five were recorded as atrial fibrillation, five were recorded as fatal haemorrhage, four were recorded as elevated heart rate, three events were recorded as ruptured aorta, and one event was recorded as respiratory distress. A total of seven (17%) cardiac and respiratory events were recorded as fatal. Season, track condition, race distance, and weight carried were associated with MSI at $p < 0.20$ and were included in the multivariable model.

The results of the multivariable Poisson regression model of variables significantly associated with MSI are presented in Table 3. Race distance and track condition were significantly associated with MSI. After adjusting for race distance, the rate of MSI was significantly lower on 'dead' and 'slow' tracks compared with 'good' tracks. After adjusting for track condition, the rate of MSI was significantly greater for horses in longer races (≥1671 m) compared with horses in races of ≤1200 m (Table 2). There were no significant interactions in the final model. The Pearson goodness of fit statistic for the final model was $p = 0.56$, indicating no evidence of poor model fit.

Discussion

This is the first study to report on the incidence of failure to finish a race due to MSI in flat races in New Zealand and provide an assessment of the risk factors for MSI during a race. The incidence of MSI during flat races in New Zealand appears to be low when compared with international data. Recent work from the U.K. reported a race-day MSI incidence of 2.1 per 1000 starts,[4] whilst Cohen and others[5] reported a MSI rate of 4.1 per 1000 starts in Kentucky. Similarly, the rate of MSI fatalities during a race was lower than that previously reported in the USA (1.9 per 1000 starts) and the U.K. (0.7 per 1000 starts).[4] It is unlikely that the incidence of MSI during a race was underestimated in this study, as failure to finish a race represents a key event that prevented a horse from completing the race, which was subsequently recorded on official race-day records. Due to the nature of the recording pre-2011 (when the RIU was established) it is possible that the rate of fatalities may be underestimated in the current study. However, the rate of fatalities reported in this study was the same as that previously reported in a study of race-day fatalities in Victoria, Australia, a racing jurisdiction with similar structures and levels of reporting as found in New Zealand.

Table 2. Univariable Poisson regression for musculoskeletal injuries occurring during Thoroughbred flat races in New Zealand (2005-2011) (n = 136).[a]

Variable	Level	No. of Starts	No. of Musculoskeletal Injuries	IRR	95% Confidence Interval	*p* Value	Wald *p* Value
Race year	2005/06	29,751	18	–	–	–	0.73
	2006/07	30,574	27	1.46	0.80-265	0.21	
	2007/08	31,276	23	1.21	0.65-225	0.53	
	2008/09	33,061	20	0.99	0.52-1.89	1.00	
	2009/10	32,349	22	1.12	0.60-209	0.71	
	2010/11	31,605	26	1.36	0.74-248	0.32	
Season	Spring	49,620	40	–	–	–	0.13
	Summer	52,647	45	1.06	0.69-1.62	0.79	
	Autumn	48,484	24	0.61	0.37-1.02	0.06	
	Winter	37,865	27	0.88	0.54-1.44	0.62	
Sex	Male	104,605	73	–	–	–	
	Female	84,011	63	1.07	0.77- 1.50	0.68	0.68
Track condition	Fast	5478	6	–	–	–	0.04
	Good	73,231	67	0.83	0.36-1.92	0.67	
	Dead	44,481	24	0.49	0.20-1.20	0.12	
	Slow	32,310	15	0.42	0.16-1.09	0.08	
	Heavy	33,116	24	0.66	0.27-1.62	0.37	
Age (year)	2	6072	3	–	–	–	0.52
	3	43,228	26	1.22	0.36-4.02	0.328	
	4	56,374	38	1.36	0.42-4.42	0.129	
	5	42,439	33	1.57	0.48-5.13	0.234	
	6+	40,503	36	1.80	0.55-5.84	0.58	
Apprentice allowance	No	144,005	102	0.93	0.63-1.37	0.71	0.71
	Yes	44,611	34	–	–	–	
Race distance	≤ 1200 m	49,554	25	–	–	–	0.002
	1201-1400 m	47,914	27	1.12	0.65-1.92	0.69	
	1401-1670 m	44,587	31	1.38	0.81-2 33	0.23	
	≥ 1671 m	46,561	53	2.26	1.40-3.63	0.001	
Weight carried	46-54.5 kg	55,382	52	–	–	–	0.15
	54.6-55.5 kg	40,677	25	0.65	0.41-1.05	0.08	
	55.6-56.9 kg	38,547	27	0.75	0.21-1.19	0.22	
	57-76 kg	54,010	32	0.63	0.41-0.98	0.04	
Rating bands	50-54	46,817	29	–	–	–	0.71
	55-65	45,695	38	1.34	0.82-2 18	0.23	
	66-75	57,524	38	1.07	0.66-1.73	0.79	
	76-85	21,639	18	1.34	0.75-242	0.33	
	86-ll5	16,941	13	1.24	0.64-238	0.52	
Field size	3-9	43,560	26	–	–	–	0.71
	10-11	42,003	31	1.24	0.73-208	0.42	
	12-13	45,245	36	1.33	0.80-221	0.26	
	14-18	57,808	43	1.25	0.76-203	0.38	
Barrier	1-3	51,029	31	–	–	–	0.67
	4-6	50,625	41	1.33	0.83-212	0.23	
	7-9	44,443	33	1.22	0.75-1.99	0.42	
	10-21	42,519	31	1.20	0.73-1.97	0.47	
Race number	1	16,942	10	–	–	–	0.27
	2	18,389	11	1.01	0.43-237	0.98	
	3	18,596	10	0.91	0.38-219	0.83	
	4	18,840	10	0.90	0.37-216	0.81	
	5	19,364	23	2.01	0.96-4.23	0.06	
	6	19,732	15	1.29	0.58-287	0.53	
	7	19,925	13	1.10	0.48-252	0.81	
	8	20,507	16	1.32	0.60-291	0.49	
	9	17,487	18	1.74	0.80-3.78	0.16	
	10 +	18,834	10	0.90	0.37-216	0.81	

[a] Showing incidence rate ratios (IRR) for all Thoroughbred flat race starts (*n* = 188,616) in the 2005/06-2010/11 racing years.

Table 3. Results of multivariable Poisson regression model of the variables significantly associated with musculoskeletal injury occurring during Thoroughbred flat races in New Zealand (2005- 2011) (n = 136).[a]

Variable	Level	No. of Starts	No. of Musculoskeletal Injuries	IRR	95% Confidence Interval	*p* Value	LRT *p* Value
Total		188,615	136				
Track condition	Fast	5478	6	1.17	0.51- 2 69	0.71	0.002
	Good	73,231	67	–	–	–	
	Dead	44,481	24	0.59	0.34-0.95	0.03	
	Slow	32,310	15	0.51	0.29-0.90	0.02	
	Heavy	33,116	24	0.80	0.5 1.3	0.34	
Race distance	≤ 1200m	49,554	25	–	–	–	0.04
	1201-1400 m	47,914	27	1.11	0.64-1.91	0.71	
	1401-1670 m	44,587	31	1.35	0.229	0.26	
	≥ 1671 m	46,561	53	2.21	1.38-3.57	0.001	

[a] For all Thoroughbred flat race starts (n = 188,616) in the 2005/06- 2010/11 racing years.

The lower incidence rates reported here may relate to the training and management of racehorses in New Zealand.[8] Previous studies of racehorses in training identified associations between exercise distances accumulated in training and breaks from training and various measures of training and racing performance.[10-12] Specifically, horses with a voluntary or involuntary interruption to training before their first trial were less likely to trial, resulting in fewer overall trial starts, which was associated with a reduced chance of a race start. It may that the trainers' perception of the horse's ability and soundness to trial and race, with trainer deciding to retire a horse rather than continue to train and race it. Therefore, it is possible that the flat racing population of horses in New Zealand may be likened to the previously described "healthy horse effect" or survival bias and the lower rates of MSI observed during races. Similarly, many studies have reported associations between exercise distances accumulated in training, prior racing history and time between races, and the risk of MSI and fatalities in training and racing. However, these relationships are known to be complex and are likely to vary with different case definitions investigated. Training data were not available for the cohort of horses used in the current study but further work to address the relationship between exercise history and the rates of MSI during racing is required.

In addition to potential differences in training and racing schedule, the track surface used for races in New Zealand compared to other racing jurisdictions may explain the low rate of MSI observed in this study. Racing in New Zealand is conducted on grass tracks, whereas races in the USA are most commonly run on dirt tracks, which have higher rates of injuries compared to synthetic tracks. However, given that the rates of injuries on turf tracks in the USA are higher than those reported for synthetic tracks, it is likely that other factors in addition to track surface contribute to the higher rates observed internationally. Differences in the rules of racing, for example, permit the use of therapeutic medications in the USA, and the structure and type of racing across racing jurisdictions may also contribute to the variation in rates reported worldwide. Given this, there is a need for a collaborative approach to determine regional similarities or differences that contribute to the rate of racing MSI.

Only two exposure variables were found to be associated with the incidence of MSI during a race in the multivariable modelling. Longer race distances were found to be a risk factor for MSI, in agreement with results of studies investigating racing fractures and fatalities. An Australian study reported increased odds of racing fatalities, of which most were due to MSI, for every additional furlong raced. The authors of that study suggested the increased risk was likely due to increased exposure time for an injury to occur, and the possibility of more fatigued horses in longer races. The race distances in Australia are similar to those of New Zealand, with a maximum distance of between 3200-3600 m.

The results showed a lower risk of MSI on "dead" and "slow" tracks when compared with races run on "good" tracks. An increased incidence of MSI or risk of fatalities or fractures on "fast" or "good/

firm" tracks compared to "heavy/soft" tracks has been reported in Australia and the U.K. Within New Zealand, there appears to be an active program by track managers to avoid fast tracks with watering of tracks in summer and extensive drainage in winter to improve the consistency of the going. This may explain the low number of fast tracks observed in this dataset, which may have been a contributor to the apparent inability of the present study to identify fast tracks as a risk factor for MSI despite most of the MSI being due to fracture. The avoidance of the harder, less compliant track conditions rated as "fast" by New Zealand track managers is of interest and the drivers behind this decision require further examination.

Most of the MSI veterinary events in this study were reported as fractures, with smaller numbers of tendon and ligament and lameness and soft tissue injuries reported. A previous study reported 42% and 41% of retirements from racing in New Zealand were due to fracture or tendon injuries, respectively. However, Perkins et al. included injuries occurring in training making direct comparisons with the results of this study difficult. In 2011, the RIU took over the monitoring and management of the Stipendiary Steward's reports in New Zealand.

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Case-control study of risk factors for pasture- and endocrinopathy-associated laminitis in North American horses

This presents the results of a study authored by a special task force that designed this case controlled clinical study. The authors were M.C Coleman, J.K. Belknap, S.C. Eades, H.L. Galantino-Homer, R.J. Hunt, R.J. Geor, M.E. McCue, C.W. McIlwraith, R.M. Moore, J.F. Peroni, H.G. Townsend, N.G. White, K.J. Cummings, R. Ivanek-Miojevic and N.D. Cohen.[1]

Take home message

The present study identified several risk factors for pasture-and endocrinopathy-associated laminitis (PEAL) that may assist not only in managing and preventing this form of laminitis, but also in guiding future research into its pathogenesis.

Introduction

Laminitis is a debilitating disease in horses and commonly results in severe pain, lameness, and loss of athletic performance.[2] The economic and welfare impacts of laminitis are high because of the associated poor prognosis, severe pain, and frequency of recurrence. Furthermore, the estimated incidence of laminitis ranges from 1.5% to 34% with an estimated lifetime risk of 15%.Recognizing the impact of laminitis on horses and horse owners, veterinarians desire an improved understanding of laminitis, as evidenced in a 2009 survey conducted by the AAEP in which its members identified laminitis as the highest priority for research funding and investigation.

In the National Animal Health Monitoring System equine study performed in 2000, horse owners in the United States reported that pasture-associated laminitis and laminitis of unknown etiology were the most common forms of laminitis. Previous cohort studies have identified some risk factors (e.g., hyperinsulinemia) for PEAL in ponies, and experimental euglycemic-hyperinsulinemic clamp studies have been shown to induce laminitis in ponies and horses. However, the importance of these and other risk factors for naturally occurring PEAL has not been characterized among North American horses. Notable efforts have been made in the past decade to further the profession's understanding of this complex condition; however, much of the research on laminitis has been limited to the study of mechanistic pathways following experimental induction of the condition. Although valuable, experimental models may not fully replicate the multifactorial interactions underlying naturally occurring laminitis. Thus, observational studies of naturally occurring laminitis are necessary to advance our knowledge and understanding of the condition and associated risk factors as well as to design future investigations for its prevention and control. The objective of the study reported here was to investigate risk factors for the development of PEAL in horses and ponies in North America. It was the result of the AAEP Foundation to commission a task force to investigate ways that we could use our members to gather clinical data rather than using research models for further understanding of factors contributing to laminitis.

Methods

Case selection

The study was designed as a matched, case-control study in which participating veterinarians were asked to identify case-control sets that consisted of 1 horse with PEAL (cases) and 2 control horses without laminitis. Cases were defined as incident cases of PEAL with clinical signs detected ≤ 4 weeks prior to examination and collection of survey data (the time-frame for detection of clinical signs was extended from the initial requirement of ≤ 48 hours after detection). In addition, case horses were required to have evidence of bilateral forelimb lameness of Obel grade ≥ 2 and at least 2 of the following findings: sensitivity to hoof testers greatest in the region of the toe at the time of initial examination, a characteristic foundered stance, radiologic evidence of laminar thickening, and postmortem evidence (gross or microscopic) of laminitis. Horses were excluded as PEAL cases if they had any of the following findings: previous history of laminitis or navicular disease; laminitis associated with sepsis, a non-weight-bearing lameness, or excessive grain

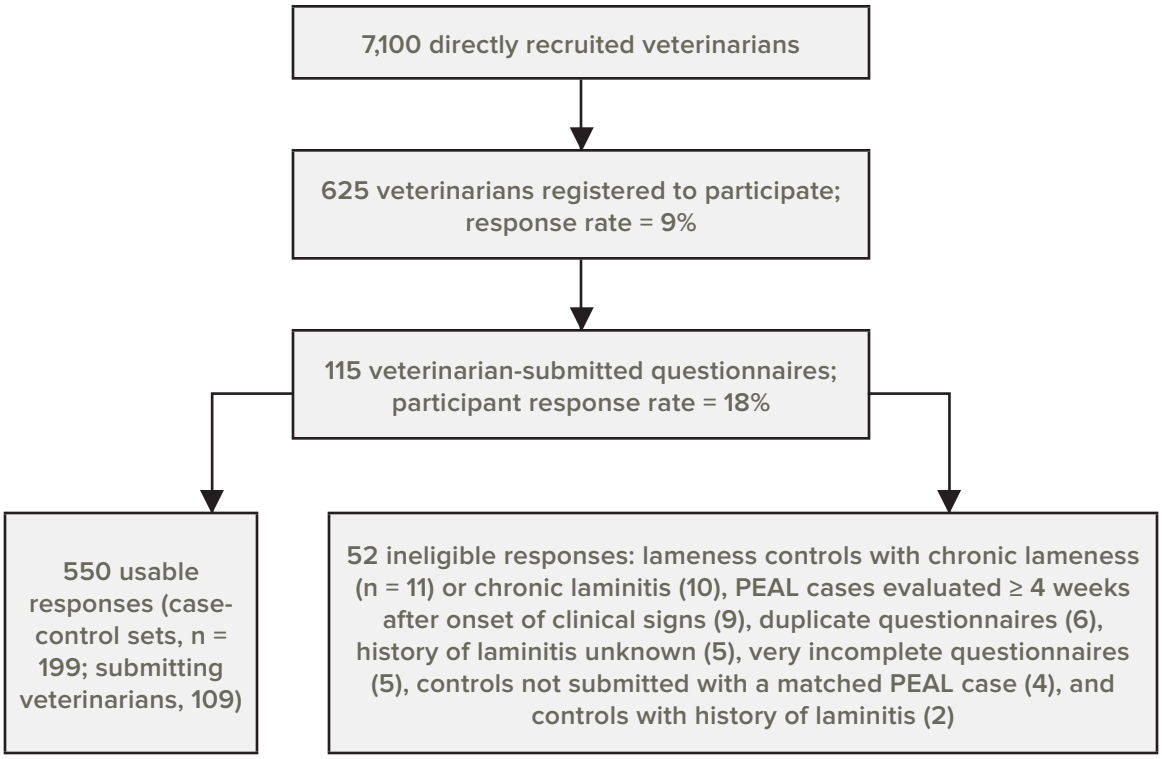

Figure 1. Flow diagram illustrating recruitment of horses with PEAL (case horses) and matched control horses (healthy control horses and lameness control horses) in a study of risk factors for development of PEAL in horses and ponies in North America.

consumption; other concurrent disease conditions of the foot; radiologic signs of chronic laminitis (e.g., extensive remodeling of the third phalanx); or gross evidence of chronic laminitis, such as divergent growth rings (founder rings) in the hoof capsule or if they were an equid other than a horse or pony.

Control selection

For each PEAL case, at least 1 horse from each of 2 control populations, healthy controls and lameness controls, was identified. A healthy control was defined as any healthy horse residing at a different farm than that of the PEAL case and ideally the next horse examined by the veterinarian for a routine wellness examination (e.g., vaccination, Coggins testing, health certificate completion, or routine dental examination). A lameness control was defined as any horse with lameness in only 1 forelimb and residing at a different farm than the PEAL case. To qualify as a lameness control, the horse's lameness must have been present for ≤ 4 weeks and must have been graded as ≥ 3 according to the AAEP's 5-point lameness grading scale.16 Horses were excluded from the control groups if they had a history of laminitis or had clinical or diagnostic findings indicative of previous laminitis (e.g., divergent hoof rings, dorsal hoof dishing, or preexisting radiologic evidence consistent with laminitis, such as rotation of the third phalanx relative to the hoof wall).

And a prior sample size estimation indicated that approximately 200 PEAL cases and 400 controls were required on the basis of a significance level of 5%, statistical power of 80%, an OR of 2 for PEAL cases relative to controls, and 2 controls/PEAL case.

Results

A total of 199 horses with PEAL that were each matched with at least 1 healthy control (n = 198) or lameness control (153) horse were included in the analysis. These 550 horses were located in 32 states and 3 Canadian provinces (Figure 2), and their cor-

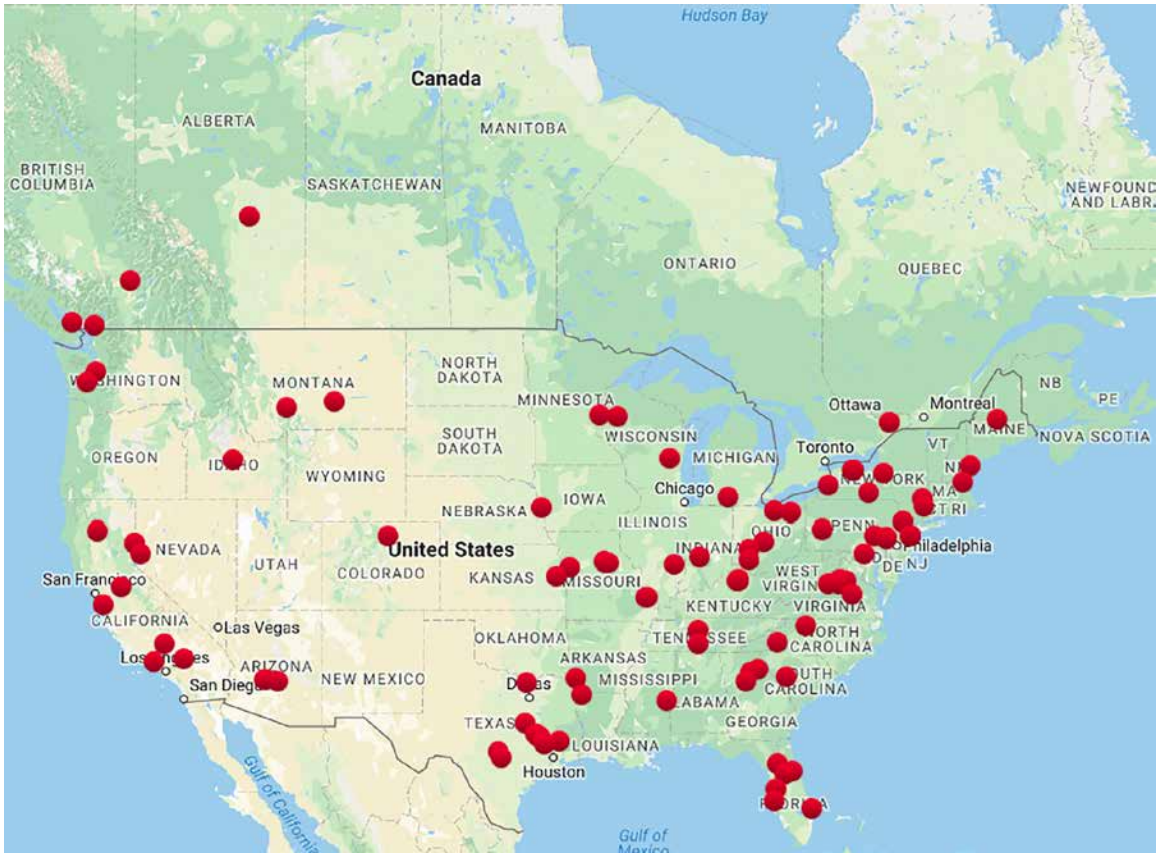

Figure 2. Geographic distribution (32 states and 3 Canadian provinces) of matched PEAL cases (n = 199) and corresponding healthy controls (198) and lameness controls (153) for the study in Figure 1.

responding data were submitted to the study over a 4-year period (2012, n = 174 [32%] horses; 2013, 177 [32%]; 2014, 91 [17%]; and 2015, 108 [20%]). Age was known and reported for 547 horses, with an age range of 1 to 34 years, a mean of 13.3 years, and a median of 13 years. Neither sex nor age was a risk factor for development of laminitis. Breeds included Quarter Horses, American Paint Horses, and Appaloosas combined (n = 237 [43%]); Thoroughbreds (70 [13%]); draft horses and European warmbloods combined (66 [12%]); Arabians (48 [9%]); gaited horses, including Tennessee Walking Horses and Saddlebreds (36 [7%]); ponies and miniature horses (29 [5%]); and Morgans (17 [3%]). Breed was not reported for 8 (1%) horses and was reported as other for 39 (7%) horses. The severity of lameness for each of the 199 horses with PEAL was assessed with the Obel grading system (grade 2, n = 121 [61%] horses; grade 3, 62 [31%]; and grade 4, 16 [8%]) The final multivariable CLR model comparing data between case horses and healthy control horses indicated that horses with an overweight body condition (BCS ≥ 7), generalized or regional adiposity (alone or in combination), or a preexisting endocrinopathy, along with horses that did not receive concentrates in their diet, had higher odds of developing PEAL. Horses that had received corticosteroids within the 30 days prior to examination were more likely to develop PEAL (OR, 5.65; 95% CI, 1.32 to 24.27) than were horses that had not received corticosteroid treatment within the 30 days prior to examination. However, corticosteroid administration within that timeframe was uncommon in all groups of horses (case horses, n = 12/198 [6%]; healthy control horses, 4/198 [2%]; and lameness control horses, 1/151 [1%]) for which this variable was reported.

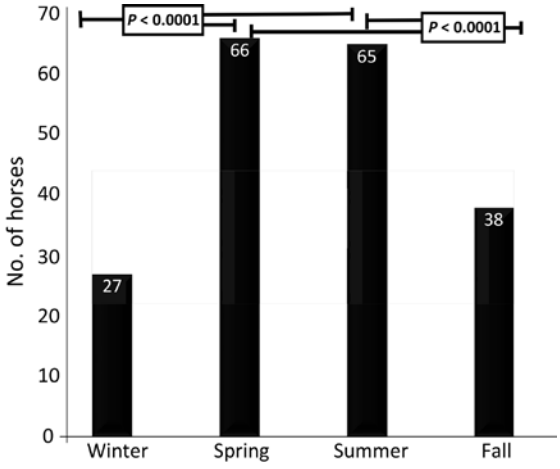

Figure 3. Seasonal distribution for the onset of clinical signs associated with PEAL as reported for 196 of the 199 (98%) case horses for the study in Figure 1. Horses were significantly (P < 0.001) more likely to develop laminitis in the spring or summer, compared with the fall or winter.

The final multivariable CLR model comparing data between case horses and healthy control horses indicated that horses with an overweight body condition (BCS ≥ 7), generalized or regional adiposity (alone or in combination), or a preexisting endocrinopathy, along with horses that did not receive concentrates in their diet, had higher odds of developing PEAL. Horses that had received corticosteroids within the 30 days prior to examination were more likely to develop PEAL (OR, 5.65; 95% CI, 1.32 to 24.27) than were horses that had not received corticosteroid treatment within the 30 days prior to examination. However, corticosteroid administration within that timeframe was uncommon in all groups of horses (case horses, n = 12/198 [6%]; healthy control horses, 4/198 [2%]; and lameness control horses, 1/151 [1%]) for which this variable was reported.

Discussion

The final multivariable CLR model comparing data for case horses and lameness control horses yielded similar results, with PEAL more likely in horses with an overweight body condition (BCS ≥ 7), generalized or regional adiposity (alone or in combination), or a preexisting endocrinopathy. There were no substantial bivariable interactions between any pairs of variables in either model. To our knowledge, the present study represents the first reported observational study of veterinarian-diagnosed incident cases of PEAL in North America. The use of incident cases in the present study was crucially important because identified risk factors were more likely to have been causal, whereas studies of prevalent cases identify factors that might be causal or that might be associated with surviving with the disease or recurrence of it. Therefore, the risk factors for PEAL identified in the present study may assist not only in managing and preventing this form of laminitis, but also in guiding future research into its pathogenesis.

Results of the present study of incident cases of PEAL supported the causal association of obesity and laminitis because the obesity preceded the onset of laminitis. This strong association indicated that the risk of laminitis might be reduced by controlling obesity or modifying the underlying determinants of obesity. Careful feeding and management practices aimed at reducing body weight and adiposity should be considered. Although this concept is not new,[3] results of the present study contributed evidence of the association of body morphometrics with the odds of developing laminitis and should be compelling information for convincing veterinarians and horse owners of the risks that obesity and increased adiposity pose relative to laminitis. In human medicine, causes of obesity not associated with increased caloric intake or decreased energy expenditure have been identified, resulting in novel methods of obesity control and prevention.[4,5] Similarly and as noted previously, additional strategies other than dietary management and exercise might be needed in some cases to prevent obesity in horses.

Endocrinopathies in this population was surprising, but may have resulted from reporting bias (e.g., uncertainty regarding medical history prior to evaluations for laminitis or variability in interpreting the questionnaire and whether further diagnostic testing was warranted before a horse was considered to have an endocrinopathy). More importantly, it was possible that laminitis might have been the first clinical sign of an endocrinopathy recognized by horse owners or veterinarians. These findings indicated that early recognition of endocrinopathies is vital

to allow earlier intervention with medical treatment or husbandry management strategies to reduce the likelihood of affected horses developing PEAL.

Corticosteroid administration to horses has been implicated as inducing laminitis; however, no direct evidence of a causal association has been identified. Although the use of corticosteroids in horses is widespread, the incidence of corticosteroid-induced laminitis was low in an observational study investigating risk factors for development of laminitis and in experimental studies of disease. Although the multivariable model comparing PEAL cases to healthy controls in the present study yielded a 13-fold greater odds of PEAL among horses that had received corticosteroids within the 30 days prior to examination relative to horses that had not, the validity and magnitude of this association must be considered with caution. Prior corticosteroid use did not remain in the final multivariable model comparing PEAL cases to lameness no controls, suggesting that the association observed with healthy controls could have been confounded by another variable. More importantly, corticosteroid administration was uncommon in all groups of horses, and the small numbers rendered our estimates of the magnitude of an effect unstable, as reflected in the wide 95% CI. In addition, respondents' recall of corticosteroid administration may have been greater for horses with laminitis than for horses in the control groups, creating a potential for marked recall bias. Nevertheless, the authors believe that the results of the present study indicated that this topic merits further investigation through a well-designed, large-scale, hypothesis-driven observational study.

Finally, as with any epidemiological study, the effects of confounding were considered. Cases and controls were matched on the basis of input from responding veterinarians in an effort to control for confounding on this variable. Bias introduced by measured variables were accounted for in the multi-variable model; however, the association with PEAL may have been confounded by other factors that were not measured or considered.

In conclusion, the present observational study revealed several important risk factors that might contribute to the development of PEAL. A strategy to reduce the incidence of PEAL could involve further elucidating the determinants, identifying risk factors that are modifiable by medical management or husbandry changes, and educating horse owners and veterinarians about these factors and interventions. In addition to continued investigation of screening tests, treatments, and other interventions that can ameliorate insulin dysregulation and obesity, studies are warranted to identify determinants of obesity and adiposity as well as interventions for modifiable risk factors. It is also plausible that earlier recognition and treatment of endocrinopathies might contribute to reducing the incidence of the devastating disease of laminitis.

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Damage accumulation modeling and rate dependency of spinal dura mater

This is a summary of an article by Drs. Nicole L. Ramo, Snehal S. Shetye, and Christian M. Puttlitz published in the Journal of Engineering and Science in Medical Diagnostics and Therapy.

Take home message

Mechanical damage in dura mater plays an important role in spinal cord injury, however, constitutive characterizations of the tissue have not modeled damage. This study was the first to model damage in spinal cord dura mater, and the results showed distinct rate-dependent behaviors, particularly when exposed to strain-rates above that experienced during normal voluntary neck motion suggesting the possible existence of a protective mechanism.

Introduction

As the strongest of the meningeal tissues, the spinal dura mater plays an important role in the overall behavior of the spinal cord-meningeal complex. It follows that the accumulation of damage affects the dura mater's ability to protect the cord from excessive mechanical loads. Unfortunately, current computational investigations of spinal cord injury etiology typically do not include post-yield behavior. Therefore, a more detailed description of the mate-

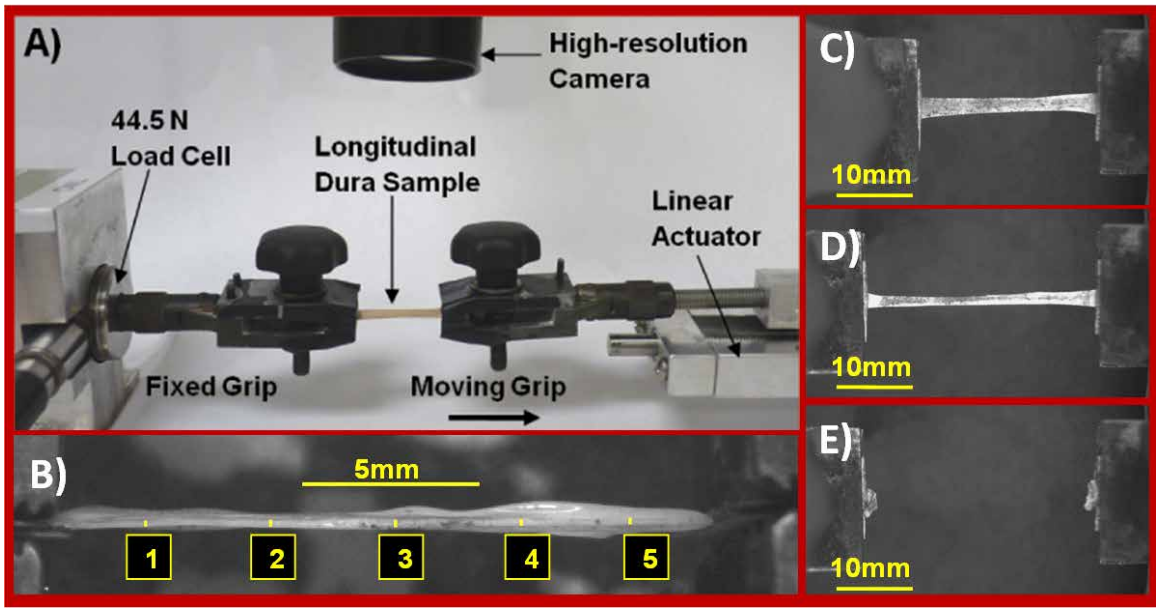

Figure 1: (A) Uniaxial testing apparatus with labeled components; (B) Five thickness measurements were made via analysis of images taken with grips turned at a 90° orientation from the testing configuration; A representative tension to failure test showing the sample at (C) 0.5N preload, (D) prior to mid-substance failure, and (E) immediately following mid-substance failure.

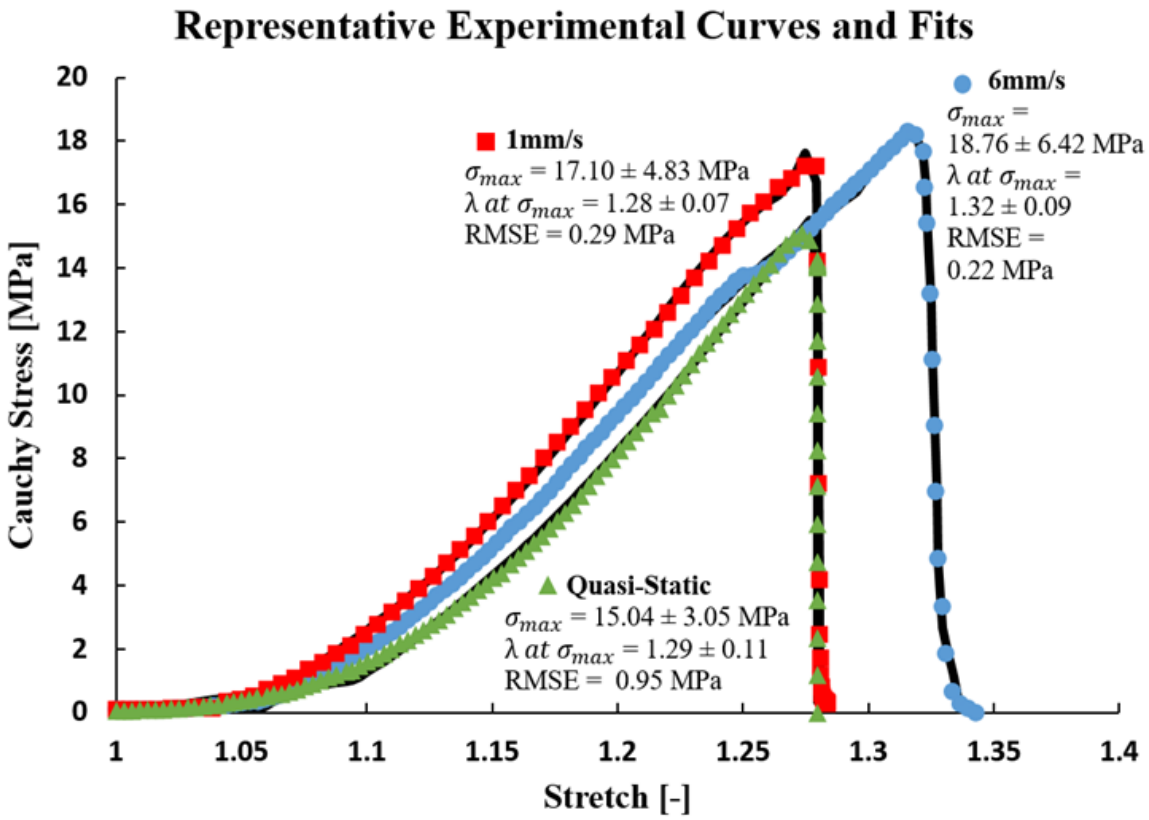

Figure 2: Representative experimental stretch-stress curves (colored symbols) from each strain-rate group demonstrate the elastic non-linearity that is characteristic of hydrated fibrous soft tissues. The group averages for maximum failure stress, stretch at maximum stress, and the model fit (black curve) root mean square error are also given.

rial behavior of the spinal dura mater, including characterization of damage accumulation, is required to comprehensively study spinal cord injuries. The aim of this study is to characterize the damage accumulation behavior of the spinal dura mater under uniaxial loading.

Methods

Longitudinal (i.e. cranial-to-caudal long-axis) samples of ovine cervical dura mater were tensioned-to-failure at one of three strain rates (quasi-static, 0.05/sec, and 0.3/sec) (Figure 1). The resulting stress-strain data were fit to a hyperelastic continuum damage model to characterize the strain-rate dependent sub-failure and failure behavior. The constitutive damage model is a piece-wise function with separate terms representing damage to the collagen fibers and to the matrix.[1]

Results

The constitutive model fit the data well, with errors of 1.5%, 3%, and 6% for the 0.3/sec, 0.05/sec, and quasistatic strain rates, respectively (Figure 2). Significant differences in several model parameters demonstrates that the damage behavior of the fibrous and matrix components of the dura mater are strain-rate dependent. Specifically, fiber stiffness and nonlinearity were increased at higher strain rates. Additionally, at higher strain rates, the initiation to matrix damage was delayed and the tissue exhibited greater ductility and held more strain energy before failure.

Conclusions

The results show distinct damage behaviors for the matrix and fiber constituents, and that the damage

effects vary with applied strain-rate. These differences suggest a possible protective mechanism occurring at strain-rates above what the tissue experiences during normal voluntary neck motion. Given these findings, it is imperative that the formulation presented herein be implemented into finite element computational models of the spinal cord meninges in order to improve the accuracy of simulations of spinal cord dynamics and injury/damage scenarios.

Acknowledgments

This work was supported in part by the National Institutes of Health [grant number EB012048] and a scholarship from The Force and Motion Foundation.

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The importance of subchondral bone in the pathophysiology of osteoarthritis

This is a summary of an article by Drs. H.L. Stewart and C.E. Kawcak, and was published as part of a collection of articles in One Step at a Time: Advances in Osteoarthritis, in collaboration with Frontiers in Veterinary Science.[1]

Take home message

Subchondral bone plays a critical role in the pathogenesis of osteochondral disease across veterinary species. The subchondral bone is highly adaptable, with the ability to model and remodel in response to loading stresses experienced by the joint. Repetitive stress injuries within the joint can result in primary or secondary pathologic lesions within the subchondral bone, which have been recognized to contribute to the development and progression of osteoarthritis. Recent advances in diagnostic imaging, particularly volumetric imaging modalities have facilitated earlier identification of subchondral bone disease. Despite these advancements, limitations in our knowledge about subchondral bone makes treatment and prevention of these conditions challenging. The purpose of the report is to review our current understanding of subchondral bone and its relationship to osteoarthritis across veterinary species, with a specific focus on the research that has been performed in horses. It can be concluded that our current understanding of subchondral bone is advancing, and future experimental, clinical and pathologic studies will provide additional insight about subchondral bone and its relationship to joint disease.

Introduction

As our understanding of the underlying pathophysiology of osteoarthritis grows, we have begun to recognize that osteoarthritis is a disease of not just articular cartilage, but of the osteochondral unit. The osteochondral unit is composed of articular cartilage, calcified cartilage, and subchondral and trabecular bone, which work synergistically to support functional loading of the joint. Subchondral bone has received particular attention in recent years, as derangements in this essential tissue have been recognized for its contribution to the development and progression of osteoarthritis. This review discusses the anatomy, physiology, and biomechanical principles that guide subchondral bone function; and then delves into the specific conditions of subchondral bone, specifically subchondral bone disease, repetitive stress injury and chronic fatigue injury. The review concludes with some general principles of diagnosis and a brief discussion of treatment and prevention strategies.

Conclusion

Substantial insight has been gained about the biomechanical influences of the joint on the subchondral bone, with the relationship between subchondral bone injury and articular cartilage loss and the development of degenerative joint disease only beginning to be elucidated. Continued investigation of the adaptive and maladaptive changes within the subchondral bone by researchers and clinicians alike will continue to yield valuable information about the behavior of this unique component of the joint. Further discovery of the delicate balance of factors that maintain the integrity of the subchondral bone and homeostasis within the joint will surely enhance and direct our understanding of subchondral bone disease in across both veterinary and human patients.

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Assessment of a novel equine tarsocrural experimental joint disease model using recombinant interleukin-1 beta and arthroscopic articular sampling of the medial malleolus of the tibia on the standing sedated horse

This is a summary of an article by Drs. Nelson, King and Frisbie published in 'The Veterinary Journal' 2017; 229:54-59.[1]

Take home message

The equine tarsocrural synovitis model established a transient synovitis and lameness. Also, the standing needle arthroscopic surgical technique to collect articular cartilage and synovial membrane tissue enabled a feasible method capable of evaluating of intra-articular therapies without requiring horse sacrifice.

Introduction

Synovitis is a critical component of equine joint disease. Experimental synovitis models, including the injection of recombinant interleukin 1 beta (reIL-1β), induce transient disease and are used to investigate new joint therapies.[2] However, synovitis models have not been described in the tarsocrural joint.

While these models have potential to be non-terminal, there are no descriptions of standing techniques to harvest articular tissues, which are important for evaluating disease-modifying effects of emerging joint therapies. An 18-gauge arthroscope has been used for diagnostic stifle evaluation in standing horses.[3] This technique was well tolerated and avoided a postoperative convalescence period. Therefore, we developed a technique using this needle arthroscope to collect articular cartilage and synovium samples to improve the breadth of joint tissue evaluation without requiring general anesthesia or horse sacrifice.

The aims of the study were to determine subjective and objective pain parameters in the reIL-1β synovitis model and to describe the surgical biopsy technique, including the location and quantity of tissues obtained.

Methods

The institutional animal care and use committee at CSU approved the protocols for this non-terminal study of 24 horses aged 4 to 10 years. The reIL-1β (50 ng) was injected into a randomly assigned tarsocrural (synovitis) joint. The contralateral (control) joint was injected with the same volume of PBS. Horses were divided into 4 treatment groups to investigate a joint therapeutic with the tarsocrural

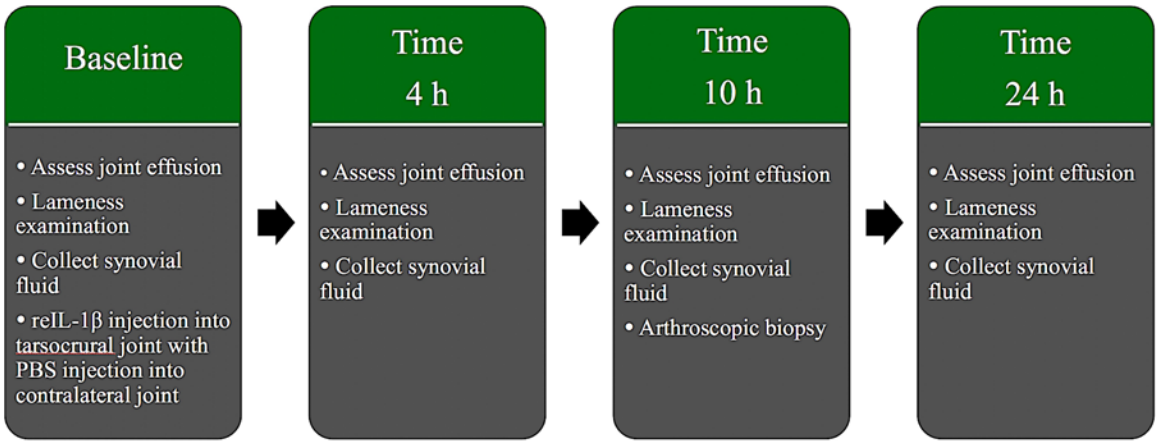

Figure 1. Timeline of data collection performed using this study design.[1]

synovitis model. Only results from joints unexposed to the treatments are reported in this publication. Results of the therapeutic are reported elsewhere.[4]

Data collection time points were established at baseline and then 4 hours, 10 hours and 24 hours after induction of synovitis (or controls)(Figure 1). Joint effusion was graded subjectively on a 0-4 scale (0 = none, 4 = severe) and lameness assessments were performed using a force platform (Bertec Corp.), inertial sensor system (Equinosis Q) and subjective assessment (AAEP scale) from a blinded investigator.[5,6] After lameness evaluation, synovial fluid was collected, and the amount harvested was recorded. Synovial fluid was to be analyzed for prostaglandin E2 and glycosaminoglycan concentrations and cytologic profiles.

Arthroscopic biopsies were collected using an 18-ga arthroscope (Biovision Tech.) and Ferris Smith rongeurs (Figure 2). Horses were sedated, the tarsal region anesthetized using a peroneal tibial nerve block and placed in standing stocks in preparation for aseptic surgery. Samples of articular cartilage from the medial malleolus of the tibia (2, each 3 mm^2) were harvested and analyzed for chondrocyte viability and mRNA expression. Other analyses included quantification of glycosaminoglycans and total DNA content. Two samples of synovial membrane were also collected per joint (each 3 mm^2) and processed for histology and mRNA analysis. Successful collection of tissue was defined as having enough tissue or synovial fluid to perform all planned analyses. Surgical time was defined as the time from synovial fluid collection to closure of the instrument portal. Horses were evaluated for at least 2 weeks following completion of the study and were then returned to the seller. Statistical analysis of the data was performed using a repeated measure mixed model ANOVA. Statistical significance was defined as $P<0.05$.

Figure 2. Image documenting surgical access ports for collection of articular cartilage from the medial malleolus and synovial membrane in standing sedated horses.[1]

Results

In the joints with synovitis, median (IQR) scores at baseline 0 (0-0) were significantly different from all other time points (T4: 2(2-3); T10: 3(3-3); T24: 3(3-4); all $P < 0.001$).

Peak vertical reaction forces in synovitis joints were significantly lower (increased lameness) than control joints at T4 and T10 ($P<0.0001$) but were not different by T24. Stance duration was lower in synovitis joints at T10 ($P<0.05$) compared with controls. Inertial sensor variables at baseline were significantly different from T10 (PDmin and PDMax: $P<0.001$) but not at T24.

Synovial fluid volumes averaged about 2 mLs at each time point, regardless of classification as control or synovitis limb, and all joints provided enough fluid to perform all planned assessments.

Of the 24 horses used, four joints from four different horses had a moderate amount of hemorrhagic fluid exit the instrument portal at the conclusion of surgery that was self-limiting. All horses tolerated the procedure well and no horses reacted during arthroscope insertion or tissue collection. Mean (+/- s.d.) surgical time was 13 +/- 7.6 min per joint (range: 3-35 min). Arthroscopy enabled access to the medial malleolus of the tibia as well as a substantial

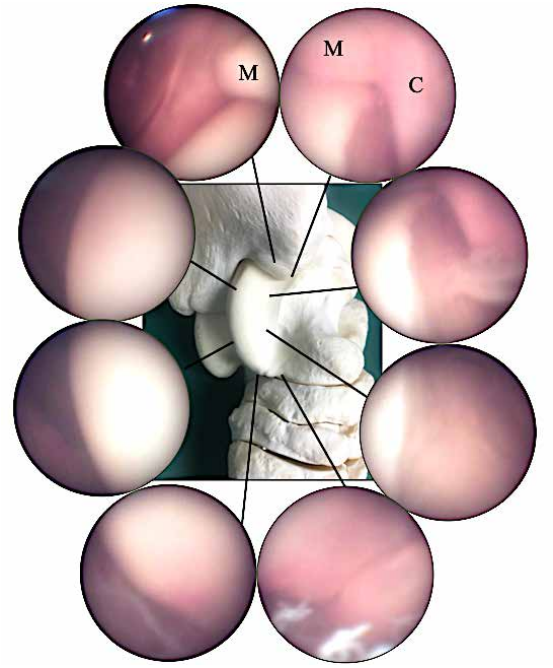

Figure 3. Figure depicting arthroscopic access for tissue harvest in the dorsal aspect of the tarsocrural joint. M, medial malleolus; C, short collateral ligament.[1]

portion of the medial trochlear ridge of the talus (Figure 3).

All planned articular cartilage and synovial membrane samples were able to be collected from all horses and were sufficient to perform all planned assessments. No horses had postoperative complications involving the surgical procedures.

Conclusions

The reported arthroscopic technique and synovitis model provided a platform for a non-terminal study and facilitated investigation of symptom and disease modifying effects of a joint therapy. This methodology could be considered in testing emerging joint disease treatments.

Acknowledgements

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Autologous and allogeneic equine mesenchymal stem cells exhibit equivalent immunomodulatory properties in vitro

This is a summary of an article by Drs. Aimee Colbath, Steven Dow, Wayne McIlwraith and Laurie Goodrich and Ms. Nikki Phillips published in Stem Cells and Development.[1]

Take home message

Autologous and allogeneic equine bone marrow-derived mesenchymal stem cells (BMDMSCs) have equivalent immunomodulatory properties in vitro. This suggests allogeneic equine BMDMSCs may be a valuable stem cell source for the treatment of musculoskeletal disease in the horse.

Introduction

Although there are multiple sources of stem cells in the horse, BMDMSCs are commonly used for musculoskeletal injury[2] and have been found to have increased chondrogenic abilities when compared to adipose-derived MSCs.[3] However, expansion of MSCs has multiple clinical disadvantages. Currently, BMDMSCs require 2-4 weeks for culture expansion before treatment may be pursued. This process is expensive and time consuming. Additionally, there is some evidence that BMDMSCs from diseased or older donors may be of decreased quality.[4,5]

Allogeneic (non-self) BMDMSCs may provide an effective alternative to autologous ("self") BMDMSCs by providing an "off-the-shelf" treatment, allowing for immediate treatment and careful selection of superior cells from young, healthy donors. However, the immune properties of allogeneic stem cells must be examined prior to the incorporation of these cells into clinical practice.

Previous studies have found allogeneic BMDMSCs capable of immune suppression in vitro.[6-8] In addition, these cells have been used clinically with a low rate of joint flare and clinical success.[9,10] However, we are unaware of any study that has directly compared the immune properties of allogeneic and autologous BMDMSCs in vitro. Therefore, the aim of this study was to compare the immunomodulatory properties of autologous and allogeneic equine BMDMSCs and to identify the mechanism for the immunosuppressive properties of allogeneic BMDMSCs.

Methods

We conducted studies to assess the immunological properties of equine allogeneic BMDMSCs compared with those of autologous BMDMSCs. For assessment of inherent immunogenicity, the relative ability of allogeneic and autologous BMDMSCs to stimulate spontaneous proliferation of equine lymphocytes was compared. This was performed by co-incubating polymorphic mononuclear cells (PBMCs) with BMDMSCs for 4 days and assessing lymphocyte proliferation using carboxyfluorescein succinimidyl ester (CFSE) (Cell Trace™; Thermo Fischer Scientific), and analysis by flow cytometry.

The immunosuppressive activity of autologous and allogeneic BMDMSCs was evaluated by adding autologous or allogeneic BMDMSCs to activated lymphocytes and assessing suppression of lymphocyte proliferation and IFNγ production using CFSE staining and intra-cellular IFNγ expression by flow cytometry. Fifty-six allogeneic and 12 autologous combinations were evaluated.

In addition, assays were performed to elucidate the mechanism(s) by which equine mesenchymal stem cells (MSCs) suppress lymphocyte function. Potential mechanisms evaluated included production of prostaglandin E_2 (PGE_2), nitric oxide, transforming growth factor-beta, and indoleamine 2,3-dioxygenase. Inhibitors for each of the pathways were added to co-cultures of stimulated PBMCs and BMDMSCs and lymphocyte proliferation was measured using CFSE by flow cytometry.

Once the mechanism of immune suppression was identified as prostaglandin E_2 (PGE_2) mediated, PGE_2 levels were measured in the supernatants of untreated MSCs and PBMC cocultures as well as co-cultures treated with a PGE_2 inhibitor. Data was collected using a commercially available ELISA (PGE_2 Parameter Assay Kit, R&D systems®) per the manufacturer's recommendations.

Results

Immunogenicity testing revealed autologous and allogeneic BMDMSCs both induced mild but equivalent levels of spontaneous lymphocyte activation in vitro. Increased lymphocyte proliferation was only found at ratios of 1 autologous MSC per 10 PBMCs, or 1 allogeneic MSC per 10 or 50 PBMCs ($P<0.05$) (Figure 1).

When immune suppressive ability was compared, a statistically significant and equivalent immune suppression was noted for autologous and allogeneic BMDMSCs at 1 MSC per 10 PBMCs (Figure 2). Likewise, IFNγ expression by PBMCs decreased in a dose dependent manner with the addition of allogeneic and autologous MSCs, and allogeneic and autologous BMDMSCs had equivalent IFNγ suppression (Figure 3).

Finally, we found that incubation of MSCs with activated PBMCs in the presence of an inhibitor of the cyclooxygenase pathway, indomethacin, resulted in a significant reversal of the immune suppressive effects of allogeneic MSCs (Figure 4). This indicates that allogeneic BMDMSCs use the cyclooxygenase pathway as a mechanism of immune suppression of lymphocyte proliferation. PGE_2 levels in supernatants from MSCs/PBMCs cocultures treated with indomethacin showed a significant decrease in PGE_2 ($P<0.05$) confirming indomethacin effectively blocked the PGE_2 pathway.

Conclusions

Autologous BMDMSCs are extensively used for the treatment of equine tendonitis, desmitis and osteoarthritis in the horse. Although allogeneic BMDMSCs could be a more convenient, cost-effective, and potentially increase the quality of cells by donor

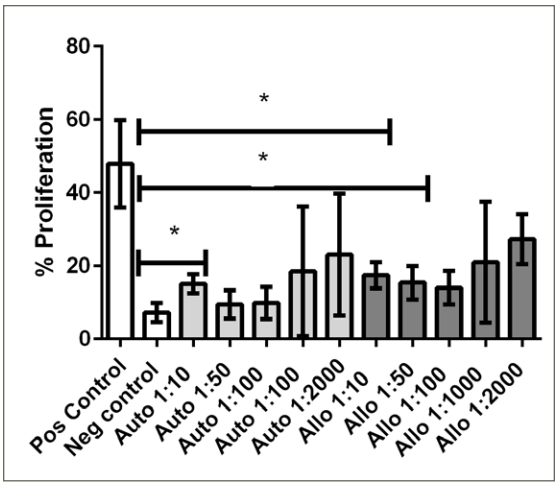

Figure 1. Lymphocyte proliferation assay: Relative immunogenicity of allogeneic and autologous MSCs. Allogeneic and autologous BMDMSCs are nonimmunogenic at low ratios of MSCs:PBMCs. No difference was detected between the immunogenicity of autologous and allogeneic MSCs at any ratio. However, a small degree of immunogenicity was noted when autologous MSCs were added at a ratio of 1:10, and allogeneic MSCs were added at ratios of 1:10 and 1:50.

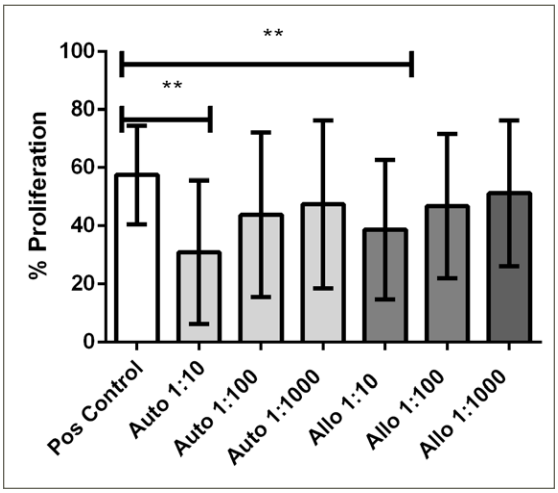

*Figure. 2. Immunosuppression assay (lymphocyte proliferation). Allogeneic and autologous MSCs were immunosuppressive, shown by a decrease in lymphocyte proliferation at a ratio of 1 MSC per 10 PBMCs. No difference was found between the immune suppressive abilities of allogeneic versus autologous MSCs. P values < 0.01 are marker by **.*

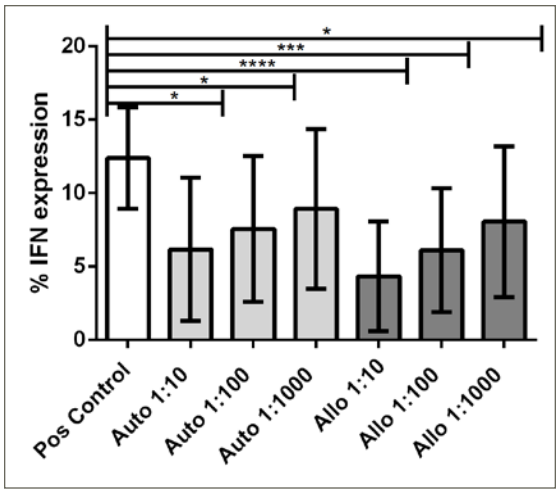

*Figure 3. Immunosuppression assay (IFNγ expression). IFNγ expression decreased with increasing ratios of BMDMSCs to PBMCs. The greatest decrease in IFNγ expression was observed at a ratio of 1 MSC per 10 PBMCs. P values <0.05 are marked by *. P values < 0.001 are marked by ***, and P values < 0.0001 are marked by ****.*

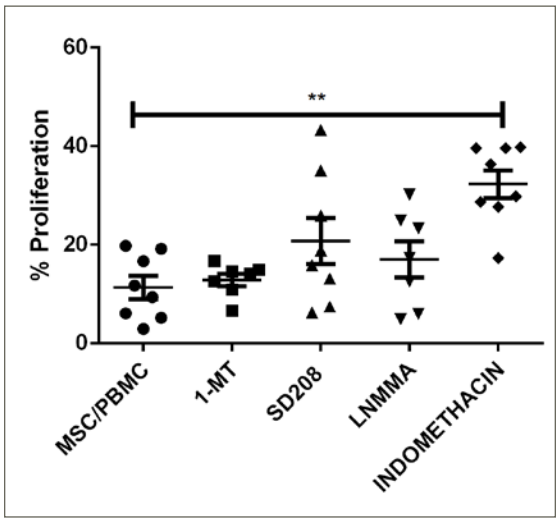

*Figure 4. Immune mechanism assay. Inhibition of potential mediators of immunosuppression, revealed a reversal of immunosuppression only when co-cultures were treated with indomethacin (an inhibitor of the PGE_2 pathway). P values <0.01 are marked by **.*

selection, little is known about their immune properties compared to autologous BMDMSCs.

To determine whether allogeneic or autologous BMDMSCs elicited an immune response from PBMCs, cells were co-cultured and lymphocyte proliferation assessed. We found a small, but equivalent amount of lymphocyte proliferation at high ratios of MSCs to PBMCs. In contrast, when MSCs were co-cultured with stimulated PBMSCs, MSCs were noted to be immunosuppressive causing a decrease in lymphocyte proliferation. Previous in vitro studies using human and equine BMDMSCs support our findings demonstrating an increase in the immunosuppressive properties of MSCs preactivated with IFNγ and a decrease in production of inflammatory cytokines when MSC media was used to treat stimulated PBMCs.[11,12]

Our study demonstrated the source of the MSCS (allogeneic or autologous) is not an important variable in determining the degree of immune suppression in vitro. This finding could have significant clinical implications, as allogeneic BMDMSCs may be a more convenient and less expensive product for the treatment of musculoskeletal disease in the horse. This study suggests that further in vivo studies are warranted to compare the behavior of allogeneic and autologous cells within the normal and inflamed joint.

The pathway of MSC immunosuppression has been investigated in the human, murine, and canine.[13-15] Our study found that only PGE_2 was an important mediator of immunosuppression by allogeneic BMDMSCs. This finding is in agreement with a previous study that investigated the role of interleukin-6, nitric oxide, and PGE_2 as mediators of immunosuppression by allogeneic BMDMSCs.[6]

Based on our findings, we suggest that further research should be conducted in vivo to compare the relative clinical benefits of the anti-inflammatory and immunomodulating properties of allogeneic and autologous BMDMSCs. In conclusion, allogeneic and autologous BMDMSCs appear to be equally immunosuppressive in vitro. It also appears that equine MSCs principally use the cyclooxygenase pathway for suppression of T cell function.

Acknowledgements

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Culture conditions that support expansion and chondrogenesis of middle-aged rat mesenchymal stem cells

This is a summary of an article by Dr. John Kisiday, Mr. John Schwartz, and Drs. Suwimol Tangtrongsup, Laurie Goodrich, and Dan Grande that was published in Cartilage.[1]

Take home message

Rats are used for early preclinical testing for cartilage tissue engineering therapies, and their relatively short lifespan is well suited to study the effect of aging. However, for bone marrow mesenchymal stem cells (MSCs), rat MSCs have been reported to experience a rapid decline in chondrogenic potential with aging that is inconsistent with MSCs from humans and other species. This study refutes those data by identifying culture conditions in which middle-aged rat MSCs undergo robust chondrogenesis.

Introduction

The ability of mesenchymal stem cells (MSCs) to heal articular cartilage defects in the aging population has been questioned by evidence that the chondrogenic potential of MSCs can decrease with age.[1] This question can be addressed *in vivo* using rats, which are commonly used for early preclinical testing of cartilage tissue engineering therapies, and have a relatively short lifespan. However, age-related declines in chondrogenic potential have been reported to be particular strong for rat MSCs as conventional chondrogenic conditions of pellet cultures in defined medium has failed to support chondrogenesis from animals beyond young adulthood.[2,3] Using MSCs from middle-aged rats, the objective of this study was to reconsider limitations associated with aging of rat MSCs, in culture conditions involving a scaffold, and in both serum-free and -supplemented chondrogenic medium.

Methods

MSC isolation and culture

Bone marrow was flushed from the femurs of 14-15 month old Wistar rats, and the nucleated cells were seeded at a concentration of 0.75 x10^6 cells/cm^2 in alphaMEM 15% fetal bovine serum (FBS). The cultures were trypsinized after 5-6 days, and reseeded at 10 x10^3 cells/cm^2 in alphaMEM + 10% FBS + 5 ng/ml FGF2 (expansion medium), and then trypsinized after 2 days. The collected cells were further expanded by seeded at 6 x10^3 cells/cm^2 on tissue culture plastic (TCP) or fibrinogen-coated TCP.[4] The effect of two days of exposure to chondrogenic medium (described below) was compared to continuous culture in expansion medium.

Chondrogenic culture and analysis

MSCs were encapsulated in 1.5% agarose at 10x10^6 cells/ml. Baseline chondrogenic medium consisted of high-glucose DMEM, 1% ITS+, 37.5µg/ml ascorbate-2-phosphate, 100 nM dexamethasone. Samples were cultured in the presence or absence of 10 ng/ml TGFβ, or with TGFβ plus 5% FBS. After 15 days of culture, samples were evaluated for GAG (DMMB) and hydroxyproline (DMBA) accumulation. Histological sections were stained for GAG (toluidine blue) or type II collagen.

Results

Monolayer expansion

Colony-forming culture resulted in approximate 1-4 million MSCs per animals. After 7 days of monolayer expansion on TCP or fibrinogen, the total number of population doublings on fibrinogen surfaces (2.5) was modestly higher than TCP (1.9); therefore, all subsequent expansion was conducted on fibrinogen surfaces. MSCs that were cultured in chondrogenic medium for two days, and then switch to expansion medium resulted in nearly twice as many population doublings as expansion medium only.

Chondrogenesis

MSCs were expanded through one passage prior to seeding into agarose and culturing in chondrogenic media. In serum-free chondrogenic medium, GAG and hydroxyproline accumulation were not

significantly different between 7 male and 7 female donors. Next, using MSCs from 4 animals, chondrogenesis in serum-free chondrogenic medium was compared to negative controls that did not contain TGFβ, and chondrogenic medium supplemented with 5% FBS. After 15 days of culture, GAG accumulation in negative controls was below the detection limit of the DMMB assay, while hydroxyproline accumulation was extremely low (~0.005 µg/mg wet weight). Hydroxyproline accumulation in serum-free chondrogenic medium was more than 20-fold higher than negative control cultures. With serum-supplementation, GAG and hydroxyproline accumulation increased 2.7- and 3.4-fold over serum-free cultures ($P<0.002$). Histological analysis indicated that not all encapsulated MSCs accumulated a GAG- and type I collagen-rich pericellular matrix. The increase in matrix accumulation with serum-supplementation coincided with a higher frequency of cells surrounded by pericellular matrix.

Conclusions

The use of rats to study age-related changes in chondrogenic potential is not supported by the current literature that has shown a severe decrease in chondrogenesis beyond young adulthood. This assumption is challenged by the current study in which evidence of chondrogenesis of middle-aged rat MSCs in agarose cultures was reliably detected from male or female donors. For many years, serum-free medium has been widely used to induce MSC chondrogenesis in vitro.[4] Here, the addition of serum to serum-free culture increased the frequency of robust differentiation and overall matrix accumulation. With serum-supplementation the accumulation of GAG (~2 µg/mg ww) was approximately similar to young adult equine MSCs that were cultured in the same manner, while hydroxyproline accumulation in rat cultures (~0.18 µg/mg ww) was approximately 50% of equine cultures.[5] These data suggest that the propensity of aging rat MSCs to undergo chondrogenesis is approximately similar to other species.

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Effect of culture duration on chondrogenic preconditioning of equine bone marrow mesenchymal stem cells in self-assembling peptide hydrogel

This is a summary of an article by Drs. John Kisiday, Suwimol Tangtrongsup and Aimee Colbath that was published in the Journal of Orthopaedic Research.[1]

Take home message

Bone marrow mesenchymal stem cells (MSCs) are isolated and expanded *in vitro* in an undifferentiated state. Therefore, for therapies in which MSCs are expected to repair articular cartilage the cells must receive specific cues that induce differentiation to a chondrocyte-like phenotype. This study investigated the potential to rapidly generate chondrocyte-like equine MSCs *in vitro*. In chondrogenic medium, equine MSCs differentiated to a chondrocyte-like phenotype within days. However, after removal from chondrogenic medium the cells strongly expressed a marker of hypertrophy, which is suggestive of endochondral ossification. These data indicated that minimal induction of chondrogenesis is not sufficient for cartilage repair, and support the concept that chondrocyte-like MSCs are better suited for bone repair.

Introduction

Undifferentiated MSCs that have not received chondrogenic cues prior to implantation have not proven capable of healing cartilage defects. It has been postulated that the induction of MSC chondrogenesis prior to implantation, hereafter referred to as 'chondrogenic preconditioning', is necessary to result in a stable, chondrocyte-like phenotype that secretes and maintains cartilage-like repair tissue *in vivo*.[1] Therefore, the objective of this study was to evaluate protocols for inducing equine MSC chondrogenesis *in vitro* using a new approach that results in suspensions of cells that are suited for injectable therapies. Undifferentiated MSCs into self-assembling peptide hydrogel, and then culturing in chondrogenic medium containing dexamethasone and transforming growth factor beta (TGFβ).[2] Following preconditioning culture, commitment to chondrogenesis was evaluated by isolating the MSCs from the peptide hydrogel, seeding into agarose hydrogel, and culturing in the absence of TGFβ.

Methods

Equine MSCs were isolated from bone marrow aspirates taken from 5 young adult horses and culture-expanded through ~8 population doublings in expansion culture. Next, MSCs were encapsulated in the self-assembling peptide hydrogel KLD12 using previous established techniques[3] at $10x10^6$ cells/ml and cultured in chondrogenic medium (high-glucose DMEM, 1% ITS+, 37.5µg/ml ascorbate-2-phosphate, 100 nM dexamethasone, 10 ng/ml TGF-β1). After no more than 5 days of culture, MSCs were released from the peptide by disrupted the hydrogel with micropipetting. The cell/peptide suspension was treated with 0.25% trypsin/EDTA for 5 minutes, and then expansion medium plus 0.1% collagenase for 45 minutes. The cell-peptide suspension was incubated for 30 minutes in tissue culture flasks to allow the released cells to adhere, and the adherent cell population was collected as an individual cell suspension and seeded into 1.5% agarose at $10x10^6$ cells/ml. MSCs from preconditioning culture were cultured in chondrogenic medium **without** TGFβ. Agarose control cultures were created in a similar manner using undifferentiated MSC at the start of priming cultures and maintained in TGFβ-free or 10 ng/ml TGFβ medium. All cultures were evaluated for GAG and hydroxyproline accumulation, type II collagen immunohistochemistry, and gene expression of types I, II, and X collagen 17 days after the start of preconditioning culture. Induction of immunogenicity with chondrogenic preconditioning was determined by major histocompatibility complex class II (MHC-II) expression, which was quantified using flow cytometry.

Results

GAG and hydroxyproline accumulation in positive controls was ~15-fold higher than negative controls. One day of chondrogenic preconditioning did not significantly stimulate GAG accumulation over negative controls. Two days of chondrogenic preconditioning resulted in GAG accumulation that was not significantly different from positive controls, while three day of preconditioning resulted in GAG accumulation that was ~80% greater than positive controls. Hydroxyproline accumulation was largely similar to GAG accumulation. Four and five days of preconditioning did not improve matrix accumulation. Type II collagen staining was reflective of matrix accumulation. Therefore, the remainder of the study focused on three days of chondrogenic preconditioning. For MSCs that were preconditioned for 3 days and cultured in negative control medium for 14 days, types I and II collagen expression were comparable to positive controls, while type X collagen expression was ~65-fold higher than positive controls. Potential induction of immunogenicity with preconditioning culture was indicated by MHCII expression, which was nearly absence in undifferentiated MSCs, and ~7% positive immediately after preconditioning culture.

Conclusions

This study demonstrated the potential for effective and efficient chondrogenic preconditioning, which is consistent with reports that temporary exposure to TGFβ is sufficient to induce MSC chondrogenesis or sustain ECM accumulation.[1-7] Bone marrow MSCs have been reported to exhibit indicators of hypertrophy with the progression of chondrogenesis *in vitro*,[8,9] or with subcutaneous implantation,[10] which lends caution that chondrogenic preconditioning may direct MSCs to endochondral ossification. This potential was evidence in the current study given the high level of type X collagen expression by preconditioned MSCs. In addition, while undifferentiated MSCs are considered safe for allogeneic applications, chondrogenic preconditioning may induce immunogenicity.

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Effect of rear wheel suspension on tilt-in-space wheelchair shock and vibration attenuation.

This is a summary of a paper published by Ms. Molly Hischke and Dr. Raoul Reiser in Physical Medicine & Rehabilitation (PM&R).[1]

Take home message

Rigid manual tilt-in-space wheelchairs respond to rough surfaces in a similar manner to other rigid wheelchairs, exposing the user to potentially harmful impacts and vibrations. The newly designed aftermarket suspension system reduces some aspects of shock and vibration exposure, potentially improving the quality of life for those needing to use manual tilt-in-space wheelchairs.

Introduction

Tilt-in-space wheelchairs are designed to address the adverse effects associated with pressure management, spasticity, respiratory and digestive complications, sitting tolerance, pain, edema, postural realignment, pressure sores, and hypotension that accompany prolonged sitting.[1] The tilt-in-space design allows the seat of the wheelchair to be rotated independent of the frame. In order to do so, tilt-in-space wheelchairs tend to be heavier than traditional manual wheelchairs. To reduce their weight, suspension is not incorporated into manual tilt-in-space wheelchairs. QuadshoX LLC (Fort Collins, CO) has created a patented method to attach a spring-damper unit to the rear wheel (Figure 1) with hopes of reducing secondary injuries to wheelchair users associated with shock and vibration exposure. The aim of this study was to investigate the shock and vibration reducing capabilities of the newly available aftermarket rear wheel suspension system for manual tilt-in-space wheelchairs.

Methods

Ten healthy non-wheelchair users volunteered for the study. Subjects were pushed by the same trained investigator over four different obstacles while using a Quickie IRIS® Tilt-in-Space manual wheelchair (Sunrise Medical, Phoenix, AZ). The subjects traversed the obstacles with the wheelchair as

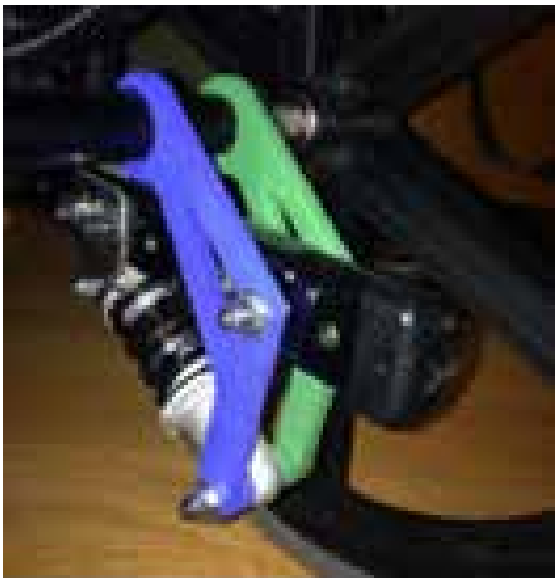

Figure 1. Suspension system on the rear axle of the tilt-in-space wheelchair. A second, identical spring-damper unit is attached just inside the rear wheel on the opposite side of the chair.

manufactured and with the QuadshoX suspension kit. Surfaces included a/an 1) exterior door threshold, 2) truncated domes, 3) 2 cm descent, and 4) 2 cm ascent. Two solid wheels of different diameters, Primo Cheng Shin Tires (Cheng Shin Rubber, Yuanlin, Taiwan), were also studied: a 0.381-meter (small) diameter wheel and a 0.508-meter (large) diameter wheel. Rigid chair trials were completed first, with suspension trials ~2 weeks later. Three acceptable trials were collected in each condition. Suspension chair trials needed to be within 0.2 sec (on average) of the matched rigid chair trials as determined by hand timing. A tri-axial accelerometer was mounted to the rear of the wheelchair seat pan with signals sampled at 2000 Hz. Peak resultant accelerations were analyzed from surface 1, 3-4, root mean square (RMS) resultant accelerations were analyzed from

surface 2, and vibration dose value (VDV) and total power were analyzed from all surfaces 1-4. The ISO 2631-1 establishes methods to investigate shock and vibration exposure.[2] The standards state frequency weightings are applied to frequencies to most harmful for human exposure (4-12 Hz for seated subjects). Therefore, frequency weighted (FW) and un-weighted peaks were analyzed.

Results

The use of suspension decreased the un-weighted peak acceleration at the rear wheel when it impacted the door threshold, when it traversed the 2 cm ascent, and when the rear wheel was ascended 2 cm ($p<0.05$). The use of suspension decreased FW peak accelerations at the rear wheel when it impacted and left the door threshold by 10-25%, and when the rear wheel descended 2 cm by 19-34% ($p<0.05$). The use of suspension did not significantly change the un-weighted accelerations when the rear wheel left the door threshold, and when the rear wheel traversed the 2 cm decent. There were no significant differences in FW peak accelerations at the rear wheel during the 2 cm ascent with the use of suspension. There were no significant differences in weighted and un-weighted peak accelerations at the front caster wheel over any of the four obstacles.

With suspension, RMS and total VDV significantly decreased 14% and 10-22% respectively ($p<0.05$). There were no significant differences between the rigid and suspended chair in total vibration power in frequency octaves most harmful in human exposure (4-12 Hz). The results of wheel diameter impacting peak accelerations, RMS, VDV and total power were inconclusive because there were significant differences in time spent over the obstacles.

Conclusions

The results indicate the aftermarket rear wheel suspension reduces some aspects of shock and vibration exposure, specifically at the rear wheel. Numerous health risks are correlated with shock and vibration exposure such as low back pain, neck pain, discomfort, and muscle fatigue. To our knowledge, there is no set amount of reduction in shock and vibration exposure decreasing the health risks with exposure. As of now, the recommendation is to reduce shock and vibration exposure as much as possible. The reductions in shock and vibration with the use of the aftermarket rear wheel suspension may decrease the health risks, such as pain and muscle fatigue. The results may be utilized by clinicians and wheelchair users for proper selection as well as aid in the future development of wheelchair suspension.

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Equine models for the investigation of mesenchymal stem cell therapies in orthopaedic disease

This is a summary of an article by Drs. Colbath, Frisbie, Dow, Kisiday, McIlwraith and Goodrich published in Operative Techniques in Sports Medicine.[1]

Take home message

This article reviews equine models of post-traumatic osteoarthritis, focal cartilage healing and tendonitis used for evaluating mesenchymal stem cell therapies. These models are essential for investigating the efficacy of MSCs and have yielded promising results.

Summary

The horse as a model for orthopedic disease in humans

Although small animal laboratory models have been used extensively for testing MSCs in musculoskeletal disease,[2-4] these models are considered anatomically inferior to equine models. Horses' cartilage thickness, joint size and joint forces are more appropriate for translational models.[5] Further, the equine superficial digital flexor tendon is particularly appropriate as a translational model of tendonitis with similarities to the human Achilles tendon.[6,7] Equine experimental studies may include controlled exercise using a treadmill, and ample synovial fluid or tendon tissue is available for biochemical and biomechanical testing.[8-11] In addition, horses are used for athletic endeavors and suffer from naturally occurring disease including osteoarthritis, desmitis, tendonitis, meniscal injury and osteochondritis dessicans.[12-15] The ability to assess naturally occurring disease and the availability of technical equipment including arthroscopy, CT and MRI make this population particularly relevant for pre-clinical testing. In addition, pain and lameness may be assessed both subjectively and objectively using force plates and/or inertial sensor systems.[16] Histological analysis can be performed both for models of tendonitis and osteoarthritis.

Specific experimental models of post-traumatic osteoarthritis include a well-established radial carpal osteochondral fragment model. In addition, two impact models of osteoarthritis have been described. These include an impact to the medial femoral condyle or palmar aspect of the metacarpus.[17,18] Models of superficial digital flexor tendon injury include surgical removal of a window of tendon[19] or column of tendon,[20] transcutaneous radiofrequency coblation,[21] burr-induced mechanical injury,[22] or collagenolytic enzymes.[23-25]

Cellular therapies involving MSCs for treatment of musculoskeletal disease have expanded rapidly in the last several years. The above-mentioned equine models of musculoskeletal injury play an important role in addressing some of the questions regarding MSC mechanisms and efficacy. In particular, equine models may be useful in determining optimal cell delivery source, cell dose and the best way to pre-condition MSCs prior to intra-articular or intra-tendinous injection.

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In vitro effects of high-intensity laser photobiomodulation on equine bone marrow-derived mesenchymal stem cell viability and cytokine expression

This is a summary of an article by Drs. F.J. Peat, A.C. Colbath, L.R. Goodrich, M.R. King and Ms. L.M. Bentsen published in Photomedicine and Laser Surgery.[1]

Take home message

This study demonstrated the ability of high-intensity laser irradiation to increase the expression of anti-inflammatory and vascular endothelial growth factor cytokines from equine mesenchymal stem cells (MSCs) *in vitro*. Although the *in vivo* behavior of irradiated MSCs is yet to be studied, photobiomodulation to increase cellular expression of cytokines that regulate inflammation and promote angiogenesis may become a clinically useful technique. Therapeutic interventions utilizing energy from monochromatic light are extremely dose-dependent, thus translation of *in vitro* laser protocols from the laboratory to the clinical setting will require quantification of the energy dose reaching target tissues in the *in vivo* environment. Further investigation of the potential for beneficial effects on MSCs from 1064 nm laser photobiomodulation to enhance their therapeutic properties is warranted.

Introduction

This study examined the influence of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser irradiation on equine bone marrow-derived mesenchymal stem cell (MSC) viability, proliferation, and cytokine expression *in vitro*. Photobiomodulation of cells using monochromatic light is a technique designed to influence cellular processes. Previous studies have shown dose-dependent effects of low-level laser irradiation on cell proliferation and cytokine expression in a range of cell types and species. Evidence for the influence of 1064 nm wavelength near-infrared irradiation on MSCs is sparse, and high-energy doses have shown inhibitory effects.

Methods

MSC cultures from six horses were exposed to 1064nm irradiation with an energy density of 9.77 J/cm^2 and a mean output power of 13.0 W for 10 sec. MSC viability and proliferation were evaluated through flow cytometry and real-time live cell analysis. Gene expression and cytokine production in the first 24 h after irradiation were analyzed through polymerase chain reaction (PCR), multiplex assay, and enzyme-linked immunosorbent assay.

Results

Twenty-four hours after irradiation, irradiated MSCs demonstrated a significant increase in expression of interleukin (IL)-10 and vascular endothelial growth factor (VEGF) compared with control MSCs. No difference in viability was detected between irradiated and control MSCs. Irradiated cells demonstrated slightly lower proliferation rates, but remained within 3.5% confluence of control cells.

Conclusions

Under the specified irradiation parameters used in this study, equine MSCs remained viable and expressed increased concentrations of IL-10 and VEGF. IL-10 has an anti-inflammatory action by inhibiting the synthesis of proinflammatory cytokines at the transcriptional level. This response to 1064 nm irradiation shows promise in the photobiomodulation of MSCs to enhance their therapeutic properties.

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Modulating the oxidative environment during mesenchymal stem cells chondrogenesis with serum increases collagen accumulation in agarose culture

This is a summary of an article by Drs. Suwimol Tangtrongsup and John Kisiday that was published in the Journal of Orthopaedic Research.[1]

Take home message

The objective of this *in vitro* study was to characterize the generation of oxidative stress with chondrogenesis of mesenchymal stem cells (MSCs), and determine whether oxidative stress supports or suppresses neo-cartilage synthesis. Oxidative stress was evident when MSC chondrogenesis was induced in conventional serum-free medium. Adding serum to the differentiation medium greatly decreased oxidative stress, and coincided with an increase in collagen synthesis. Therefore, reducing oxidative stress is an effective means to promote neo-cartilage synthesis by chondrogenic MSCs.

Introduction

The ability of bone marrow MSCs to undergo chondrogenesis has fostered enthusiasm for tissue engineering approaches to resurface damaged or diseased articular cartilage. Current strategies for inducing MSCs chondrogenesis are largely focused on growth factors that stimulate differentiation, although it has been postulated that additional measures to support the growth and maintenance of neo-cartilage will increase the likelihood of success.[1] Oxidative stress, as indicated by intracellular levels of reactive oxygen species ROS, has been shown to reduce matrix synthesis in chondrocyte cultures.[2-3] Therefore, the objectives of this study were to characterized levels of intracellular ROS during chondrogenesis in serum-free culture, and determine whether lowering levels of ROS coincided with improved matrix synthesis.

Methods

Equine MSCs were isolated from bone marrow aspirates and culture-expanded through ~8 population doublings in alphaMEM + 10% fetal bovine serum (FBS) and 2 ng/ml FGF2. After expansion, MSCs were encapsulated in 1.5% (w/v) agarose at $12x10^6$ cells/ml, and cultured in chondrogenic medium (high-glucose DMEM, 1% ITS+, 37.5µg/ml ascorbate-2-phosphate, 100 nM Dexamethasone, 10 ng/ml TGF-β1) for up to 15 days. To study the effect of serum on ROS production and chondrogenesis, 5% FBS was added to chondrogenic medium. *Analysis* – In agarose cultures, CellROX® reagent were used to evaluate intracelluar ROS, qualitatively using a fluorescent microscope. Extracellular matrix accumulation by quantifying the accumulation of sulfated glycosaminoglycan (GAG) and hydroxyproline, by DMMB and DMBA dye binding assay, respectively. Cell viability was evaluated using the CellTiter Blue (CTB) assay. GAG, hydroxyproline and cell viability data were normalized by wet weight. Gene expression of type I and II collagens was evaluated using real-time PCR. Total glutathione (GSH), an endogenous antioxidant, was quantified using commercial GSH/GSSG-Glo™ assay.

Results

In serum-free medium, staining for ROS increased with time in culture, appearing to reach a maximum on day 6 that was sustained through 12 days of culture. Compared to ITS, the addition of 5% FBS greatly decreased the intensity of ROS staining at all timepoints. Glutathione decreased ~12-fold between days 1 and 6 of chondrogenic culture, and was not affected by the presence of FBS. After 15 days of culture, FBS supplementation increased hydroxyproline accumulation ~80%; otherwise, measures of matrix accumulation, cell viability, and collagen gene expression were largely unaffected.

Conclusions

In cell culture, it is known that serum starvation can increase the production of ROS,[4-6] which was consistent with elevated levels of ROS in chondrogenic MSCs with time in serum-free culture. The reduction

in glutathione during early MSCs chondrogenesis suggests that increases in intracellular ROS are at least in part due to downregulation of endogenous antioxidants, which has been previously reported for MSCs chondrogenesis and superoxide dismutase.[7] FBS proved to be an effective antioxidant based on the lowering of intracellular ROS relative to serum-free culture. The most significant effect of FBS was a ~80% increase in collagen accumulation without a concomitant increase in proteoglycan content, which is consistent with reports that ROS can differentially affect the synthesis of collagen and proteoglycans.[8] In conclusion, this study identified temporal changes in the oxidative environment during MSCs chondrogenesis, and the potential benefit of adding antioxidants to existing methods of inducing differentiation. Further, the evaluation of serum as an antioxidant here may be particularly important for tissue engineering strategies that seek to induce chondrogenesis *in vivo* as serum is a component of synovial fluid.

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Not missing the future: a call to action for investigating the role of regenerative medicine therapies in pediatric/adolescent sports injuries

This is a summary of an article by Drs. T.M. Best, A. Caplan, M. Coleman, L.R. Goodrich, J. Huard, L.D. Kaplan, B. Noonan, P. Schoettle, C. Scott, and H. Stiene published in the Sports Medicine Reports.[1]

Take home message

This article summarizes a meeting intended to shed more light on regenerative medicine therapies in youth sport.

Introduction

Regenerative medicine encompasses the use of stem cell and other cell-based therapies, growth factors, and biologics in the management of each individual's innate capabilities for tissue repair and regeneration to optimize a therapeutic outcome. These therapies are increasingly being used in sports injuries, for the most part without clearly defined evidence base. The goal of this meeting is to educate practitioners and the public, and to pioneer a means of accumulating data on the safe and effective way to use regenerative medicine therapies in pediatric and adolescent.

Conclusion

In this meeting, seven actions were endorsed by the group. Action one advises caution when treating youth as the research continues. While platelet rich plasma (PRP) use for musculoskeletal injuries has been deemed relatively safe in adults, potential long-term effects on youth have not yet been thoroughly researched. The second action recommends improvements in regulatory oversight. While these therapies are relatively safe, they are often provided without regulatory oversight. There are many clinics offering unregulated cell-based therapies, especially with sports related injuries. Many of these clinics are registering clinical trials on ClinicalTrials.gov to increase their credibility. There is an urgent need for this lack of regulation to be addressed and resolved. Action three calls to expand governmental and other research funding as this would help create the evidence base to guide the already widespread use of the cell-based therapies in young adults. Action four calls for creation of a system of registries. These registries will help establish use and outcome data which would provide key information to fill knowledge gaps in the field. Action five calls for development of a multi-year policy and agenda and to build support for it. This will be important going forward, as it will help keep the industry regulated and strictly enforced. Action six encourages the building of a multidisciplinary consortium. This will help advance the evidence base and promote regulation in a systematic way. Finally, action seven calls for development and pursuit of a clear collective impact agenda.

Regenerative medicine therapies offer a very potent way to assist with the treatment of sports injuries, but the data on their effectiveness and long-term safety is limited, especially in young adults.

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Use of platelet-rich plasma immediately after an injury did not improve ligament healing, and increasing platelet concentrations was detrimental in an in vivo animal model

This is a summary of an article by Drs. R.F. LaPrade, L.R. Goodrich, J.N. Phillips, G.J. Dornan, T.L. Turnbull, M.L. Hawes, K.D. Dahl, A.N. Coggins, J. Kisiday, D. Frisbie, and J. Chahla published in the American Journal of Sports Medicine.[1]

Take home message

Limited information in basic science and clinical trials exists to determine if ligament healing may be accelerated with the use of biological adjuvants, such as platelet-rich plasma (PRP). However, in this study in a rabbit model, the use of PRP did not enhance healing.

Introduction

This article addressed the use of platelet-rich plasma (PRP) in the effectiveness of healing ligament injuries, specifically in the medial collateral ligament (MCL). New Zealand White rabbits were used as a model as the healing of their MCL closely reflects that of a human. MCL injuries are the most common knee ligament injuries and can usually heal with nonoperative treatment. Often however, the mechanical and histological properties have been reported to not return to normal. For this reason, PRP has been advocated to advance the healing of the MCL. This is because PRP contains inherently high concentrations of beneficial growth factors that have been demonstrated to improve tissue healing, especially during the inflammatory phase of healing. While the use of PRP injection for injured ligaments is used worldwide, there are many conflicting reports in the literature and debate continues surrounding the effectiveness and role of PRP in accelerating ligament healing. Potential factors that may affect the effectiveness of PRP include platelet concentration, leukocyte count, timing of the treatment, and activation of platelets within PRP.

The purpose of this study was to determine whether a single dose of PRP at different platelet concentrations could accelerate healing and correspondingly improve histological characteristics and biomechanical properties when injected immediately postoperatively in the injured MCL model. The central hypothesis was that PRP would accelerate healing in an MCL injury model after acute trauma and correspondingly enhance the histological and biomechanical properties when compared with platelet-poor plasma (PPP) or saline.

Methods

Eighty skeletally mature New Zealand White rabbits were used. The MCL was torn midbody to stimulate a grade 3 tear. After an acute injury of the MCL, the administration of autologous PRP at three different platelet concentrations (0 million/uL, PPP; 0.6 million/uL, 2 x PRP, and 1.2 million/uL 4 x PRP) were performed and compared with a saline injection control in the contralateral knee. Histological analysis and a biomechanical endpoint characterization were utilized to assess ligamentous healing and compared it to a sham surgery group.

Results

The most important finding of this study was that one single dose of either PPP or 2x PRP at the time of the injury did not accelerate ligament healing. Additionally, 4x PRP demonstrated a significant negative effect on ligament strength as well as collagen orientation at 6 weeks after an injury. Thus, the hypothesis was not supported. This raises concern that the current practice of treating knee ligament injuries, specifically MCL tears, with PRP immediately after an injury or surgery may not improve healing at low doses of PRP but could be harming ligament healing at higher PRP doses.

Conclusion

One single dose of PPP and 2× PRP at the time of injury did not improve ligament healing. In addition, 4× PRP negatively affected ligament strength and histological characteristics at 6 weeks after an injury.

This study helped provide guidance on a common issue in clinical practice. However, as is clear from the findings in this study, further studies to determine the timing and dosing frequency of PRP to treat ligament healing are required.

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An investigation of shock wave therapy and low-intensity pulsed ultrasound on fracture healing under reduced loading conditions in an ovine model

This is a summary of an article by Drs. Benjamin Gadomski, Kirk McGilvray, Jeremiah Easley, Ross Palmer, Jin Jiao, Yi-Xian Qin, and Christian Puttlitz published in the Journal of Orthopaedic Research in 2018.[1]

Take home message

Shockwave therapy and low-intensity pulsed ultrasound demonstrated the ability to increase osteoblast and decrease osteoclast numbers while accelerating bone formation rates during mechanically unloaded fracture healing.

Introduction

The inherent reduction in mechanical loading associated with microgravity has been shown to result in dramatic decreases in the bone mineral density (BMD) and mechanical strength of skeletal tissue.[1-3] In addition to this elevation in fracture risk, previous work has demonstrated that skeletal fracture healing is severely inhibited by the microgravity environment experienced during spaceflight.[3;4-7] Shock wave therapy (SWT) and low-intensity pulsed ultrasound (LIPUS) have been used to treat nonunion and delayed healing of bony fractures in humans by generating low-level mechanical stresses at the fracture site and inducing subsequent cellular and molecular responses involved in the healing cascade.[8-12] Due to their noninvasive nature, compact equipment size, ease of administration, low complication rate, and high efficacy, SWT and LIPUS have become well-accepted and commonly used clinical therapeutic techniques and make them ideal candidates to increase the rate of bony fracture healing during scenarios such as spaceflight.[12] The objective of this study was to interrogate the efficacy of these two therapies as countermeasures to the inhibited fracture healing experienced during partial gravity unloading in a previously developed large animal (sheep) model with the hypothesis that bone formation and strength would be increased following treatment.[1]

Methods

A total of twenty-eight skeletally mature, female, Rambouillet Columbian ewes (age > 6 years) were included in this study. Animal use approval was granted by the Colorado State University Animal Care and Use Committee (Approval #11-2938A). Nine animals were randomly allocated to an Earth-based mechanically unloaded (0.25G) group and underwent hindlimb metatarsal unloading as described by Gadomski et al.[1] Briefly, a trans-biarticular external skeletal fixation device (IMEX, Longview, TX) was implanted on the right hindlimb such that the metatarsal bone was isolated from mechanical loading. These animals were then exposed to mechanical unloading of the metatarsal bone for a period of 3 weeks (21 days), at which point a 3.0mm ostectomy was created and stabilized with a fixation plate. An additional full gravity group of 9 animals (1G Group) was included in which an identical 3.0mm diaphyseal ostectomy was created, plated, and casted, allowing full loading to be transmitted through the metatarsal bone.

SWT was administered to five animals from each of the two groups (0.25G-SWT and 1G-SWT) 6 days following the creation of the ostectomy with an applied energy of 0.63 mJ/mm^2, a frequency of 5Hz, and a total shock count of 4,000 (Storz Medical AG, Tägerwilen, Switzerland) (Figure 1). LIPUS was administered to four animals from each group (0.25G-LIPUS and 1G-LIPUS) two days following surgery for 20 minutes per day, five days per week for the remainder of the four-week healing period by applying a 200-µsec burst of 3.3MHz sine waves repeated at 1 kHz with an average intensity of 20 mW/cm^2. The data from ten animals of a previous study were included as control data in which reduced (0.25G-C, n=5) and full gravity (1G-C, n=5) control groups un-

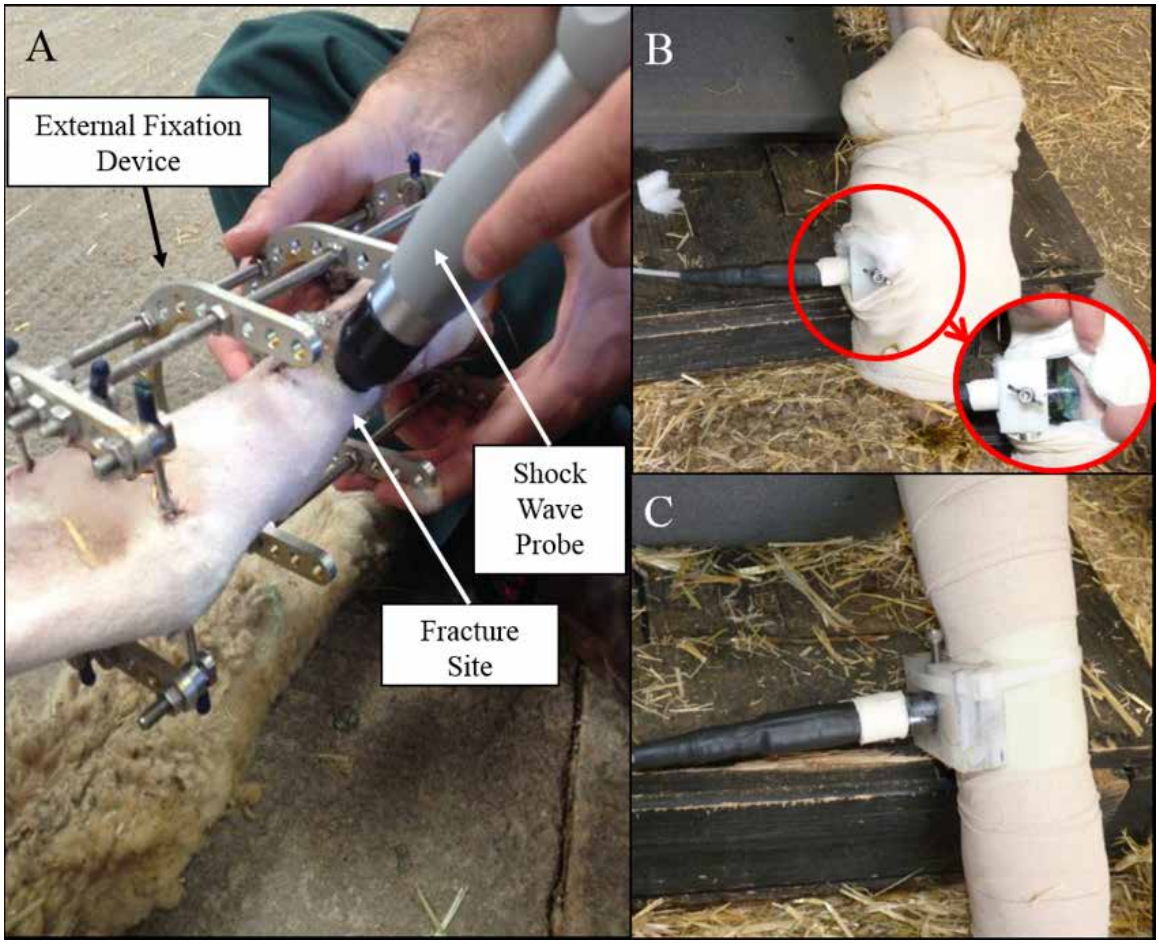

Figure 1. (A) SWT was administered around the circumference of the fracture site of the 0.25G-SWT and 1G-SWT groups 6 days following the creation of the ostectomy. (B) The LIPUS ultrasound probe was secured to the external fixation frame via a polyethylene fixture to ensure identical placement against the skin overlaying the metatarsal diaphysis between treatment sessions for 0.25G-LIPUS animals. (C) The ultrasound probe was inserted through a pre-drilled hole in the fiberglass cast of 1G-LIPUS animals to attain skin contact and secured via a polyethylene fixture.

derwent the surgical protocols previously described with no administration of treatment (including sham treatments).[4] All groups were euthanized after a 28-day healing period. Non-destructive biomechanical four-point bending tests, micro-computed tomography (μCT), and histomorphometric analyses were performed on all specimens.

Results

Results of the 4-point bending tests demonstrated no statistically significant differences in stiffness within the 0.25G groups or 1G groups. Significant alterations in bone volume fraction and bone mineral density (BMD) within the fracture gap were not observed following SWT or LIPUS treatment as compared to control specimens for the 0.25G or 1G gravitational groups. Callus BMD of 0.25G-SWT specimens was significantly greater ($p<0.05$) than that of 1G-SWT specimens. Callus bone volume (BV) was not significantly elevated in 0.25G-SWT or 0.25G-LIPUS groups as compared to 0.25G-C specimens; however, callus BV was significantly reduced in 1G-LIPUS specimens as compared to 1G-C and 1G-SWT specimens ($p<0.05$).

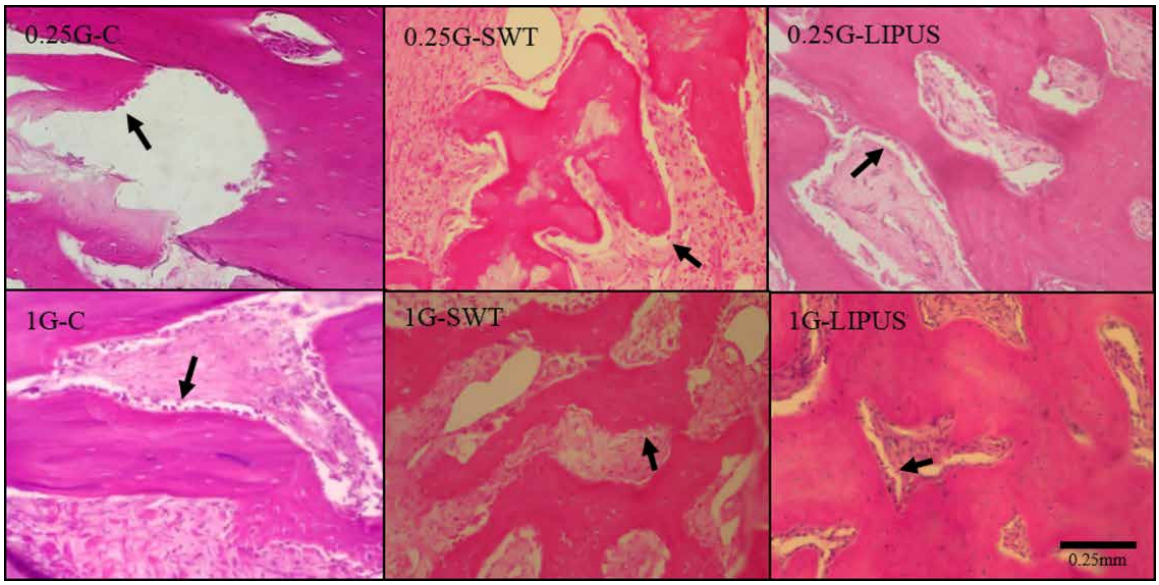

Figure 2. Osteoblast (indicated by arrows) numbers within the periosteal callus were significantly elevated (p<0.05) in 0.25G specimens treated with SWT (26.91 + 3.98, 0.25G-SWT, top middle) and LIPUS (31.63 + 5.01, 0.25G-LIPUS, top right) as compared to those that received no treatment (14.37 + 2.01, 0.25G-C, top left). (Bottom) No significant alterations were observed between 1G groups (26.89 + 5.68, 29.59 + 3.61, and 35.38 + 5.48 for the 1G-C, 1G-SWT, and 1G-LIPUS groups, respectively). Data are presented as mean + standard deviation.

Histomorphometric analyses demonstrated significantly elevated callus bone area in 0.25G-LIPUS and 1G-LIPUS specimens as compared to other groups ($p<0.05$). Osteoblast numbers (Figure 2) were increased 87% and 120% in 0.25G-SWT and 0.25G-LIPUS specimens as compared to 0.25G-C specimens on the periosteal surface, respectively, and 152% and 244% on the endosteal surface, respectively ($p<0.05$). Osteoblast numbers were significantly elevated in 1G-LIPUS specimens as compared to 1G-SWT and 1G-C groups, with increases of 18% and 23% in 1G-SWT and 1G-LIPUS specimens as compared to 1G-C specimens, respectively ($p<0.05$). Periosteal osteoclast numbers were significantly reduced by 82% and 86%, respectively, in 0.25G-SWT and 0.25G-LIPUS specimens as compared to 0.25G-C specimens ($p<0.05$). Mineralizing surface was significantly increased by 53% and 92%, respectively, in 0.25G-SWT and 0.25G-LIPUS specimens as compared to 0.25G-C specimens, while bone formation rate was significantly elevated 88% and 80%, respectively, as compared to 0.25G-C specimens ($p<0.05$).

Conclusions

These data provide strong evidence that SWT and LIPUS elevate osteoblast numbers and bone formation rates as well as decrease osteoclast numbers in a large animal model of partial gravity unloading; however, our hypothesis was only partially confirmed. While bone formation rates were increased following LIPUS and SWT treatment under partial-unloading, no increase in 4-week mechanical strength was observed. It is possible that an increase in fracture healing (i.e. callus mechanical competence) would be experienced at longer timepoints under reduced loading conditions given the increase in osteoblast numbers and bone formation parameters following both treatments.

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Biomechanical and histologic evaluation of the effects of underwater treadmill exercise on horses with experimentally induced osteoarthritis of a carpal joint

This is a summary of an article by Drs. Moorman VJ, Bass L, King MR. published in The American Journal of Veterinary Research in 2018.[1]

Take home message

Overall improvements in thoracic limb function, joint range of motion, and synovial membrane integrity indicate that exercise in an UWT is a viable therapeutic option for the management of carpal joint osteoarthritis in horses.

Introduction

Joint disease has been defined as a complex imbalance in the homeostatic mechanisms of degradation and repair, with the inflammatory cascade playing a crucial role in the progressive catabolic process.[1] Numerous intra-articular treatments have been used to affect pathological manifestations of joint disease in horses by decreasing the degree of inflammation within the affected articulation.[2-4] However, little attention has been focused on the various forms of physical rehabilitation that could aid in modifying joint disease and it's progression in horses.[5] Rehabilitative approaches have become effective treatment options for reducing or limiting harmful compensatory gait abnormalities in humans.[6,7] Rehabilitation programs that address osteoarthritis and musculoskeletal injuries in humans often incorporate some form of aquatic exercise. The purpose of the study reported here was to quantify clinical, biomechanical, and articular effects of exercise in an UWT, compared with results for simulated hand walking, in horses with unilateral experimentally induced carpal joint osteoarthritis. We hypothesized that aquatic therapy for horses with carpal joint osteoarthritis would enhance neuromuscular function, reduce thoracic limb gait abnormalities associated with carpal joint pain and inflammation, and improve histologic characteristics of affected carpal joints, which would provide further objective support for the use of aquatic rehabilitation.

Conclusion

Exercise in an UWT significantly reduced synovial membrane inflammation and resulted in significant clinical improvements with regard to symmetric thoracic limb loading, uniform activation patterns of select thoracic limb muscles, and return to baseline values for carpal joint flexion, compared with results for horses with simulated hand walking.

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Equine manual therapies in sport horse practice

This is a summary of an article by Dr. Kevin K. Haussler published in Veterinary Clinics Equine: Equine Sports Medicine in 2018; 34: 375-389.[1]

Take home message

Manual therapies can provide detailed diagnostic and therapeutic approaches to assess and manage neuromuscular coordination and strength in sport horses. Soft tissue or joint mobilization is indicated to help limit the effects of joint immobilization and to restore proprioceptive mechanisms. Equine chiropractic research has shown positive effects for pain relief, improving flexibility, reducing muscle hypertonicity, and restoring spinal motion symmetry

Introduction

Manual therapies involve the application of the hands to the body, with a diagnostic or therapeutic intent. In horses, a diverse array of manual techniques such as touch therapies, massage, joint mobilization, and manipulation (i.e., chiropractic) have been applied with a primary therapeutic intent (e.g., reduce pain or stiffness).[1-3] However, all of these therapies also have important diagnostic value in assessing musculoskeletal pain and dysfunction that is not possible with other more traditional physical examination approaches or imaging modalities. In sport horse practice, the primary issues that limit performance are chronic repetitive use injuries associated with long active athletic careers of pushing physical and psychological limits of both horse and rider. Chronic, poorly-localized pain and stiffness combined with slower reflexes or altered muscle timing contribute to poor performance issues and increase the risk of acute injury and inflammation. Manual therapies can provide detailed soft tissue, osseous and articular evaluation techniques and unique methods to assess neuromuscular coordination and strength in sport horses that are not possible with routine lameness evaluation or neurologic tests.

Identification of Rehabilitation Issues

Any medical, surgical or rehabilitation plan is only as good as the diagnosis that it is based upon. As veterinarians, we are typically very good at establishing or defining diagnoses based on a known pathology or on anatomic localization (i.e., pathoanatomic diagnosis). At times, we may even slide into the misguided approach of "treating the diagnostic image" without giving full consideration to determining the clinical relevance of the diagnostic imaging findings relative to the presenting or continued clinical signs of the patient. At the other end of the diagnostic-treatment spectrum are those owners and practitioners that are solely focused on the function of the horse (i.e., is the horse able to do its job) despite the accumulation of known musculoskeletal injuries and chronic, multi-limb lameness over a long active athletic career. Striving to find a balance between applying both structural and functional approaches is ideal for managing the athletic demands and injuries in sport horses.

From the functional perspective, general rehabilitation issues to be addressed in equine athletes include, in progressing order:

1) Pain management
2) Proprioceptive deficits
3) Stiffness
4) Weakness or fatigue
5) Neuromuscular control

Pain management is always the first step in rehabilitation as it is not possible or ethical to ask a patient to exercise or do stretching when they are in pain. The body's normal protective mechanisms will not

allow you to fully contract a muscle attached to an acutely stained tendon or to freely move a joint with acute synovitis. Nociceptive input by itself induces many other neurologic reflexes (e.g., withdrawal reflex, crossed-extensor reflex, etc.) that function acutely to protect the body from further injury. However, chronic nociceptive input leads to peripheral and central sensitization (i.e., wind-up) that has widespread neurologic and musculoskeletal effects that make clear distinctions between pain or lameness, altered proprioception or body awareness (i.e., somatoesthesia), and altered gait patterns difficult to interpret.

As horses move into the proprioceptive and flexibility phases of rehabilitation, more focus is placed on how the horse is perceiving its environment through its sensory system and able to navigate through that environment with its motor system. This integration is often referred to as neuromuscular or neuromotor control and relies heavily on afferent signaling from proprioceptors, which include muscle spindle fibers in muscles, Golgi tendon organs, and many other soft tissue mechanoreceptors located in joint capsules and fascial planes. The motor component includes active and passive structures. The active structures that can be addressed with rehabilitation include all motor pathways from the motor cortex in the cerebrum for control of movement, the cerebellum for balance and coordination, down to the timing and strength of muscle contractions. Passive structures include the joint capsules, ligaments, and the superficial and deep fascial layers that cover and envelop muscles and neurovascular bundles. All of the sensory and motor components and active and passive structures must function optimally for the horse to be able to progress in a defined rehabilitation or training program to build endurance and strength required for sport-specific demands.

Pain Management

The goal of most rehabilitation programs is the early initiation of movement to begin the process of restoring normal joint motion, strength and coordination. Acute pain and inflammation are typically managed with NSAIDs, cold therapies (i.e., ice), restricted exercise and compression wraps, if indicated, in an effort to protect local tissues and to limit excessive joint movement. Once the initial acute inflammatory phased has begun to subside in 3-5 days, then gentle, slow passive soft tissue or joint mobilization is indicated to help limit the effects of joint immobilization and to restore proprioceptive mechanisms.[4,5] Joint mobilization is usually applied in a graded manner, with each grade increasing the range of joint movement. Grades 1 to 2 joint mobilization involve inducing small degrees of joint motion around the neutral joint axis (i.e., resting joint position) and then beginning to move the joint up to 50% of normal joint range of motion for a specified articulation. If passive joint motion is too painful, then applying light pressure and inducing motion of the overlying skin and subcutaneous tissues may help to improve lymphatic flow and increase mechanoreceptor stimulation in an effort to inhibit nociceptive signaling via local and spinal cord mechanisms.[6] Manual lymph drainage has been described for use in the management of lymphedema in horses; however, no controlled studies exist evaluating its effectiveness.[7] The reparative process of tissue healing includes collagen synthesis and fibrous tissue proliferation. Significant fascial restrictions or adhesions can limit injury recovery if proper mechanical stimulation and restoration of fascial glide of both superficial and deep tissues is not achieved. Skin rolling techniques and deep tissue massage provide increased level of mechanical stimulation of connective tissues, which may be required in patients with extensive fibrosis or soft tissue adhesions.[2] Prolonged joint immobilization or forced stall rest are often counterproductive to maintaining musculoskeletal health.

Chronic pain often induces sensitization or wind-up which produces generalized pain that is poorly localized and is often disassociated from the initial inciting injury. In humans, massage therapy, joint mobilization and manipulation are often used to address chronic pain syndromes and compensatory gait mechanisms (i.e., antalgic gait). In horses, massage therapy has been shown to be effective for reducing stress-related behavior[8] and lowering mechanical nociceptive thresholds within the thoracolumbar region.[9] The use of acupuncture evaluation techniques to localize reactive loci within superficial soft tissues is useful for assessing overall nociceptive thresholds and diagnosing myofascial

pain. Acupressure or ischemic compression techniques can be used to treat local muscle pain or hypertonic bands (i.e., trigger points).[10] Two randomized, controlled clinical trials using pressure algometry to assess mechanical nociceptive thresholds (MNTs) in the thoracolumbar region of horses have demonstrated that both manual and instrument- assisted spinal manipulation can reduce back pain (or increase MNTs).[9,11]

Stiffness

Neck or back stiffness is a common cause of poor performance in sport horses. Stiffness localized to a specific limb articulation is typically due to joint capsule fibrosis or periarticular adhesions. Stiffness can also be produced by pain and muscle guarding associated with osteoarthritis or dorsal spinous process impingement. Muscle spasms or hypertonicity are common clinical findings in horses with neck of back pain or stiffness.[2] Detailed palpation techniques provided by manual therapy techniques can help to localize the source of stiffness to the various tissue types and possible pathophysiology of the clinical complaint.

All of the individual articulations of the proximal and distal limbs can be mobilized to assess the quality and quantity of joint motion. As isolated joints are moved through full flexion and extension and accessory motions of internal and external rotation or translation, the ease of joint movement and any restrictions or painful responses are noted. A full description of the techniques for joint mobilization are beyond the scope of this chapter, but a simple example of assessing internal and external rotation of the coffin joint demonstrate asymmetries in the end range of motion. Gentle rotation of the hoof internally and externally helps to determine the quality and quantity of passive axial rotation of the coffin joint as the pastern region is stabilized proximally (Figure 1).

Active stretching involves using the patient's own movements to induce a stretch, whereas passive stretches are applied to relaxed muscles or connective tissues during passive soft tissue or joint mobilization.[3,12] In horses, active stretches of the neck and trunk are often induced with baited (i.e., carrot) stretches with the goal in increasing flexion, extension or lateral bending of the axial skeleton.[13] Asking horses to produce active stretching of the limbs is often difficult; therefore, passive stretches are most commonly prescribed in horses.[12] In horses, passive stretching exercises of the limbs and axial skeleton have anecdotal effects of increasing stride length and joint range of motion and improving overall comfort.[12] In a noncontrolled study, passive thoracic limb stretching lowered wither height due to possible relaxation of the fibromuscular thoracic girdle.[14] However, a randomized controlled trial in riding school horses evaluating the effect of two different

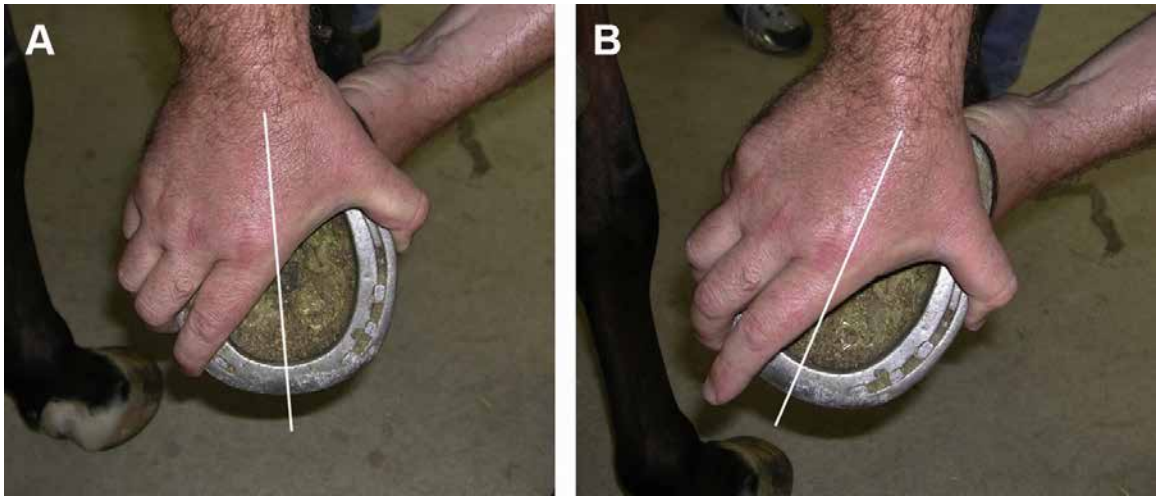

Fig. 1. Joint mobilization of the coffin joint in internal (A) and external (B) rotation. Note the reduced or asymmetric end range of motion induced during external rotation, compared with internal rotation (white lines).

8-week passive stretching programs reported no significant changes in stride length at the trot but had a detrimental effect of decreasing joint range of motion within the shoulder, stifle, and hock articulations.[15]

The focus of recent equine chiropractic research has been on assessing the clinical effects of spinal mobilization and manipulation on pain relief, improving flexibility, reducing muscle hypertonicity, and restoring spinal motion symmetry. Spinal mobilization has been shown to be effective at increasing spinal flexibility in ridden horses without clinical signs of back pain.[9] Manipulation may preferentially stimulate receptors within deep intervertebral muscles, while mobilization techniques most likely affect more superficial axial muscles. Only one study has compared mobilization to manipulation in horses and spinal manipulation induced a 15% increase in displacement and a 20% increase in applied force, compared to mobilization.[16] At most vertebral sites studied, manipulation increased the amplitudes of dorsoventral displacement and applied force, indicative of increased spinal flexibility and increased tolerance to pressure in the thoracolumbar region of the equine vertebral column.

Manually-applied forces associated with chiropractic techniques are able to produced substantial segmental spinal motion.[17] Additional studies have assessed the effects of equine chiropractic techniques on increasing passive spinal mobility (i.e., flexibility)[9,16] and reducing longissimus muscle tone.[18] The effect of manipulation on asymmetrical spinal movement patterns in horses with documented back pain suggest that chiropractic treatment elicits slight but significant changes in thoracolumbar and pelvic kinematics and that some of these changes are likely to be beneficial.[19,20]

Equine osteopathic evaluation and treatment procedures have been described in textbooks and case reports, but no formal hypothesis-driven research exists.[21,22] A case series of 51 horses with chronic lameness or gait abnormalities that were poorly localized were treated with osteopathic techniques under sedation and had reported positive results in the majority of case from 6 to 12 months after treatment.[23]

Weakness

Weakness (i.e., lack of muscular strength) is a common but poorly recognized or easily localized disorder. The etiology of weakness is often neurologic-based but clinically we often attribute weakness to muscular disorders due to the lack of epaxial muscle development, inability to perform advanced training techniques, asymmetrical movement patterns (e.g., not bend to the left) or difficulty in clearing a jump. The most common cause of weakness is reflex inhibition due to soft tissue or orthopedic pain. A lame horse is that is unable or unwilling to place full weight bearing on a limb also has distinct changes in muscle activation (i.e., timing and amplitude of contractions). Muscles that have altered timing can include individual muscles that turn on too early or stay active too long or do not turn on at all. Muscles also have changes in the number of motor units activated, which directly correlates to the amplitude or strength of muscle contraction. A horse with a painful back often has accompanying muscle hypertonicity of varying degrees, which alters the resting muscle tone and threshold for muscle activation. A common misconception is that a hypertonic or muscle spasm is a “strong” muscle; however, due to chronic activation it is often a very weak muscle with altered on-and-off timing that increases the risk of injury. Chronic pain often induces recruitment of peripheral or proximal limb muscles which we interpret clinically as altered gait patterns. Neurogenic atrophy can be noted locally within a segmentally-innervated myotome and varying degrees of disuse atrophy may be noticed more regionally over the lateral neck or dorsal trunk in horses with chronic neck or back pain.

Motor control

Manual forces are used to induce passive stretching, weight-shifting or activation of spinal reflexes, which help to increase flexibility, stimulate proprioception and strengthen core musculature.13,24 Soft tissue mobilization has the additional effect of stimulating regional or systemic changes in neurologic signaling related to pain processing and motor control. Joint mobilization and manipulation can provide effective management of pain and neuromuscular deficits associated with musculoskeletal injuries, alterations in postural control, and locomotory issues related to antalgic or compensatory gait. In re-

sponse to chronic pain or stiffness, new movement patterns are developed by the nervous system and adopted in an attempt to reduce pain or discomfort. Long after the initial injury has healed, adaptive or secondary movement patterns may continue to persist, which predispose adjacent articulations or muscles to injury. Activation of proprioceptors, nociceptors, and components of the muscle spindles provide afferent stimuli that have direct and widespread influences on components of the peripheral and central nervous systems that directly regulate muscle tone and movement patterns. The various forms of manual therapy are thought to affect different aspects of joint function via diverse mechanical and neurologic mechanisms.

Conclusions

The goals of neuromuscular rehabilitation are to:

1) identify the individual muscle or muscle groups involved;

2) diagnose the underlying cause of muscular dysfunction (or neurologic or muscular disease);

3) define the rehabilitation issue relevant for that horse on that day (i.e., timing or amplitude);

4) develop and implement a focused rehabilitation plan to address the specific needs of the individual patient; and

5) provide objective outcome measures to assess accomplishment of goals and eventual return to optimal function.

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