2006 Equine Research Report
Published by the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana.

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Associate Dean for Research and Advanced Studies: Thomas R. Klei, PhD

Director, Equine Health Studies Program: Rustin M. Moore, DVM, PhD, DACVS

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Our Mission: The LSU Equine Health Studies Program will become a premier equine biomedical center in the 21st century through leading-edge research of equine diseases, contemporary instruction of professional veterinary students and veterinarians in advanced studies programs, and enhanced continuing education of the horse-owning public and private equine practitioners, with the ultimate goal of providing state-of-the-art diagnostic and therapeutic capabilities for critically ill and injured horses, and optimal clinical service to horsemen in Louisiana and the surrounding region.
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Welcome to the 2006 Research Report from the LSU School of Veterinary Medicine’s (SVM) Equine Health Studies Program (EHSP)! The purpose of this report is to document activities pertaining to scientific studies conducted at the LSU-SVM that help advance equine health and well-being. The contents reflect equine biomedical scientific investigations completed between 2004 and 2005.

The report demonstrates the extensive and diverse equine biomedical research performed by EHSP multidisciplinary faculty, advanced studies students, veterinary students, undergraduate students and technical staff. The works in this third issue demonstrate the program’s continued commitment to advancing the health, well-being and performance of horses through basic and applied biomedical research. The report also provides information highlighting our state-of-the-art research facilities and equipment, our published scientific manuscripts and abstracts, as well as grants and contracts awarded.

Our state-of-the-art laboratory facilities and equipment represent major advances to the EHSP resulting from acquisition of funds from the Louisiana Governor’s Biotechnology Initiative Grants Program, Louisiana Board of Regents Enhancement Grants Program and recurrent funding through the State Legislature resulting from Louisiana racetrack slot machine revenue. Through continued improvements and advancements, our research facilities are becoming second-to-none, which facilitates leading-edge research by faculty, staff and students by providing an enriching, stimulating and conducive atmosphere for scientific discovery.

The economic impact of the Louisiana equine industry on the State’s economy is over $2.5 billion annually. A 2005 study by the American Horse Council demonstrated that Louisiana’s horse industry ranks fifth nationally in terms of its economic contribution to the Gross Domestic Product. We have a tremendous responsibility to the equine population and horse-owning community that we serve. It is a responsibility that we embrace and continually strive to fulfill through ongoing cutting-edge research and state-of-the-art clinical service.

The important findings of the research outlined in this report would not have been possible without the availability and use of horses. All scientific works are conducted following Federal Guidelines for the Humane Care and Use of Animals. The availability of horses to be carefully and compassionately used for advancing equine health is vitally important for the discovery of more effective methods for prevention and treatment of illnesses and injuries afflicting horses. These horses are not taken for granted; their lives are valued, their dignity preserved and they are treated with the utmost humane care.

We do not use client-owned horses for research purposes. Retrospective analyses of medical records are sometimes used, however, to assemble information on a series of cases with a specific illness or injury. The information obtained from these records is utilized to determine factors that might prove useful to improve the outcome of horses in the future with similar conditions through enhanced treatments or management strategies.

We are proud of the accomplishments we continue to make and look to the future with optimism and excitement! We continue to set our sights on establishing the EHSP as one of the premier equine biomedical programs in the world.

Sincerely,

Rustin M. Moore, DVM, PhD, DACVS
Professor and Director
Dean’s Message

The Louisiana State University School of Veterinary Medicine is again extremely proud to present this Research Report of our Equine Health Studies Program. This multidisciplinary program continues to bring recognition to the School for its outstanding achievements and remarkable scientific productivity in equine research, clinical service, instruction, and outreach.

This 2006 Report captures the scientific activities of the program from 2004 and 2005 as the faculty and staff continue to focus their efforts in studies involving a broad range of conditions, including laminitis, colonic function, orthopedics and biomechanics, infertility in the mare, and small airway disease. As a career equine clinician prior to my time in administration, I continue to be extremely impressed with the advances in equine medicine and surgery and the rate of new discoveries underpinned by leading edge technologies. Indeed it is an exciting time to see the frontiers of science move forward and to have our Equine Health Studies Program right in the middle of it.

At this writing, the Equine Health Studies Program finds itself in transition. Most readers would be aware that our program director Dr. Rusty Moore resigned in the Fall of 2006 to become the head of the Clinical Sciences Department at The Ohio State University College of Veterinary Medicine. Dr. Moore had brought the program forward in outstanding fashion and his leadership and tireless commitment will be missed. The future is bright, however, as we continue our search for a new director, with exceptional programmatic momentum and robust resources in place to continue on a positive trajectory.

Our commitment to improve the health and welfare of the horse through research and clinical developments is unwavering. Stay tuned—our Equine Health Studies Program is moving forward at a rapid pace. With continued facility enhancements, state-of-the-art equipment, and instrumentation interfaced with outstanding faculty and staff, there is little doubt that we are on a projection to become a preeminent program.

Sincerely,

Peter F. Haynes, DVM, DACVS
Dean, School of Veterinary Medicine
2004-05 Faculty

Abolghasem Baghian, Assistant Professor, Veterinary Microbiology & Parasitology
Dr. Baghian received his M.S. in Microbiology from Southeastern Louisiana University in 1981, and he received his Ph.D. in 1985 from Arizona State University. Dr. Baghian was a postdoctoral researcher at the LSU School of Veterinary Medicine, where he later became an instructor and is currently an assistant professor. Dr. Baghian’s research focuses on investigating the structure and function of herpes simplex virus glycoprotein K and the structure and function of Kaposi’s sarcoma associated herpesvirus (KSHV) glycoproteins gH, gL, and gB to those other herpesviruses.

Steven A. Barker, Professor, Veterinary Physiology, Pharmacology & Toxicology
Dr. Barker is a professor of veterinary physiology, pharmacology and toxicology at the LSU School of Veterinary Medicine in the Department of Comparative Biomedical Sciences. He received his B.S. in 1971, his M.S. in 1973, and his Ph.D. in 1978, all from the University of Alabama. Dr. Barker is also the director of the Analytical Systems Laboratory.

Ralph E. Beadle, Professor Emeritus, Equine Medicine
Dr. Beadle was born and raised in Montana. He completed his pre-veterinary and veterinary education at Colorado State University, where he was awarded a D.V.M. in 1967. He spent the next five years at the University of Georgia, where he worked in the Equine Clinic and obtained a Ph.D. in Veterinary Physiology. After a period of two years spent at Michigan State University, he has been at LSU for the rest of his professional career. During the first seven years at LSU, he was in the Department of Veterinary Physiology, Pharmacology and Toxicology, where he taught both physiology and pharmacology. From that time until September of 1999, he was in the Department of Veterinary Clinical Sciences, where he worked in the Medicine Section of the Equine Clinic. He retired in September 1999, but since that time has continued to be involved in the activities of the Department of Veterinary Clinical Sciences as a professor emeritus. His research interests involve non-sweating horses, horses with recurrent airway disease, and horses affected with acute and chronic laminitis.

Aloisio C. D. Bueno, Clinical Instructor, Equine Surgery
Dr. Bueno, originally from Brazil, obtained his veterinary medical degree from Unoeste University and then completed two years of work in a private equine practice in Brazil. He then completed a one-year internship in large animal medicine and surgery, followed by a two-year M.S. program at the LSU School of Veterinary Medicine. Dr. Bueno then completed a one-year fellowship in large animal surgery at Oregon State University before going to the University of California-Davis for a three-year residency in equine surgery. Upon completion of his residency, Dr. Bueno returned to LSU as a clinical instructor of equine surgery and provides the majority of the equine emergency surgery service. Dr. Bueno is investigating the pathophysiology, prevention and treatment of laminitis.

Daniel J. Burba, Professor, Equine Surgery
Dr. Burba was born near Punxsutawney, Pa., on a dairy farm. He and his parents moved to Grayson, a small town in eastern Kentucky, when he was 13. His family raised Quarter Horses, and he still owns Quarter Horses and competes in team penning in the southern regional organization with family who live in Florida. He completed his pre-veterinary studies at Morehead State University in Kentucky. He received his D.V.M. from Auburn University in 1986, and then completed a large animal internship (1987) and equine surgical residency (1990) at Oklahoma State University. He is board certified by the American College of Veterinary Surgeons. His clinical interests include lameness and orthopedic surgery and laser surgery. His research interests include musculoskeletal injuries, such as joint disease.

Ann Chapman, Visiting Assistant Professor, Equine Medicine
Dr. Chapman was born and raised in Harriburg, Penn. She received her DVM from the LSU School of Veterinary Medicine in 2001. After working in private practice for one year, she began her combination Equine Internal Medicine Residency/Graduate program at LSU in 2002. Dr. Chapman completed her residency in 2005 and became a Diplomate of the American College of Veterinary Internal Medicine (Large Animal). Dr. Chapman performs Mobile Equine Medicine Consultation throughout the state of Louisiana and Equine
Ambulatory Medicine to the local Baton Rouge community.

Sharon Chirgwin, Assistant Professor, Research
Dr. Chirgwin was born and raised in Australia. She obtained a B.S. with Honors, majoring in Biochemistry and Zoology, from James Cook University, in Townsville, Queensland. Dr. Chirgwin then completed a Ph.D. in Molecular Parasitology at the Queensland Institute of Medical Research, before joining the laboratory of Dr. Thomas Klei at LSU, where she worked on both human and horse parasites. Her research interests include the molecular characterization of the early infection events of parasitism.

Doo Youn Cho, Professor, Veterinary Pathology
Dr. Cho is a professor of veterinary pathology in the Department of Pathobiological Sciences at the LSU School of Veterinary Medicine. Dr. Cho is also the section chief for necropsy/surgical biopsy in the School’s Veterinary Teaching Hospital and Clinics. He received his D.V.M. in 1966 and his M.V.Sc. in 1970, both from Seoul National University in Korea. In 1973, he received his M.S., and in 1978, he received his Ph.D., both from Kansas State University.

Laise Rosa Rodrigues Costa, Visiting Assistant Professor, Equine Emergency and Critical Care
Dr. Costa was born and raised in Sao Paulo, State of Sao Paulo, Brazil. She completed her veterinary degree at the School of Veterinary Medicine, FMVZ, Sao Paulo State University, UNESP-Botucatu in December 1987. After almost two years working as a large animal clinician at UNESP-Botucatu in Brazil, Dr. Costa came to the LSU School of Veterinary Medicine for an Internship in Equine Medicine and Surgery. She then moved to Lexington, Ky., where she obtained her M.S. degree at the University of Kentucky in May 1994, studying the immune response to equine infectious anemia virus. During the ensuing two years, Dr. Costa worked for the Veterinary Medical Teaching Hospital - Equine Medicine Service at the University of California-Davis and obtained a grant to study genetic variability of Corynebacterium pseudotuberculosis isolates affecting horses, cows, sheep and goats. In 1996, Dr. Costa returned to LSU for an equine internal medicine residency, and she became Board certified in Large Animal Internal Medicine in 1999. She worked for six months as a clinical instructor in Equine Medicine at LSU and then at the University of Georgia for one month. Dr. Costa initiated her Ph.D. program in the spring of 2000. While pursuing her Ph.D. in the Department of Pathobiological Sciences, Dr. Costa worked as a clinical fellow in equine medicine through the Department of Veterinary Clinical Sciences. She obtained funding for several clinical studies and five research projects involving equine airway disease. Dr. Costa has also been involved in several activities concerning neonatology and intensive care.

Susan C. Eades, Professor, Equine Medicine
Dr. Eades graduated from the LSU School of Veterinary Medicine, then completed an internship in large animal medicine and surgery, and a residency in large animal internal medicine at the University of Pennsylvania’s New Bolton Center. She then moved to Athens, Ga., and completed a Ph.D. in Veterinary Physiology at the University of Georgia. Her doctoral studies concentrated on intestinal vascular and nonvascular smooth muscle physiology and pharmacology. Upon completion of her Ph.D., Dr. Eades began as an assistant professor of large animal medicine at the University of Georgia College of Veterinary Medicine, where she remained through 1997. She returned to LSU in 1997 as an associate professor of equine medicine. Dr. Eades’ clinical interests include equine internal medicine; however, she has a special interest in cardiology and ultrasound. Her research interests include intestinal disease and laminitis.

Bruce E. Eilts, Professor, Theriogenology
Dr. Eilts is originally from the Minneapolis/St. Paul area in Minnesota. He graduated from high school in West St. Paul, Minn., and then attended the University of Minnesota as a pre-veterinary medicine student. He obtained a B.S. in veterinary science in 1975 and his D.V.M. in 1977, both from the University of Minnesota. He was in private practice for one year before returning to the University of Minnesota to obtain an M.S. in theriogenology in 1982. After two and a half years in private practice in southern California, he came to LSU as an assistant professor in 1984. He became board certified in the American College of Theriogenologists in 1986. His main clinical interest is basic reproduction management in the horse, and his main research interest is intrafollicular insemination in the mare.
**Timothy P. Foster, Research Assistant Professor, Molecular Virology and Cell Biology**

Dr. Foster was born in San Francisco, Calif., and obtained a B.S. degree in Biochemistry and a B.S. degree in Microbiology/Zoology from LSU in 1995. In 1999, he received a Ph.D. in Veterinary Medical Sciences with an emphasis in Biochemistry and Molecular Virology from the LSU Departments of Biochemistry and Veterinary Microbiology and Parasitology. Dr. Foster is a research assistant professor in the Division of Biotechnology and Molecular Medicine at the LSU School of Veterinary Medicine. His primary interests are deciphering the molecular interplay between host cells and various pathogens, as well as translational investigations that transition primary bench work science rapidly into the clinical environment.

**Dennis D. French, Professor, Veterinary Science**

Dr. French, originally from Chatfield, Minn., obtained his B.S. and D.V.M. degrees from the University of Minnesota in 1976 and 1979, respectively. He is a Diplomate of the American Board of Veterinary Practitioners, certified in equine practice. His clinical interests include equine herd health and sport horse medicine. His research interests include equine parasitology, immunology, and exercise physiology in horses. Dr. French is currently a professor of veterinary science at the LSU School of Veterinary Medicine, and provides equine ambulatory services for the Veterinary Teaching Hospital & Clinics. Dr. French is a past president of the Louisiana Veterinary Medical Association. Dr. French and his family are active in many equine and equestrian activities throughout the state.

**William G. Henk, Professor, Veterinary Anatomy and Cell Biology**

Dr. Henk is a professor of veterinary anatomy and cell biology in the department of Comparative Biomedical Sciences at the LSU School of Veterinary Medicine. He is also the chief of the Electron Microscopy Laboratory. Dr. Henk received his B.S. in 1967, his M.Ed. in 1971, and his Ph.D. in 1977, all from the University of Georgia.

**Jeremy D. Hubert, Assistant Professor, Equine Surgery**

Dr. Hubert was born in Wales but grew up on a ranch in Zimbabwe, where he received his veterinary degree (BVSc.). After two years of mixed animal practice in Zimbabwe and the United Kingdom, he completed an internship in equine medicine and surgery at LSU. This was followed by a year in equine practice in the U.K. before embarking on a combined equine surgery residency and M.S. program, which he completed in July 1999. He became board certified by the American College of Veterinary Surgeons in 2000. He worked as a clinical instructor in Equine Surgery for one year and accepted a position as an assistant professor of equine surgery at LSU in October 2001. His clinical interests include upper respiratory tract disease, as well as lameness and orthopedics. He is currently involved in scientific investigations involving extracorporeal shockwave therapy, bone density, and the role of eosinophils in gastrointestinal tract disease.

**Jill R. Johnson, Professor, Equine Medicine**

Dr. Johnson is a native of South Dakota. She graduated from veterinary school at the University of Minnesota, then stayed on and completed a M.S. degree in Veterinary Surgery and Radiology. She joined the faculty of the LSU School of Veterinary Medicine in 1977. She is a specialist in internal medicine (Diplomate, American College of Veterinary Internal Medicine) and equine practice (Diplomate, American Board of Veterinary Practitioners). Past research activities have centered on immuogenetics and immunology. Current research activities include evaluation of methods of quantifying exercise training using the global positioning system (GPS) and development of tissue culture models to study laminitis and chronic obstructive pulmonary disease using microgravity methods.

**Thomas R. Klei, Boyd Professor, Parasitology and Veterinary Science**

Dr. Klei obtained his B.S. and Ph.D. degrees in biology and zoology from Northern Michigan University and Wayne State University in 1965 and 1971, respectively. He then completed postdoctoral training at the National Institute of Health. He joined the faculty at the LSU School of Veterinary Medicine in 1975. He became a Boyd Professor in Parasitology and Veterinary Science at LSU in 1992. Dr. Klei has conducted leading-edge investigations into and has contributed greatly to our current understanding of equine parasitology. Dr. Klei is currently serving as the associate dean for Research and Advanced Studies at the LSU School of Veterinary Medicine.
Konstantin G. Kousoulas, Professor, Veterinary Virology
Dr. Kousoulas is a professor of veterinary virology in the department of Pathobiological Sciences at the School of Veterinary Medicine. He is also a professor of poultry science and an adjunct professor of biological sciences. Dr. Kousoulas is the director of the LSU School of Veterinary Medicine’s Division of Biotechnology & Molecular Medicine. He received his B.S. in 1975 from Fairleigh Dickinson. In 1977, he received his M.S. and in 1981, he received his Ph.D., both from Pennsylvania State University.

Mandi J. Lopez, Assistant Professor, Equine and Comparative Orthopedics
Dr. Lopez was born and raised in the Pacific Northwest. She attended veterinary school at the University of California, Davis and then completed an internship at Kansas State University prior to going to the University of Wisconsin, where she completed a residency in large animal surgery and obtained both her M.S. and Ph.D. degrees. Her area of interest and expertise is comparative orthopedic research and surgery. Dr. Lopez is board-certified by the American College of Veterinary Surgeons. She came to LSU in January 2004 and heads the Laboratory for Equine and Comparative Orthopedic Research.

Sara K. Lyle, Clinical Instructor, Theriogenology
Dr. Lyle was born and raised in Gainesville, Fla. She obtained her B.S. in Chemistry at Duke University and her D.V.M. from the University of Florida. She completed a residency in theriogenology in 1989 and a M.S. in reproduction in 1991 at the University of Florida. She is board certified by the American College of Theriogenologists. Her clinical interests include mare infertility and assisted reproductive technologies. Her research interests include reproductive immunology (equine) and assisted reproductive technologies in horses.

Charles T. “Chuck” McCauley, Assistant Professor, Equine Surgery
Dr. McCauley joined the equine faculty in the Department of Veterinary Clinical Sciences in February 2006. Dr. McCauley is an assistant professor of equine surgery. Most recently, he was employed in a busy private referral practice in northeast Texas. Dr. McCauley completed his B.S. in Microbiology and his D.V.M. at Texas A&M University. He successfully completed an internship and residency in food animal medicine and surgery at Oklahoma State University. In addition, Dr. McCauley also completed a residency in large animal surgery (equine emphasis) at Purdue University. He is double boarded by the American Board of Veterinary Practitioners (Food Animal Practice) and the American College of Veterinary Surgeons (Large Animal Surgery).

Rebecca S. McConnico, Assistant Professor, Equine Medicine
Dr. McConnico is originally from north central Ohio, where she lived for 18 years. She obtained her B.S. in Animal Science from the University of Arkansas, her D.V.M. from Louisiana State University, and her Ph.D. and clinical residency in large animal internal medicine from North Carolina State University. She is board certified in Equine Internal Medicine and her clinical interests are in equine critical care and internal medicine. Her research interests include inflammatory disease of the equine large intestine and infectious diseases and the effects on mucosal physiology and permeability.

Colin F. Mitchell, Assistant Professor, Equine Surgery
Dr. Mitchell, originally from Perth, Scotland, received his veterinary medical degree from the University of Edinburgh. He then completed an internship at the University of Prince Edward Island prior to entering a combined three-year equine surgery residency and M.S. graduate program at the University of Minnesota, which he completed in June 2004. He then remained on the hospital staff at the University of Minnesota, where he worked as the equine emergency clinician/surgeon until July 2005, when he joined the LSU School of Veterinary Medicine. He is board certified by the American College of Veterinary Surgeons. His clinical interests include soft tissue surgery and ultrasound. His research interests include assessment of gastrointestinal motility.

Mustajab Mirza, Visiting Assistant Professor, Equine Medicine
Dr. Mirza received his DVM from the University of Agriculture Faisalabad Pakistan in 1992. He received his MS degree from LSU in 1998. Dr. Mirza’s primary interests are repair of long bone fractures and pathogenesis of colics in equids. He primarily provides after-hours emergency services for the LSU Equine Clinic.
Rustin M. Moore, Professor, Equine Surgery
Dr. Moore, professor of equine surgery, currently serves as director of the Equine Health Studies Program and service chief of Equine Medicine and Surgery. He is originally from West Virginia and earned his B.S. from West Virginia University. He obtained his D.V.M. and Ph.D. from The Ohio State University and completed his equine surgical residency at the same institution. He is board certified by the American College of Veterinary Surgeons. Dr. Moore began at the LSU School of Veterinary Medicine in October 1994. Some of his clinical interests include lameness, colic and its associated complications, and surgery. Dr. Moore’s research focuses on vascular and nonvascular smooth muscle physiology, and pharmacology and the pathophysiology and treatment of colic, laminitis, endotoxia and heaves.

Claudio C. Natalini, Assistant Professor, Veterinary Anesthesiology
Dr. Natalini is originally from Rio de Janeiro, Brazil, where he attended the Universidade Federal Fluminense and graduated in veterinary medicine in 1984. From 1985 to 1986, Dr. Natalini enrolled in a residency program in veterinary surgery and medicine at the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. He worked from 1986 to 1992 as a staff surgeon/anesthesiologist at UFRGS. In 1991, Dr. Natalini completed the M.S. program in veterinary anesthesiology at Universidade Federal de Santa Maria (UFSM), Brazil. In 1992 he became an assistant professor of veterinary anesthesiology at UFSM. In 1994 he obtained his board certification from the Brazilian College of Veterinary Surgeons and Anesthesiologists (CBCAV) and served as CBCAV secretary for one year. In 1996, Dr. Natalini enrolled in a Ph.D./residency program at the University of Minnesota, earning his degree in 2000 working with opioid spinal mediated analgesia in the equine. In 2002, Dr. Natalini joined the Department of Veterinary Clinical Sciences at the LSU School of Veterinary Medicine. Dr. Natalini’s clinical interests are small and large animal pain management with emphasis in spinal analgesia and local anesthesia. His research interest includes the pharmacology and physiology of spinal administration of analgesic in horses.

Kathy L. O’Reilly, Associate Professor, Veterinary Immunology
Dr. O’Reilly was born in Corona, Calif. She obtained her B.S. and M.S. in Microbiology from the University of Wyoming in 1977 and 1982, respectively. Following a year working as a research scientist at the Wyoming State Diagnostic Laboratory and then in the University of Wyoming Department of Biochemistry, Dr. O’Reilly completed her Ph.D. in Veterinary Science (Immunology) at the University of Wisconsin-Madison in 1989. Dr. O’Reilly completed postdoctoral training at in the Colorado State University Department of Pathology, where she studied the immune response to feline immunodeficiency virus and feline leukemia virus. Dr. O’Reilly joined the faculty within the Department of Pathobiological Sciences (then the Department of Veterinary Microbiology and Parasitology) at the LSU School of Veterinary Medicine in 1992 and has an adjunct appointment in the Department of Biological Sciences. Her research interest is in immune responses to intracellular pathogens of animals; specifically mechanisms of cell-mediated immunity and immune evasion, including cellular interactions and control of cells responding during infection. Her current research focuses on the bacterial pathogen *Bartonella hesealae* in the feline reservoir, respiratory bovine coronavirus, and the development of in vitro models studying lung disease in cattle and horses.

Dale L. Paccamonti, Professor, Theriogenology
Dr. Paccamonti, originally from Kankakee, Ill., completed his undergraduate and veterinary education at Michigan State University, receiving his D.V.M. in 1981. After four years in a mixed practice in Chestertown, Md., he pursued advanced training at the University of Florida, where he completed a residency in theriogenology and received his M.S. degree in 1988. Dr. Paccamonti is a Diplomate in the American College of Theriogenologists. He joined the faculty at the LSU School of Veterinary Medicine in 1988, where he is currently a full professor of theriogenology in the Department of Veterinary Clinical Sciences. Dr. Paccamonti’s primary research interests include the study of infertility in mares, assisted reproduction techniques in horses, factors affecting sperm motility in stallions, semen cryopreservation in stallions, and the process of fetal maturation and parturition in mares. He also collaborates in reproductive research in other domestic species. In addition to research endeavors, his duties include teaching theriogenology to third and fourth year veterinary students. He shares responsibility for clinical theriogenology cases in all species presented to the Veterinary Teaching Hospital & Clinics.
Daniel B. Paulsen, Professor, Veterinary Pathology
Dr. Paulsen received his B.S. in 1975, his D.V.M. in 1977, and his M.S. in 1978, all from Kansas State University. In 1989, he received his Ph.D. from Oklahoma State University. Dr. Paulsen’s major research interests are bovine respiratory disease with emphasis on Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus, bovine virus diarrhea, and bovine respiratory coronavirus; pathogenesis, bacterial genetics, respiratory immunity and vaccinology; toxicologic pathology associated with inhaled toxins and effects of inhaled substances on the pathogenesis of asthma; and application of immunohistochemical techniques in equine respiratory disease and laminitis and in cancer biology.

Gary A. Sod, Clinical Instructor, Farm Animal Health Management
Dr. Sod received his M.A. in Mathematics from the University of California at Berkeley in 1975. He earned his Ph.D. in Applied Mathematics from that same institution in 1976. The next 12 years were spent doing research in mathematical and computational physics resulting in the writing of a monograph on numerical methods in fluid dynamics and 42 journal publications. Dr. Sod served as an adjunct professor in the Department of Mechanical Engineering and a professor in the Department of Mathematics at Tulane University from 1985 through 1997. Dr. Sod then attended the LSU School of Veterinary Medicine and obtained his D.V.M. in 2001. He has since completed an equine internship and food animal medicine and surgery residency at LSU and is now a clinical instructor with the Farm Animal Health Management service. Dr. Sod received the American College of Veterinary Surgeons Resident Research Publication Award in 2004 and the Mark S. Bloomberg Memorial Resident Research Award from the Veterinary Orthopedic Society in 2004 and 2005. Dr. Sod is enrolled in a large animal surgery residency in conjunction with the Sawtooth Equine Service in Idaho. His research interests include biomechanics and the design of orthopedic implants specific to the equine patient.

Changaram S. Venugopal, Professor, Veterinary Physiology & Pharmacology
Dr. Venugopal is a veterinarian who graduated from Kerala Veterinary College and Research Institute of Kerala University. After practicing as a veterinarian on the Kamadhenu Dairy Farm for five years, he pursued and received his M.Sc. degree in neuropharmacology from Calicut University, India. He received his M.S. degree in cardiovascular pharmacology and his Ph.D. in pulmonary pharmacology from Massachusetts College of Pharmacy and Allied Health Sciences in a cooperative program with Harvard University in Boston. Then he worked as a postdoctoral fellow at Harvard Medical School before joining the faculty at LSU School of Veterinary Medicine in 1981. He received his New Investigator Award grant from the National Institutes of Health in 1983 and the Beecham Award for Research Excellence in 1985. His research interests include the physiology and pharmacology of vascular and nonvascular smooth muscle physiology and pharmacology, and the pathophysiology of summer pasture associated obstructive pulmonary disease.

Ashley M. Stokes, Research Assistant Professor
Dr. Stokes was born in Baton Rouge, La., and moved to Tuscaloosa, Ala., to complete her bachelor’s degree from the University of Alabama. She returned to Baton Rouge to work in Oceanography for LSU for three years before her veterinary training. She completed the D.V.M./Ph.D. program at the LSU School of Veterinary Medicine in the Department of Comparative Biomedical Sciences in 2001 and 2003, respectively. She completed a one-year post-doctorate research fellowship in the summer of 2004 where she continued her doctoral work on the vascular pathophysiology of equine laminitis. As a research assistant professor within the EHSP, Dr. Stokes has continued to focus her efforts in cardiovascular physiology with special emphasis on equine diseases.

H. Wayne Taylor, Professor, Veterinary Pathology
Dr. Taylor is a professor of veterinary pathology in the department of Pathobiological Sciences at the LSU School of Veterinary Medicine. He is also a veterinary pathologist and the director of the Louisiana Veterinary Medical Diagnostic Laboratory. Dr. Taylor received his D.V.M. from Auburn University in 1967. In 1969, he received his M.S. from the University of Missouri, where he also received his Ph.D. in 1971. Dr. Taylor is a Diplomate of the American College of Veterinary Pathologists.
The Equine Health Studies Program is one of four recognized priority research programs in the LSU School of Veterinary Medicine. Horses and equestrian activities are an important economic and recreational commodity in Louisiana and the surrounding region. Approximately 200,000 horses are owned by an estimated 60,000 people in the state, with a total direct economic impact of the equine industry in Louisiana of 2.5 billion dollars annually. Scientific investigation into the prevention and treatment of equine disease is critical to maintaining the health, well-being and performance of horses, and thus, is important for sustaining the equine industry. Substantial resources, including multidisciplinary, interdepartmental faculty, technical staff, facilities and equipment, provide an excellent environment for either graduate or clinical advanced studies.

**Graduate Programs**

Students in the LSU School of Veterinary Medicine’s interdepartmental Equine Health Studies Program can obtain Master of Science (M.S.) and Doctor of Philosophy (Ph.D.) degrees in Veterinary Medical Sciences through the School’s academic departments: Comparative Biomedical Sciences, Pathobiological Sciences and Veterinary Clinical Sciences.

**Current Research Interests**

- Gastrointestinal tract disease (colic)
  - Intestinal ischemia-reperfusion
  - Ulcerative disease
  - Intestinal motility disorders
  - Inflammatory bowel disease
- Effect of gastrointestinal tract inflammation on mucosal permeability
- Effect of NSAIDs on colonic mucosal permeability
- Summer pasture-associated obstructive pulmonary disease/COPD and other respiratory tract diseases
- Laminitis
- Nonvascular smooth muscle physiology, pharmacology, and pathobiology
  - Gastrointestinal
  - Bronchial
  - Uterine
- Vascular smooth muscle physiology, pharmacology, and pathobiology
- Analgesia and pain management
- Inflammatory mediators, including nitric oxide, endothelin and cytokines
- Medication surveillance
- Synovitis and arthritis
- Acupuncture
- Parasitology
- Endotoxemia
- Virology
- Use of global positioning system technology for equine epidemiologic studies
- Mare reproductive physiology, infertility and placentitis
- Improving freezing methods for stallion semen
- Advancing the onset of the breeding season in mares
- Intrafollicular insemination of mares
- Equine embryo biotechnology
- Assisted reproduction techniques in horses
- Endotoxin-induced late gestation abortion in mares
- Musculoskeletal injuries and other diseases causing poor performance
- Comparative orthopedics
- Effects of extracorporeal shock wave therapy on bone, tendon, ligament and nerve
EHSP Grants and Contracts

2005


2004


Leise BS, Curtis LA, Moore RM, Eades SC: Digital hemodynamics and pharmacokinetics of acepromazine administered intramuscularly in horses. LSU-SVM Department of Veterinary Clinical Sciences Organized Research Funds, October 2003.

Lyle SK, Blackmer JM: Development of a three-dimensional culture system for equine endometrial and chorionic cells. LSU-SVM Department of Veterinary Clinical Sciences Organized Research Funds, October 2003.


Bueno AC, Moore RM, Eades SC: Comparison of heparinized autogenous whole blood and perfluorocarbon solution (Oxyceyte™) as the perfusate for maintaining the disarticulated equine digit using an extracorporeal system: Development of a model to isolate the digit and study the pathophysiology of laminitis in horses. $11,546. Equine Health Studies Program, July 2004.


McConnico RS, Chirgwin S, Hubert JD, Taylor HW, Eades SC, Klei TR: Pathophysiology of equine cyathostomin infection during larval immigration into the intestinal lumen. $10,000. LSU-SVM USDA 144 Formula Funds, July 2004.


Orandle MS: Microvascular brain endothelial cell infection with EIAV neuroinvasion and persistence. $6,800. Equine Health Studies Program, July 2004.


EHSP Selected Scientific Publications

2005


**2004**


EHSP Selected Published Scientific Abstracts

2005


Moore RM: Diagnosis and treatment of joint and bone infection in horses. Proceedings Anais do II Simpósio Internacional do Cavalo Atleta - IV Semana do Cavalo, Belo Horizonte, Brazil, Universidade Federal de Minas Gerais, 29-38, 2005.


2004


How You Can Support the EHSP and Enhance the Health, Well-Being and Performance of Horses

There are many ways individuals or companies can help support the Louisiana State University School of Veterinary Medicine Equine Health Studies Program. The EHSP is “dedicated to the health, well-being and performance of horses through veterinary research, education and service.” In order to fulfill our mission of becoming one of the premier equine biomedical centers in the country, we have initiated a campaign to generate funds to enhance all aspects of our program.

The LSU School of Veterinary Medicine is a relatively young institution, with only 31 years of graduates. Our endowment is comparatively small, so each gift is extremely special to us and will make an important and immediate impact on our programs. Our fundraising efforts have been principally through private, charitable, tax-deductible gifts, as well as some other special events and activities. All gifts are tax-deductible and can be pledged with a portion being given annually over a period of a few years. We hope that you will give consideration to assisting us with our fund raising efforts for facility enhancements, endowed/distinguished professorships and chairs, and/or scientific investigation.

An endowed gift is a permanent gift. The principal is invested and returns annual interest. Part of the annual interest is reinvested to increase the principal, and part is used for the purpose intended (such as a professorship/chair or research activities). Endowed funds are usually named for the benefactor or for a designated honoree. Some examples of how your endowed gifts can advance the EHSP and its research, education and service missions include professorships, chairs, research, and facility construction.

Professorships and Chairs: The state of Louisiana has a matching program for Endowed Professorships and Endowed Chairs. The School currently does not have any Endowed Chairs and only three Endowed Professorships, none of which are in the area of equine clinical or biomedical science. An Endowed Chair in equine biomedical sciences would be distinguished by being the first and only endowed chair in the School of Veterinary Medicine. These endowed positions are vital to move our instructional and investigational programs forward. The individuals in these positions will serve as leaders of teams of equine clinicians and investigators that conduct leading-edge scientific investigations to improve prevention and treatment of equine diseases.

Equine Biomedical Research: Private gifts can provide funds for conducting leading-edge scientific investigation into the cause, prevention and treatment of illnesses and injuries afflicting horses. With the limited amount of state and federal funding available for equine scientific investigations, it is vital to the health, well-being and performance of horses that we provide funds through private, charitable gifts to investigate and improve our ability to successfully prevent and treat illnesses and injuries of horses that can be performance-limiting, career-ending and even life-threatening.

General EHSP Support Fund: Gifts can be made into the General EHSP Support Fund (a non-endowed account), which is used to purchase new or replacement equipment in the Equine Clinic for scientific investigations. Additionally, these funds are often used for continuing educational activities for the horse-owning public and private equine veterinarians who rely upon us for consultation and referral services. These funds also are used to assist with other educational, promotional and fundraising activities.

Memorial Gifts and Naming Opportunities: Your gift may be used to honor or memorialize a beloved horse, family member, or friend. Naming opportunities exist for endowed gifts such as scholarships, professorships, and chairs. Construction projects such as the Equine Intensive Care Unit, Equine Isolation Unit, Equine Reproduction Unit,
Equine Lameness and Performance Evaluation Unit and research laboratories offer a wide variety of naming opportunities.

Again, any gift will be very special to the LSU School of Veterinary Medicine’s Equine Health Studies Program and will make a dramatic and immediate impact on our teaching, service and scientific investigation programs. We thank you for your generosity and support. To learn more about how your gift will assist the EHSP with its mission, please visit our website (www.LSUEquine.com) or contact Ky Mortensen via telephone (225-578-9590) or e-mail (kmortensen@vetmed.lsu.edu).
EHSP Facilities and Equipment

The Equine Health Studies Program is an interdepartmental, multidisciplinary equine biomedical program within the Louisiana State University School of Veterinary Medicine that is dedicated to the health, well-being and performance of horses through veterinary research, education and service. A diverse group of faculty, advanced studies students and staff conduct leading-edge scientific investigations involving equine health and disease utilizing state-of-the-art facilities and equipment. The program maintains a herd of 90 horses and ponies for use in scientific investigations and instructional activities. Three research barns collectively containing over 40 stalls and several pastures and paddocks are available for housing horses used in scientific studies. The EHSP research facilities include the Equine Physiology & Pharmacology Laboratory, the Equine Performance Evaluation Laboratory, the Equine Cell & Tissue Culture Laboratory and the Laboratory for Equine and Comparative Orthopedic Research. Additionally, the clinical facilities and equipment within the Veterinary Teaching Hospital and other core research facilities and resources within the School of Veterinary Medicine support the research activities of the EHSP.

Equine Physiology & Pharmacology Laboratory

Numerous faculty and graduate students conduct scientific investigations involving equine physiology, pharmacology and pathophysiology, including but not limited to vascular and nonvascular smooth muscle physiology/ pathophysiology/ pharmacology (digital and intestinal vasculature, bronchial, uterine and intestinal smooth muscle); the effects of intestinal ischemia-reperfusion injury, nonsteroidal antiinflammatory drugs and parasitism on mucosal physiology and permeability; effects of endotoxin, experimental laminitis and medications on systemic and local digital hemodynamics; reproductive physiology related to mare and stallion fertility; effects of medications on behavior and activity; and effects of drugs and delivery systems for analgesia and pain management. The laboratory contains 24 organ baths integrated with force transducers and polygraphs to measure tension on tissues (vascular smooth muscle, nonvascular smooth muscle, cardiac muscle, skeletal muscle and tendon) in response to inflammatory mediators or pharmacologic agents. For studies of tissue permeability and effects of pharmacological agents on tissue integrity, we have 12 Ussing chambers mounted within the main laboratory. Dual channel Doppler flow and laser Doppler ultrasound flow meters and probes to measure blood flow and tissue perfusion in several species are available. The lab also contains three 8-channel and six 4-channel polygraphs to record data from both force and pressure transducers. Additional instrumentation is available, including electromyography, electrocardiography, and equipment for cutaneous analgesia nerve conduction velocity studies. Two motion chambers are available for assessing the effects of medications on activity and behavior of horses.

Equine Performance Evaluation Laboratory

The Equine Performance Evaluation Laboratory (EPEL) is equipped with a high-speed treadmill for exercising horses at speeds that mimic racing conditions. The EPEL is also equipped with a TekScan digital pressure system that incorporates pressure sensors in specially designed horseshoes to evaluate static and dynamic weight bearing to assess lameness. Equipment is available for dynamic endoscopic examination. Polygraphs and pressure transducers are available for measuring airway pressures and impedance. Equipment is available for electrocardiographic and echocardiographic evaluations of the heart before and after intense exercise on the treadmill. A speed of sound ultrasound machine for assessing bone Ussing chambers for gastrointestinal tract permeability studies.
density is available and used to assess the effect of growth, training, injury, and treatment modalities on bone density of the third metacarpal bone of horses. A focused extracorporeal shock wave therapy (ESWT) unit is available and is used to evaluate the effects of ESWT on healing of tendon, ligament and bone and on its functional (analgesia) and morphologic effects on nerves. Funds have been recently obtained to acquire a kinematic gait analysis system and a force plate, which will be installed in the EPEL in the near future.

**Equine Cell and Tissue Culture Laboratory**
The Equine Cell and Tissue Culture Laboratory is well equipped to support tissue culture activities. The laboratory has three laminar flow biohazard hoods, four CO₂ incubators, nine Synthecon bioreactors, a phase contrast microscope with digital image capture capabilities, two centrifuges, refrigerators and ultra-low temperature freezers. In concert with other centralized faculties in the School of Veterinary Medicine, including electron and confocal microscopy and molecular biology, investigators have a wide range of state-of-the-art equipment and facilities to employ tissue culture as a research tool. Current projects involving the laboratory include the growth of laminar cells for use as an in vitro model of laminitis, bone marrow stromal cells for use in experimental tendon healing, colonic and cecal epithelium for use in the study of bacterial factors in laminitis, bronchoepithelial cells for the study of summer pasture-associated obstructive pulmonary disease, endometrial cells for the study of endometritis and other conditions affecting mare fertility, and corneal epithelial cells for the study of herpes virus infections of the eye. Our laboratory pioneered the use of rotating wall vessels for growing cells under microgravity conditions, which yield three-dimensional tissue assemblies for the study of various equine diseases.

**Laboratory for Equine and Comparative Orthopedic Research**
The Laboratory for Equine and Comparative Orthopedic Research is the newest addition to the EHSP. The laboratory is specifically designed and equipped for translational orthopedic research from the molecular/genetic level to the structural biomechanical level. The laboratory is equipped with the most modern equipment for molecular/genetic work, including an MJ Research Chromo4 Detector and DNA Engine 200 for DNA fragment amplification and Quantitative PCR, a UVP hybrilinker for blot analysis and a Synergy HT multi-detection microplate reader for ELISA assays. Housed within the laboratory is a Leica DM 4000 light microscope with fluorescent, polarizing and phase contrast capabilities. The microscope is equipped with the latest in digital image capture equipment and software. Additionally, there is a PathScan Enabler to obtain ultra high quality images from 1 x 3 inch glass slides. A custom-designed servohydraulic axial torsional Material Testing System with a Flex Test SE Controller and equipped with a Multiple Gage Length Axial Extensometer makes nearly any level of mechanical tissue testing possible from the tissue and bone level to joint and whole limb testing. Presently, several state-of-the-art diamond saws are available for both orthopedic hard and soft tissue microscopic and ultrastructural sample preparation. A section of the laboratory is devoted to histologic preparation of both calcified and decalcified tissue samples requiring special processing for leading-edge orthopedic research. Areas of research focus include the pathophysiology of hip...
dysplasia, the development and implementation of novel orthopedic devices, cranial cruciate disease, synovial fluid prognostic markers for joint disease, the effects of shock wave therapy on bone, minimally invasive treatments for bone spavin, and genetic markers for orthopedic disease. The Laboratory for Equine and Comparative Orthopedic Research has been established and designed to facilitate a strong association between clinical and basic orthopedic research for advancement of orthopedic knowledge across species and disciplines.

**Veterinary Clinical Facilities and Equipment**

The LSU Veterinary Teaching Hospital & Clinics are staffed by nationally and internationally recognized veterinary specialists (internal medicine, surgery, anesthesiology, and radiology) and highly-skilled veterinary technicians, and are furnished with state-of-the-art equipment necessary to provide advanced diagnostic and therapeutic services to private referral veterinarians and the animal-owning public. The hospital facilities include two equine anesthesia induction/recovery rooms and surgical suites with modern equipment; a modern, centralized, climate-controlled 10-stall equine intensive care unit for critically ill and injured horses; an isolation unit for horses with infectious/contagious disease; and diagnostic/therapeutic procedure rooms.

Diagnostic imaging capabilities include digital radiography, ultrasonography, computed tomography and nuclear scintigraphy facilities and equipment. Plans are underway to acquire magnetic resonance imaging. Endoscopy equipment is available for assessment of the upper respiratory tract, urogenital and gastrointestinal systems. Laparoscopic equipment is available for diagnostic and therapeutic applications.

Orthopedic-related equipment available in the hospital includes two arthroscopy units, ASIF equipment and implants for fracture repair, surgical lasers (Nd:YAG, diode and CO₂), and an extracorporeal shockwave therapy unit. An Equine Lameness and Performance Evaluation (75’ x 125’ covered pavilion) provides a modern facility for evaluation of gait, locomotion and lameness in equine clinical patients.

**Equine Reproduction/Theriogenology Laboratory**

The Equine Reproduction/Theriogenology Laboratory has complete facilities for the evaluation, chill-transport, and cryopreservation of spermatozoa, including light and phase-contrast microscopes and a computer-assisted spermatozoal analysis system (Spermvision™). We have three ultrasound machines with 5-7.5 MHz linear array transducers and a 5-7.5 MHz sector array transvaginal transducer for oocyte collection by follicular aspiration or twin reduction by aspiration. A fourth ultrasound system equipped with a 3.5 sector and 5-7.5 microconvex array are available for transabdominal imaging. An Olympus endoscope is also available for hysteroscopic examination and for hysteroscopic low-dose insemination. Laparoscopy is available and used for oviductal insemination and for minimally invasive placement of intrauterine catheters. We maintain a close collaborative relationship with the Equine Biotechnology Laboratory, which is part of the LSU Agricultural Center. This facility has tissue culture laboratories and micromanipulators that make possible such advanced assisted reproductive techniques as intracytoplasmic spermatozoal injection and nuclear transfer (“cloning”).

**Equine Molecular Biology Research Laboratory**

The Equine Molecular Biology Research Laboratory is a new addition to the EHSP. The laboratory is equipped to support the molecular biology aspects of research conducted by the EHSP investigators. The missions of this laboratory...
are to perform basic, cutting-edge research in molecular biology to elucidate normal equine physiology, as well as the pathophysiology of equine disease; to train scientists, students, and visitors at all levels; and to develop new instruments and methods in equine molecular biology. This laboratory provides refrigerated centrifuge and micro-centrifuges, electrophoresis/transilluminator equipment, refrigerators and ultra-low temperature freezers, and PCR thermocycler equipment located in a molecular biology-dedicated laboratory space capable of multiple simultaneous studies. We also have direct access to the Division of Biotechnology and Molecular Medicine within the LSU School of Veterinary Medicine for quantitative Real-Time PCR, Primer Express primer/probe design, Quantity One for DNA fragment visualization and analysis, SDS-PAGE analysis, MagnaPure automated nucleic acid extraction, and microarray spotters and readers. Seminars and hands-on learning are key components of training provided by the laboratory to students, staff, faculty, and visiting scientists. Current investigations utilizing this laboratory include study of key mediators in equine laminitis, placentitis, gastrointestinal disease, summer pasture-associated obstructive pulmonary disease, bone healing, mechanisms of pain sensation and modulation, and stem cell biology. Plans are in place to expand the capabilities of this laboratory in light of the increasing importance of the molecular biological approach to the investigation of equine health and disease.

**BIOMMED – Biotechnology and Molecular Medicine**

The Division of Biotechnology and Molecular Medicine (BIOMMED), a division within the LSU School of Veterinary Medicine, is organized into three different Laboratories: 1) GeneLab; 2) Viral Vectors Laboratory (VVL); and 3) Protein and Antibody Production and Purification Laboratory (PAPPL). **GeneLab**: GeneLab produces synthetic oligonucleotides including biotinylated, fluoresceinated, phosphorylated and phosphorothioate (antisense) oligonucleotides up to 200-bases long. Additional molecular biology services include cloning and automated sequencing of genes, real-time quantitative PCR, automated preparation of chromosomal and plasmid DNAs, cDNA library construction, and microarray production and analysis. **VVL**: This laboratory provides custom baculovirus, adenovirus, vaccinia virus, herpes, and other recombinant virus construction for heterologous gene expression, and vaccine and gene therapy studies. **PAPPL**: This laboratory concentrates on the production and purification of proteins and antibodies. The laboratory produces monospecific antibodies in rabbits and mice using conventional immunization methodologies using purified protein immunogens as well as genetic immunization methods. Antibodies are concentrated and purified using standard methodologies. The laboratory also provides on a limited basis the production and characterization of monoclonal antibodies.

GeneLab operates a new bioinformatics module, which is equipped with three Macintosh G4 computers and two DELL PC computers. A new WEB-based system providing direct communication between researchers and GeneLab staff has recently been purchased. Available software includes: Primer Express (PE Biosystems) for the design of TaqMan probes for real-time PCR, MacVector (Genetics Computer Group, Inc.) for analysis of DNA and protein sequences, Oligo (Molecular Biology Insights, Inc.) for the design of PCR primers, Quantity One (BioRad, Inc.) for the visualization and analysis of images such as those produced by ethidium bromide agarose electrophoresis of DNA fragments, SDS-PAGE analysis of proteins, immunobLOTS, etc. GeneLab also has additional software for assembly of large DNA sequences (Sequencer), analysis of blots (alpha Innotech) and microarrays (Alpha Innotech 6000). Automated ordering for synthetic DNA and other reagents is assisted via a WEB-based Information System (DNA LIMS). BIOMMED has three automated sequences (ABI3777, 310, 310), three real-time PCR equipment (PE 7900, 7400), LightCycler (Roche), two MagnaPure automated nucleic acid extractors (Roche), microarray OmniGrid spotter (Gene Machines), microarray reader (AlphaReader 6000, Alpha Innotech), imager station (Alpha Innotech Fluorochrome 8000), five PCR (thermocyclers, a New Brunswick Fermentor, AktaExplorer Chromatography System, Cyclone phosphorimager, two four-column DNA synthesizers (ABI), one Synergy peptide synthesizer (ABI), and other equipment.

**Immunology Laboratory**

Three laboratories (~1200 sq ft) located on the third floor of the LSU School of Veterinary Medicine are designated for the Immunology Laboratory. These labs contain necessary equipment for immunological assays and the in vitro cultivation of lymphocytes, including laminar flow biosafety cabinets, CO₂ incubators, microscopes, water baths, a pH meter, low speed centrifuges, mixers, stir plates, refrigerators and freezers. One of the laboratories is dedicated specifically for molecular biology procedures and contains all of the equipment and materials for the isolation of and analysis of RNA,
DNA and proteins. Separate refrigerators and freezers for molecular biology samples are found in this laboratory. Spectrophotometers, pH meters, electronic balances, refrigerators and freezer and other small equipment items are also available in this laboratory.

**Flow Cytometry Facility**
The Flow Cytometry Facility is a core laboratory located on the third floor of the LSU School of Veterinary Medicine. The facility features a Becton Dickinson FACScan flow cytometer capable of measuring two light scatter parameters and three fluorescence emissions. Immunophenotyping, cell cycle analysis, apoptosis studies, and measurements of cellular function are examples of applications, which are performed routinely in this laboratory. There is also a newly acquired FACS Aria Dual Laser Flow Cytometer, which is capable of high performance cell sorting of up to 30,000 cells per second and separation of one to four distinct cell populations. Additionally, multicolor immunophenotyping and cell functional assays can expand to seven-color analysis. This unique centralized facility provides analytical capabilities for investigators throughout the LSU System, including the LSU Agricultural Center and LSU Pennington Biomedical Research Center. Both PC and Macintosh computers are utilized in data acquisition and analysis and are all connected to the School of Veterinary Medicine and LSU networks.

**Microscopy Center**

The Center features three powerful microscopes. The laser capture and microdissection microscope (PALMZeiss MicroBeam-Axiovert 200 System) allows researchers to dissect out parts of tissue on a slide and transfer it to a container for genetic, gene expression and proteomic analysis, which enables researchers to determine what genes are present and what genes and proteins are being expressed. The scanning laser confocal microscope (LEICA TCS SP2 AOBS) provides excellent quality three-dimensional reconstructions from cells and relatively thick sections of tissues, which enable researchers to examine cells and cell components in three-dimension and allows researchers to conduct co-localization studies to mark multiple proteins within the cell simultaneously. The environmental scanning electron microscope (FEI Quanta 200) provides a detailed evaluation of the surfaces with or without dehydrating the samples. This microscope also has an energy dispersive x-ray spectrometer that allows researchers to determine elemental composition.

**Analytical Systems Laboratories**
The Analytical Systems Laboratories are central service, comprehensive analytical laboratories, consisting of the Laboratory for Drug Residue Studies, the Equine Medication Surveillance Laboratory and the Analytical Systems Laboratory. The Laboratory for Drug Residue Studies provides instrumentation and expertise for the performance of drug and biological molecule pharmacokinetics, metabolism, tissue distribution and analytical method development. The laboratory is also equipped to conduct complete drug profiling using radiolabeled test materials. This laboratory operates under Federal Good Laboratory Practices regulations and has generated data for the Food and Drug Administration and private industry for submission for veterinary drug approvals by U.S. and foreign regulatory agencies. The Equine Medication Surveillance Laboratory has served as the official laboratory for the Louisiana State Racing Commission since 1987. The laboratory screens over 8,000 urine and blood samples per year and has developed sophisticated methodology for detection and confirmation of drugs and their metabolites. The laboratory also serves as a source of information to the racing industry and the public regarding drug pharmacology, metabolism and clearance. The Analytical Systems Laboratory houses advanced mass spectrometry and other analytical equipment that is used to support the
research of the School of Veterinary Medicine faculty and graduate students. Equipment in these laboratories includes a Micromass Quattro II LC/MS/MS (+/-) with APCI and ESP interfaces, one HP 1090 II, low-flow HPLC (1 ul/min), two 1090 HPLCs equipped with UV-diode array and fluorescence detectors, five Agilent 1100/1200 HPLC systems, a Thermo LXQ LC/linear ion-trap mass spectrometer, a HP 5973 GC/MS system and beta and gamma counters for radiolabel analyses.

The ASL recently acquired a Waters/Micromass Two-Dimensional-Capillary-Liquid Chromatography/Quadrupole-Time of Flight Mass Spectrometer, which enables separation and comprehensive structural analysis of peptides/proteins. The instrument is capable of conducting de novo sequencing of proteins and peptides as well as identifying and locating post-translational modifications. This type of equipment is essential for the rapidly growing fields of proteomics and bioinformatics for comprehensive examination of molecular events occurring in tissues in health and disease. This equipment is available for collaboration across the LSU campus and supports the research efforts of the School of Veterinary Medicine faculty and the Equine Medication Surveillance Laboratory, which uses it to identify illegal peptide and protein drug use in racehorses under its contract with the Louisiana State Racing Commission. The laboratory has developed full capabilities to conduct low- and high-throughput proteomics analysis and is enhancing its abilities to conduct small and large-scale protein purification. Appropriate and modern computer equipment and software is available for data acquisition, storage, and analysis.

Pathology, Histopathology & Immunohistochemistry
The equipment, instrumentation and personnel for gross necropsy, histologic evaluation and immunohistochemical staining are available in this facility. Equipment and for processing cryopreserved tissues, automatic immunohistochemical staining, and the computers and software (ImagePro) for evaluation of staining distribution and intensity are available for use in this core facility.

Division of Laboratory Animal Medicine
DLAM is housed within the LSU School of Veterinary Medicine and serves as a central administrative division for operating research animal holding facilities, including the LSU School of Veterinary Medicine Laboratory Animal Medicine and Life Sciences Animal Care facilities. DLAM acquires, maintains and cares for teaching and research animals housed in the facilities and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

SVM Library
The LSU School of Veterinary Medicine Library is the major health science library in the greater Baton Rouge area. The Library is a member of the Louisiana Library Network and the South Central Region of the National Network of Libraries of Medicine. It is a significant partner in the educational and research programs of the School of Veterinary Medicine. Centrally located on the first floor of the School of Veterinary Medicine, the Library currently holds over 48,000 volumes, approximately 600 current periodical titles in print, access to thousands of periodical titles electronically, and several electronic databases dealing with all aspects of veterinary medicine. Patrons have access to not only those resources unique to the SVM Library, but also the resources provided by the main campus libraries. The SVM Library’s collection includes topics of human medicine, comparative medicine, public health, the animal sciences, and other related areas.
Anesthesia/Pain Management

Subarachnoidally administered hyperbaric opioids in horses

Authors/Investigators:
Claudio C. Natalini, MV, MS, PhD, DBCVSA; Alexandre da Silva Polydoro, DVM, MS; Renata Lehn Linardi, DVM, MS

Description of the Problem:
Spinal analgesia in horses is commonly produced with epidural injections with a combination of a local anesthetic and an analgesic drug such as xylazine or morphine. Limitations of the technique include ataxia, loss of motor control of the hind limbs and recumbency. Spinal analgesia with subarachnoid injection using the same drug combination is not possible in horses as loss of motor control would be devastating. Developing a technique for subarachnoid injection of an analgesic is granted in this species.

Study Objective:
The purpose of the study was to evaluate and compare the analgesic effect of subarachnoidally administered hyperbaric morphine, buprenorphine, and methadone on pain threshold to electrical stimulation and on behavior, cardiovascular, and respiratory response variables in horses.

Approach:
Horses were assigned to receive hyperbaric morphine 0.01mg/kg, buprenorphine 0.001mg/kg, methadone 0.01mg/kg, and 10% dextrose in equal volume (5 ml). Treatments were administered at time intervals of > 5 days. Electrical stimulation was applied for three hours after subarachnoid injection over the dermatomes of the perineal, sacral, lumbar, and thoracic regions, and the avoidance threshold voltage was recorded. Heart and respiratory rate, blood gases, electrolytes, and sedation were also evaluated.

Accomplishments/Results/Conclusions:
Administration of 10% dextrose did not change the avoidance threshold. Morphine and methadone significantly increased the avoidance threshold by 10 minutes after injection, which lasted until 120 minutes after subarachnoid administration in the perineal, sacral, lumbar, and thoracic regions. Profound analgesia (avoidance threshold > 40 V) was achieved in all regions. Buprenorphine also significantly increased the avoidance threshold by 10 minutes after injection which lasted 60 minutes and was considered moderate. No significant differences for cardiovascular and respiratory variables. Heart rate, blood pressure, respiratory rate, and blood gases stayed within normal limits. No ataxia, sedation, or central nervous system excitement was observed. Hyperbaric morphine and methadone administration may prove useful for pain management of severe intensity in horses.

Benefits to/Impact on the Equine Industry:
Developing a technique of opioids use in horses brings new options for the equine practitioner to treat pain and muscle skeletal injuries in this species. Opioids not only treat pain but also allow for a faster recovery, which decreases the industry costs.

Take Home Message:
Opioid analgesic use in horses is extremely beneficial and may decrease industry costs. New studies are granted as the techniques for opioid use are different from those used in small animals and human beings and are not completely understood in the equine patient.

Acknowledgements:
Funding was provided by the Department of Veterinary Clinical Sciences at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:

Caudal epidural analgesia can be a useful pain management strategy for horses with rear limb pain.
Identification of the MDR1 gene in horses

Authors/Investigators:
Claudio C. Natalini, MV, MS, PhD, DBCVSA; Renata Lehn Linardi, DVM, MS

Description of the Problem:
There is a wide difference of therapy response between species involving several drugs in veterinary medicine. Many factors can affect drug disposition such as physicochemical properties of the drug and biological factors, including gastric and intestinal time, luminal pH, and mucosal blood flow. It has been discovered in man and other animal species that P-glycoprotein, a drug transporter expressed by the MDR1 gene, plays a role in drug disposition including absorption, distribution, metabolism, and excretion. The MDR1 gene was not described in horses until now.

Study Objective:
The objectives of this study were to identify the presence of the MDR1 gene in equine ileum, brain and spinal cord and to obtain the horse MDR1 gene sequence for the first time.

Approach:
Samples of the middle part of the equine ileum, midbrain, and cervical spinal cord were collected shortly post-mortem from horses subjected to euthanasia due to medical recommendation with no previous history of GI tract or central nervous system disease. RNA was transformed in cDNA. This cDNA was employed as a template for polymerization using a Polymerase Chain Reaction (PCR). The PCR products were analyzed by 1% agarose gel electrophoresis.

Accomplishments/Results/Conclusions:
Recovery of pure DNA fragments from agarose gel was successful, and the concentration of DNA was determined. The sequence obtained matched in 83% with rabbit sequence. The results of this study showed that the MDR1 gene is present in equine ileum, brain, and spinal cord.

Benefits to/Impact on the Equine Industry:
Understanding drug metabolism and being able to interfere with drug distribution and pharmacodynamics is a key factor in equine therapeutics. This was the first step on investigating the pharmacology of drugs that are substrate for the MDR1 gene in horses. Decreasing industry costs and increasing the efficiency of the therapeutic approaches in horses are the main benefits for the horse industry with this research project.

Take Home Message:
Future studies on the MDR1 gene in horses are granted because this gene interferes directly with important therapeutic agents used in the equine industry. Being able to understand how this gene functions in drug metabolism will enhance equine therapeutics in the future.

Acknowledgements:
Funding was provided by the Equine Health Studies Program in the Department of Veterinary Clinical Sciences at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:
Natalini, CC, Linardi, RL, Kousoulas, KG, Huang, LJT. Equus caballus multi-drug resistance p-glycoprotein 1 (MDR1) mRNA, partial cds gi|62287713|gb|AY968084.1[62287713].

Pharmacokinetics of 1% injectable methadone administered orally to horses

Authors/Investigators:
Claudio C. Natalini, MV, MS, PhD, DBCVSA; Renata Lehn Linardi, DVM, MS

Description of the Problem:
Musculoskeletal diseases are the major disorders responsible for the loss of equine performance and are the cause of a significant amount of money having to be spent in diagnosis and treatment. Consequences of musculoskeletal injury are inflammation and pain. Although opioids are the most effective analgesic for horses, their use is limited in this species due to the risk of side effects (such as excitability) when administered intravenously. Oral opioid administration has been successful in humans and small animals, but there are no reports on oral opioids in horses.

Study Objective:
The objective of this study was to measure blood serum and CSF concentrations of methadone to determine the pharmacokinetic profile of oral administration in horses.

Approach:
The sample size of four experimental units for each treatment group was calculated considering a minimum difference of 10 nanograms of methadone between groups, a power of 0.80 and a value of 0.05. Based on this calculation, 16 healthy adult horses with similar body weight were studied. Three different doses of methadone, 0.1, 0.2, and 0.4mg/kg, were orally administered. Venous blood was collected to evaluate serum levels of methadone after oral administration.

Accomplishments/Results/Conclusions:
All three doses showed high serum levels of the drug, which correlated with increase of the dose. The peak methadone concentration occurred 30 minutes after oral administration, except for the horses that received the dose of 0.2mg/kg, where the peak occurred at 60 minutes. However, there was not a significant difference between the timing of these peaks. The curve of methadone concentration was similar for the three doses, and the only significant differences between doses were found at 15 and 30 minutes, showing higher drug concentration for the highest dose. These results demonstrate a similar response for the three doses used, inferring good absorption and distribution of methadone when administered orally in horses.

Benefits to/Impact on the Equine Industry:
Animal pain has an enormous socio-economical impact on the equine industry, especially in Louisiana, a state that has a prominent racing industry. Additionally, pain causes substantial emotional distress and frustration, which is experienced by owners, trainers and veterinarians. Significant annual monetary losses are associated with painful conditions, and these conditions often result in the loss of horses or their normal athletic function. Proper pain management has been shown clinically to shorten the animal’s recovery from disorders or injuries and to be essential to the welfare of animals.

Take Home Message:
Similar to what is evident in human beings and small animals, the use of oral opioids to treat pain can be extremely cost effective in the horse industry, with lower treatment costs and less risk for the animal.

Acknowledgements:
Funding provided by the Louisiana State University School of Veterinary Medicine Department of Veterinary Clinical Sciences Organized Research Fund and the Equine Health Studies Program.

Year Completed: 2005
Gastrointestinal Tract

Mechanisms of specific and non-specific cyclooxygenase inhibitor drug-induced injury in equine right dorsal colon mucosa

Authors/Investigators:
Katrin Saile, DVM (2006); Juneja Priti; Danial B. Paulsen, DVM, MS, PhD; Michael T. Kearney, MS, MapStat; Rebecca S. McConnico, DVM, PhD, DACVIM

Description of the Problem:
In horses in the United States, colic is the second most common cause of death after old age. Non steroidal anti-inflammatory drugs (NSAIDS) such as flunixin meglumine are commonly administered to horses during colic episodes to decrease discomfort, pain and shock caused by prostaglandin release. NSAIDS may, however, contribute to the pathogenesis of right dorsal colitis because not all prostaglandins enhance the shock associated effects of these endotoxins. Some prostaglandins, such as PGE2 and PGJ2 actually have cytoprotective effects on the intestine, and stimulate epithelial repair mechanisms. PGE2 and PGJ2 have also been shown to increase the transepithelial electrical resistance.

There is a severe increase in tissue permeability in tissues treated with the COX inhibitor flunixin meglumine when compared to tissues treated with the newer specific COX2 inhibitor etodolac. Phenylbutazone, a COX inhibitor similar to flunixin meglumine, alters ion transport in the right dorsal colon, and causes apoptosis and mucosal damage. In vivo studies of the equine right ventral colon showed that flunixin meglumine decreased short circuit current (ISC) in colonic mucosa compared to control samples, and that the addition of PGE2, at certain doses, restored ISC close to control levels. Specific COX-2 inhibitors have been shown to elevate PGE2 and PGJ2 levels, and restore the transepithelial resistance in ischemic-injured equine jejunal mucosa.

Study Objective:
The objective of this study was to determine the effects of a nonspecific cyclooxygenase (COX) inhibitor (flunixin meglumine), a specific COX-2 inhibitor, and a cytoprotective prostaglandin, PGE2 on equine right dorsal colonic mucosal structure and transepithelial resistance isolated in tissue baths.

Approach:
Right dorsal colonic tissue from six horses was studied using Ussing chambers. The horses were free of gastrointestinal disease. Serosal and muscular layers of the right dorsal colon were removed using sharp dissection and the mucosa was mounted in Ussing chambers bathed in warm oxygenated Ringer solution. The tissues were allowed to calibrate for 45 minutes prior to the addition of the NSAID treatments, flunixin meglumine and NS-398. Where applicable, 16,16-dimethyl PGE2 was added in addition to the NSAID treatments. The short circuit current (ISC), a direct measure of the active ion transport, and open-circuit PD were recorded manually every 15 minutes for four hours (depending on tissue electrical current stability). Conductance and tissue resistance were calculated based on the PD and ISC using Ohm's Law (V=IR).

Data was analyzed using the SAS statistical package. The GLM procedure was used to analyze the data as a repeated measures analysis of variance in a split-plot arrangement of treatments with Treatment Group (TX_GROUP) and Horse (HORSE_ID) on the main plot, and Time (TIME) and TX_GROUP*TIME interaction as subplot factors. Response variables were short circuit current (ISC) and transepithelial resistance (RESIST). Natural log transformations were performed on RESIST raw values to stabilize variances. Formalin fixed sections were examined by light microscopy. Measurements of crypt height were made using ImagePro software, and tissues were examined for signs of apoptotic (sloughing) cells, epithelial integrity, nuclear polarity and other signs of cytopathic change.

Accomplishments/Results/Conclusions:
There was a significant decrease in transepithelial resistance (TER) in tissues treated with flunixin meglumine, compared to the control tissues. NS-398 treated tissues did not show a significant decrease in TER, compared to control tissues. PGE2 treated tissues had a TER significantly higher than control tissues. Histologic analysis showed mild to moderate changes in flunixin treated tissues, including flattened or absent epithelial cells and changes in crypt cell nuclear polarity. No changes were noted in PGE2 and NS-398 treated tissues. Crypt depth decreased with NS-398, flunixin, and PGE2 treatments. NS-398 may be a better drug to use to decrease pain associated with colic in horses because it will not decrease the TER. This decreases the chances of bacterial translocation into the bloodstream and leakage of protein into the gut. PGE2 may be useful as a cytoprotective agent in colic horses.
**Benefits to/Impact on the Equine Industry:**
PGE2 may prove to be a useful component in the treatment of colonic mucosal damage in the horse.

**Take Home Message:**
Results of this study further support previous and on-going theories that flunixin meglumine causes right dorsal colonic mucosal damage. This anti-inflammatory agent should be used with caution in horses and alternative medications to treat colic in horses should continue to be investigated and developed.

**Acknowledgments:**
Student stipend funding by the National Institute of Health-BREVS Summer Research Scholars Program, Louisiana State University School of Veterinary Medicine. Research equipment and supplies funded by the Equine Health Studies Program and Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University. The authors would like to thank the following people for their help and support: Mr. Michael Keowan, Dr. Ashley Stokes, Dr. Tara Miska, Dr. Mandi Lopez, and Mr. Michael Broussard.

**Year Completed:** 2004

**Published Manuscripts/Abstracts:**

Proceedings of the 5th Annual Equine Colic Symposium, Quebec, Canada, Aug 2005.

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**Right dorsal colonic pathophysiology in horses administered phenylbutazone**

**Authors/Investigators:**
Rebecca S. McConnico, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS

**Description of the Problem:**
Phenylbutazone (PBZ), the most commonly used non-steroidal anti-inflammatory drug for treating musculoskeletal pain in equine athletes, is often associated with adverse gastrointestinal side effects including loss of use and even death, but the exact mechanism(s) have yet to be elucidated.

**Study Objective:**
In this study, PBZ was administered to 12 adult horses to determine specific pathophysiologic events that may occur in the right dorsal colon. The overall hypothesis was that administration of PBZ to healthy horses at a standard dosing level and duration causes a decrease in right dorsal colonic blood flow, inhibition of mucosal prostaglandin E2 (PGE2) levels, and a change in production of volatile fatty acids (VFA) leading to mucosal damage and inflammation with subsequent hypoproteinemia and hypoalbuminemia.

**Approach:**
Right dorsal colonic fistulas and artery flow probes were surgically placed in 12 Thorough-bred- or Quarter-type horses between the ages of three and 15 years old. Instrumentation was performed at least 30 days prior to the first experiment to allow horses to recover from surgery. Clinical and hematological parameters, tissue levels of chemical mediators, ingesta sampling for VFA production, and colonic blood flow measurements were evaluated every 72 hours over a 28 day period in horses treated with either phenylbutazone (4.4 mg/kg BID PO) or placebo. Tissue, ingesta, and blood samples, blood flow measurements, and clinical parameters were obtained between 8 A.M. and 10 A.M. on specified days.

Clinical and serum biochemical parameters, and degree of tissue inflammation (as determined by mucosal biopsy histopathologic grading, colonic mucosal myeloperoxidase...
Gastrointestinal Tract

[MPO] activity [indirect measure of granulocyte infiltration], malondialdehyde [MDA] levels [indirect measurement of reactive oxygen metabolite involvement], and PGE2 [important in mucosal protection], ingesta analyses [volatile fatty acid [VFA] production – mucosal protection], and right dorsal colonic arterial blood flow were evaluated at 72 hour intervals over a 28 day period in a cross-over design (each horse received each treatment regimen – placebo and phenylbutazone with a 20-30 day wash-out period between experiments).

**Accomplishments/Results/Conclusions:**
Serum albumin levels were significantly decreased in PBZ-treated horses compared to control horses on days 10-28 of PBZ treatment (p< 0.05). No other clinical or hematologic abnormalities were found to show statistical significance between PBZ-treated or control groups, although two horses developed severe colitis and associated clinical signs of endotoxemia after 10 days of receiving PBZ treatment. No statistical difference was found between PBZ-treated and control samples of right dorsal colonic mucosal tissue analyzed for MPO, MDA, and PGE2 levels, as well as for specimens examined histologically for evidence of inflammation. Right dorsal colonic blood flow values were markedly increased in PBZ-treated horses compared to control horses beginning with day 1 of treatment and continuing to the end of the time periods evaluated (p < 0.0001). Differences were identified in production of short chain volatile fatty acid ratio in PBZ-treated horses compared to control horses.

**Benefits to/Impact on the Equine Industry:**
Administration of PBZ to healthy horses at standard dosing levels and duration causes an increase in right dorsal colonic artery blood flow, a change in short-chain volatile fatty acid production, and concomitant systemic hypoalbuminemia. Individual animal variability is high with regard to expression of adverse clinical signs during treatment with PBZ.

**Take Home Message:**
Phenylbutazone should be used judiciously in horses. Veterinarians should monitor their PBZ-treated patients carefully by evaluating plasma albumin or total protein levels on a weekly basis in horses treated for extended periods of time. Other forms of treatment should be considered if adverse side effects are noted.

**Acknowledgments:**
This study was funded by the Grayson Jockey Club Research Foundation.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**
Proceedings, 5th Annual Equine Colic Symposium, Quebec, Canada.

Effects of lidocaine and flunixin on permeability and secretory capacity of equine right dorsal colonic mucosa

Authors/Investigators:
Jessica L. Carey; Ashley M. Stokes, DVM, PhD; Rebecca S. McConnico, DVM, PhD, DACVIM

Description of the Problem:
Colic is a very common and often life threatening condition in the horse. Flunixin meglumine is frequently used to treat pain associated with colic, despite its inhibitory effects on the recovery of insulted intestinal mucosa. It is possible that lidocaine may have opposite effects on mucosal integrity, while also alleviating pain. If our hypothesis is correct, lidocaine and flunixin could be used in combination to treat patients with colic, while minimizing the damage to intestinal mucosa.

Cyclooxygenase (COX) inhibitors are widely used to alleviate pain associated with colic. COX inhibitors, such as flunixin meglumine, block production of prostaglandins, which are responsible for enhancing the sensitivity of pain endings in the intestine. A less common treatment for colic is intravenous lidocaine, a drug shown to have anti-inflammatory effects and inhibitory action on enteric nerves.

Study Objective:
The goal of this study was to determine the effects of lidocaine and flunixin on permeability and secretory capacity of equine right dorsal colonic mucosa. Right dorsal colonic mucosa from seven horses was collected and placed in Ussing chambers.

Approach:
Right dorsal colonic mucosal tissues were treated with lidocaine in the presence and absence of a nonspecific COX inhibitor. Short circuit current and open-circuit potential difference were recorded. Tissue resistance was calculated using Ohm’s law. Tissue samples were examined histologically. Right dorsal colonic tissue from six horses was studied using Ussing chambers. The horses were free of gastrointestinal disease, and they were euthanised for reasons other than this project. Serosal and muscular layers of the right dorsal colon were removed using sharp dissection and the mucosa was mounted in Ussing chambers bathed in warm oxygenated Ringer solution. The tissues were allowed to calibrate for 45 minutes prior to the addition treatments. The short circuit current (ISC), a direct measure of the active ion transport, and open-circuit PD were recorded manually every 15 minutes for four hours (depending on tissue electrical current stability). Conductance and tissue resistance were calculated based on the PD and ISC using Ohm’s Law (V=IR). Data was analyzed using the SAS statistical package. The GLM procedure was used to analyze the data as a repeated measures analysis of variance in a split-plot arrangement of treatments with Treatment Group (TX_GROUP) and Horse (HORSE_ID) on the main plot, and Time (TIME) and TX_GROUP*TIME interaction as subplot factors. Response variables were short circuit current (ISC) and transepithelial resistance (RESIST). Natural log transformations were performed on RESIST raw values to stabilize variances.

Accomplishments/Results/Conclusions:
Lidoacaine treated and lidocaine plus flunixen meglumine-treated tissues maintained better mucosal barrier integrity as measured by tissue conductance compared to tissues treated with flunixin meglumine alone.

Benefits to/Impact on the Equine Industry: Colic is a very common and often life threatening condition in the horse. Treatment of horses with lidocaine may aid in treating pain associated with inflammation of the intestinal tract of the horse without causing damage to the epithelial barrier as has been shown with NSAID treatment.

Take Home Message:
Treatment of horses with lidocaine may aid in treating pain associated with inflammation of the intestinal tract of the horse without causing damage to the epithelial barrier as has been shown with NSAID treatment.
Acknowledgments:
This study was funded by the Merck Merial Summer Scholars Program at Louisiana State University School of Veterinary Medicine and the LSU Equine Health Studies Program.

Year Completed: 2005

Equine Cyathostomins: Secretory and inflammatory status in the ventral colon of naturally infected and maximally treated ponies

Authors/Investigators:
Thomas R. Klei, PhD; Rebecca S. McConnico, DVM, PhD, DACVIM; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; Sharon R. Chirgwin, PhD; Sharon U. Coleman

Description of the Problem:
Larval cyathostominiosis, the most common condition attributed to cyathostomins, is characterized by a variety of signs, including acute or chronic diarrhea, peripheral subcutaneous edema, weight loss, general ill-thrift and in some instances death. The condition is generally attributed to the seasonal emergence (in the winter or spring) of large numbers of larvae from the intestinal mucosa.

Study Objective:
The objective of this study was to further test the hypothesis that the steady state cyathostomin infection causes a mucosal inflammatory response which alters epithelium secretory function.

Approach:
Twelve mature mixed breed ponies were allowed to graze a cyathostomin contaminated pasture for one year. Ponies were ranked by eggs per gram of feces (EPG levels), placed in one of two groups and housed in stalls. One group (Tx) was treated with oxibendazole (OBZ) at 20 mg/kg for five days, followed by one dose of ivermectin (IVM) at 200ug/kg. The second group (NTx) remained untreated. Necropsies were performed on ponies in group NTx four weeks after being stalled. Necropsies of Tx animals were performed seven weeks after treatment.

Accomplishments/Results/Conclusions:
Mucosal parasite burdens determined by digestion were high in the NTx group (mean 36, 403). While there was an 86% reduction in the Tx group, significant numbers of parasites remained (mean 5,168). Histologic samples indicate that eosinophil and mast cell numbers were increased in the mucosa of NTx individuals. Right ventral colonic lymph nodes (LN) were removed and pooled, and mRNA levels of IL-1,4,5,6,8 and IFN gamma and TNF alpha measured by real time RT-PCR. IL-5 and IFN gamma levels were elevated in both groups but more so in Tx individuals. Chloride secretion as reflected by short circuit current in an Ussing chamber suggests that secretory activity was increased in the Tx group.

Benefits to/Impact on the Equine Industry:
These observations suggest that while moderate to heavy infection of cyathostomins increase the numbers of inflammatory cells, it is not sufficient to increase secretory activity of the epithelium. However, the activation of hypobiotic EL3 (dormant larval stages) may alter this activity.

Take Home Message:
Results of this study suggest that antigenic stimulation of intestinal epithelium following anthelmintic treatment and the subsequent inflammatory cascade may contribute to an electrogenic chloride secretory response in parasitized equine intestine.

Acknowledgments:
This study was supported in part by funds from the Louisiana State University Equine Health Studies Program and the LSU Department of Pathobiological Sciences. Special thanks go to EHSP research associates, Ms. Catherine Koch and Mr. Mike Keowan, who went above and beyond their routine responsibilities to assist with the completion of this project during the Fall of 2005 (during the aftermath of Hurricanes Katrina and Rita).
Year Completed: 2005

Published Manuscripts/Abstracts:
Laminitis

Apoptosis in epidermal lamellar cells in clinically normal horses and CHO induced laminitic horses treated or not with buffering solution

Authors/Investigators:
Adriana H. Souza, DVM, MS; Carlos A. A. Valadao, MV, MVSc, PhD; Daniel B. Paulsen, DVM, MS, PhD; Rafael R. Faleiros, DVM, MS, PhD; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
The term apoptosis was first proposed to describe a distinct form of cell death that differs from necrosis. Unlike necrosis, apoptosis can occur physiologically and does not induce an evident inflammatory response. Apoptosis can be triggered by several factors such as cytokines, calcium, hormones, citotoxic drugs, free radicals, and viruses. In a recent study, it has been reported that the number of apoptotic cells in the equine hoof during the normal physiological state is low. In horses with laminitis, the number of apoptotic basal layer cells is increased.

Study Objective:
The objective of this study was to compare the number, type, location and distribution of apoptotic epidermal lamellar cells in clinically normal horses and those with carbohydrate-overload (CHO) induced laminitis treated with either saline solution or buffering solution (aluminum hydroxide/magnesium hydroxide).

Approach:
Formalin-fixed samples of digital lamellar tissues was collected from 23 horses divided among four treatment groups: negative control (WS, n=5); positive buffer control (WB, n=6); CHO-induced laminitis (CS, n=6); and CHO-induced laminitis with buffer treatment (CB, n=6). In the buffer treatment groups, buffer was administered immediately following the eight-hour time point. Blocks of paraffin-embedded lamellar tissue were stained for DNA fragmentation with the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. Differential immunohistochimical staining for caspase 3 and 14 were used to confirm apoptosis.

Accomplishments/Results/Conclusion:
The number of TUNEL-positive epidermal cells per 0.1 mm of primary laminae was significantly greater in the acute laminitis group (CS and CB) than in the control group (CS and CB). The TUNEL-positive basal layer cells in the CB and CS group was four-fold and six-fold greater than the WS group, respectively. The number of TUNEL-positive keratinocytes was two-fold greater than the WS group. Apoptosis of TUNEL-positive basal layer cells was confirmed by results of caspase 3 immunohistochemical staining. The TUNEL-positive keratinocytes did not stain for caspases 3 or 14. We concluded that buffer treatment decreases the intensity of the apoptotic process when compared with horses not treated; however, it was not effective to decrease it when compared with the control group, demonstrating that it did not prevent the laminitis establishment.

Benefits to/Impact on the Equine Industry:
The results of this study indicate that apoptosis may be important in the development of acute laminitis. We speculate that the treatment of cecal acidification during the naturally-acquired carbohydrate overload laminitis is likely to prevent the onset of laminitis or, at least, to decrease the intensity of the microscopic alteration of the hoof, like apoptosis of the epidermal cells.

Take Home Message:
The intracelcell buffer treatment could help to decrease the intensity of the apoptotic process when compared with horses not treated, despite the fact that it did not prevent the laminitis establishment. Further studies examining alternative dosages and administration routes may elucidate the therapeutic potential of this buffer treatment in the prevention of naturally-acquired carbohydrate overload laminitis.

Acknowledgments:
This study was supported by funds from the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine, Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES/CAPES-PROAPP of Brazilian Education and Cultural Ministry. The authors thank Maria Inês Yamazaki de Campos, Francisca de Assis Ardisson, Narcizo Batista Tel, Frank Gaza, Catherine Koch, Cheryl Crowder and Julie Millard for technical assistance.
Laminitis

Year Completed: 2005

Published Manuscripts/Abstracts:
Adriana H. Souza, DVM, MS; Carlos A. A. Valadao, DVM, PhD; Daniel B. Paulsen, DVM, PhD; Rafael R. Faleiros, DVM, MS, PhD; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS. Apoptosis in epidermal lamellar cells in clinically normal horses and CHO induced laminitic horses treated or not with buffering solution. 9th World Congress of Veterinary Anaesthesiology (WCVAA), IV Meeting of the International Veterinary Academy of Pain Management and 9th Convention of Brazilian College of Veterinary Surgery and Anaesthesiology (CBCAV), Santos, Brazil, September 12-16, 2006.

Transcription of MMP-2 and MMP-9 in horses with CHO induced laminitis treated with an intracecal buffering solution

Authors/Investigators:
Adriana H. Souza, DVM, MS; Carlos A. A. Valadao, DVM, PhD; Sharon Chirgwin, PhD; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
The acute phase of laminitis is characterized by disintegration of the basement membrane associated with morphological changes in the basal epithelial cells of the secondary epidermal lamellae. Laminin and collagen types IV and VII are key structural components of the basement membrane, and they are known targets of matrix metalloproteinase enzymes 2 and 9 (MMP-2 and MMP-9), which are believed to dissolve these substances. Under normal physiological states, controlled dissolution allows the movement of epidermal laminae past the dermal laminae as hoof growth occurs. MMPs are a family of zinc-dependent proteinases involved in physiological and pathological degradation of the extracellular matrix, basement membrane components, and molecules attaching to epidermal basal cells. It is believed that excessive activation of these enzymes is responsible for initiating the cascade of events that leads to uncontrolled dissolution of the basement membrane components and ultimately to separation of the epidermal from the dermal laminae. Degradation of the lamellar basement membrane by MMPs represents a critical early event in the pathogenesis of equine laminitis.

Study Objective:
The objective of this study was to determine the expression of MMP-2 and MMP-9 using RT-PCR analysis during development of carbohydrate overload (CHO)-induced laminitis when either a saline solution or a buffer solution composed of aluminum and magnesium hydroxide was administrated intracecally.

Approach:
Horses were randomly divided among four treatment groups: control (WS); buffer control (WB); CHO-induced laminitis (CS); and CHO-induced laminitis with buffer treatment (CB). The buffer was administered eight hours after CHO. Tissues were collected 48 hours after CHO, immediately after euthanasia. The quantification of MMP-2 and MMP-9 mRNA was made by RT-PCR. Data for RT-PCR finding was evaluated as a fold-change using a validated house-keeping gene.

Accomplishments/Results/Conclusion:
Expression of MMP-2 and MMP-9 was greater in laminitic tissues (CB, CS) than in non-laminitic tissues (WB, WS). MMP-2 expression in the CS and CB group was 2.25-fold, 1.18-fold greater than the WS group, respectively. WB group represented 0.32-fold the MMP-2 expression of the WS group. MMP-9 expression in the CS and CB group was up 17.79-fold and 5.06-fold higher than the WS group, respectively. WB group represented 0.72-fold the MMP-9 expression of the WS group. We concluded that intracecal administration of buffer solution may be useful in the management of horses fed high levels of carbohydrate.

Benefits to/Impact on the Equine Industry:
It is presumed that avoiding exotoxin liberation and consequently the activation of enzymes responsible for basal membrane dissolution could prevent the occurrence of laminitis. Our study suggests that intracecal administration of a buffer solution would be helpful to decrease the expression of MMP-2 and MMP-9 and the onset and progression of laminitis in horses with naturally-acquired carbohydrate-related laminitis.

Take Home Message:
Even though the increased MMP-2 and MMP-9 gene expressions were not completely prevented by administration of the buffer solution, the expression of both MMPs was decreased compared with controls receiving only saline after CHO. Further studies may elucidate the therapeutic potential of this buffer treatment in the prevention of naturally-acquired carbohydrate-related laminitis.
**Acknowledgments:**
This study was supported by funds from the Equine Health Studies Program of School of Veterinary Medicine of LSU, Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES/CAPES-PROAPP of Brazilian Education and Cultural Ministry.

**Year completed:** 2005

**Published Manuscripts/Abstract:**
Adriana H. Souza, DVM, MS, Carlos A. A. Valadao, DVM, MS, PhD; Daniell B. Paulsen, DVM, PhD; Rafael R. Faleiros, DVM, MS, PhD, Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS. Transcription of MMP-2 and MMP-9 in horses with CHO induced laminitis treated with an intracecal buffering solution AAEP 52nd Annual Convention, San Antonio, Texas, December 2-6, 2006.

**Effects of aluminum hydroxide/magnesium hydroxide antacid on clinical signs hematological, and biochemical alterations in horses with CHO-induced experimental laminitis**

**Authors/Investigators:**
Adriana H. Souza, DVM, MS; Carlos A. A. Valadao, DVM, MS, PhD; Jose Henrique S. Borges, DVM, MS; Renata G. Reis, DVM, MS; Andrea D.P Uribe, DVM, MS; Erica C. P. G. Bueno, DVM, MS; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Equine acute laminitis is a highly debilitating disease of the digital soft tissues. Two main theories regarding its pathophysiology state that blood flow alterations and laminar basement membrane breakdown are involved in the establishment of this severe disease. Once the hindgut flora is exposed to fermentable carbohydrate, Gram-positive bacterial overgrowth occurs resulting in increased production of lactic acid, decreased intracecal pH, killing of cecal bacteria, and induction of changes in intestinal permeability. These changes allow the access of various compounds to the systemic circulation, including delivery to the digital circulation. Activation of matrix metalloproteinases during acute laminitis leads to a breakdown of epidermal and dermal laminae interactions. This activation can be induced by exotoxins from normal cecal flora. In the same way, amine compounds produced by decarboxylation of amino acids by various bacteria may cause peripheral vasoconstriction due to their structural similarities with endogenous vasoconstrictor amines, such as serotonin and catecolamines.

**Study Objective:**
The objective of this study was to compare the clinical development of carbohydrate overload (CHO)-induced laminitis when either a saline solution or a buffer solution composed of aluminum and magnesium hydroxide was administrated intracecally.

**Approach:**
Twenty-three horses instrumented with a cecal cannula were divided among four treatment groups: negative control (WS); positive buffer control (WB); CHO-induced laminitis (CS); and CHO-induced laminitis with buffer treatment (CB). Horses were evaluated before either saline or CHO administration (T0) and at eight, twelve, 24 and 48 hours. In the buffer treatment groups, buffer was administered immediately following the eight-hour time point. Physical parameters evaluated were rectal temperature (RT), respiration (RR), facial arterial pressure (P*), capillary refill (CRT), heart rate (HR), mucous membrane (MM) color, cecal motility, and Obel grade. Hematological measurements from jugular blood and peritoneal fluid (PF) included: RBC, WBC and differential, PCV, protein, fibrinogen, and hemoglobin (Hb). Biochemistry measurements from blood and PF included AST, CK, ALP, lactate, and glucose. The density and pH of PF were also measured.

**Accomplishments/Results/Conclusion:**
All CHO-treated horses developed laminitis, but development was significantly delayed in the CB group. However, CB showed clinical alterations earlier than CS (depression, diarrhea, increased cecal motility, hyperemic MM, prolonged CRT, and signs of abdominal discomfort). Cardiorespiratory parameters were increased after 48 hours in CS and CB. RT was increased through 48 hours in CS and CB. PCV, RBCs and Hb mean values were significantly higher at 48 hours in CB. CS showed an increase in PCV and RBCs, but Hb values did not alter. There was a significant increase in WBCs eight hours post-CHO to CS and CB and values increased across time with the highest rate 48 hours post-CHO. When WBC differential was analyzed, it was possible to determine that increased values from WBC were mainly due to segmented neutrophils in both groups. Blood biochemical analysis showed a significant increase in serum AST within eight to 48 hours and 12 to 48 hours after CHO in CS and CB, respectively. There was a significant increase in CK within eight to 24 hours post-CHO in CS and CB.
Laminitis

It was not observed statistical significance blood glucose values were not significantly different but a slight hyperglycemia 12 to 48 hours in CS and CB was noted. Lactate started to increase eight hours post-CHO in CS and 12 hours in CB and remained increased through 48 hours in both groups. A significant increase in PF WBC count was observed within 24-48 hours post-CHO to CS and CB. The WBC differential showed that increase in WBC values was mainly due to segmented neutrophils, monocytes and lymphocytes. There was a significant increase in PF ALP within 12-48 hours in CS and CB. Glucose values were not significantly different, but it showed an increase in CS and CB. Lactate values were increased at 24 hours in CB and within 12-48 hours in CS. We concluded that buffer treatment did not improve some of the parameters associated with CHO administration but delayed the onset of lameness.

Benefits to/Impact on the Equine Industry:
Many therapeutic measures used to treat laminitic horses are almost always palliative or symptomatic in nature whether they are delivered locally or systemically. Often treatment regimens are developed based on clinical experience and timing or stage of the disease. Despite the data relating to cecal metabolic alterations and laminitis pathogenesis, there is no information on the use of prophylactic or therapeutic measure to re-establish or maintain cecal function. Our study suggests that a buffer solution would be helpful to minimize the signs observed in the horses administered CHO.

Take Home Message:
Although buffer treatment did not improve some of the parameters associated with CHO administration, the delay in the onset of lameness should be considered an important result of this study. Further studies examining alternative dosages and administration routes may elucidate the therapeutic potential of this buffer treatment in the prevention of naturally-acquired carbohydrate overload laminitis.

Acknowledgments:
This study was supported by funds from the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine, Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES/CAPES-PROAPP of Brazilian Education and Cultural Ministry. The authors thank Renata L. N. Jorge, Claudia A. S. Nogueira, Paulo C. da Silva and Frank Garza for technical assistance.

Year completed: 2005

Published Manuscripts/Abstract:

Serial alterations in endothelin-1 immunoreactivity, nitric oxide, insulin, glucose, and platelet-neutrophil aggregates in horses administered carbohydrate overload

Authors/Investigators:
Susan C. Eades, DVM, PhD, DACVIM; Ashley M. Stokes, DVM, PhD; Philip J. Johnson, BVSc(Hons), MS, DACVIM; Casey J. LeBlanc, DVM, PhD, DACVP; V. K. Ganjam, MA(hc), BVSc, PhD; Preston R. Buff, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Acute laminitis is a debilitating, excruciatingly painful, and often life-threatening or career-ending disease of the sensitive and insensitive laminae of the equine digit. Currently, two of the most popular mechanistic explanations for the initiation of the acute laminitic cascade include the vascular/hemodynamic and the toxic/metabolic/enzymatic theories. There has been much debate regarding which theory is the initiating event in the development of acute laminitis; however, it is likely a multifactorial disease with components from each theory involved in its initiation and propagation. Experimental models of laminitis have demonstrated the presence of vasoconstrictor substances in increased quantities relative to those present in healthy horses. Endothelin-1 (ET-1), a potent vasoconstrictor peptide produced by the endothelium, is present in increased quantities in laminar tissue from horses with CHO-induced laminitis. Furthermore, endothelium-dependent relaxation is decreased in digital vessels of horses with CHO-induced laminitis, suggesting that the nitric oxide (NO) producing capacity of the digital vascular endothelium is reduced, thereby rendering the vessels more sensitive or vulnerable to vasoconstrictive agents. Weiss, et al. demonstrated a significant increase in platelet-neutrophil aggregates in ponies administered CHO. Endothelial cell secretion of ET-1 is also increased in direct response to
hyperinsulinemia. Endothelial cell dysfunction resulting from chronic hyperglycemia ("glucotoxicity") is characterized by increased production of ET-1, reduced production of NO, and adoption of a relatively pro-thrombotic phenotype. Pass, et al. demonstrated that for maintenance of the hoof-lamellar attachment interface, keratinocytes possess a very high requirement for glucose. Clinical circumstances, such as during insulin resistance, in which the ability of cells to remove glucose from circulation might be inhibited, represent an important risk factor for laminitis in horses. From these mechanisms it is evident that alterations in the synthesis/release of ET-1 and hyperinsulinemia, and platelet activation with adherence to neutrophils could occur during the prodromal stages of CHO laminitis and thus contribute to vasoconstriction, decreased microvascular patency and sludging of blood in the laminar microvasculature.

Study Objective:
These studies were performed to evaluate serial changes in digital and jugular venous blood endothelin-1 (ET-1) immunoreactivity, nitric oxide (NO), glucose, insulin, and platelet-neutrophil aggregates during carbohydrate overload (CHO)-induced laminitis.

Approach:
Digital and jugular venous blood samples were collected at 1-hour intervals (glucose, insulin, ET-1 and NO) or 4-hour intervals (platelet-neutrophil aggregates) for 8 hours or 16 hours after laminitis was induced by administration of corn starch/pine wood slurry via nasogastric tube.

Accomplishments/Results/Conclusions:
Carbohydrate overload caused a significant increase in the concentration of ET-like immunoreactivity in the digital blood above baseline at 11 hours after CHO. Endothelin-like immunoreactivity in the digital blood was significantly greater than that in the jugular venous blood at eight, nine, 11 and 12 hours after CHO. The concentration of glucose in digital and jugular venous blood increased significantly at three, four and five hours after CHO. The insulin concentration increased significantly at five hours after CHO. Surrogate estimates of insulin sensitivity document relative insulin insensitivity. The number of platelet neutrophil aggregates in digital and jugular venous blood increased significantly at 12 hours after CHO.

Benefits to/Impact on the Equine Industry:
The present study documents increased concentrations of digital venous ET-1 concentrations after CHO. Furthermore, increases in venous insulin and glucose concentrations with relative insulin insensitivity after CHO suggest that further study of endocrine factors during CHO is of value.

Take Home Message:
Concurrent increases in ET-1, insulin, glucose, and platelet-neutrophil aggregates support a role of endothelial dysfunction in the pathogenesis of laminitis. Treatments that improve endothelial function during CHO may be beneficial.

Acknowledgments:
This study was funded by a grant from the United States Department of Agriculture National Research Initiative. the authors thank Catherine Koch, Michael Keowen, and Frank Garza for assistance.

Year Completed: 2005

Published Manuscripts/Abstracts:


Inhibition of norepinephrine and serotonin-induced contractile

Equine Health Studies Program 2006 Research Report 43
**Response of Equine Digital Vessel Rings by Ifenprodil**

**Authors/Investigators:**
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**Description of the Problem:**
Laminitis pathogenesis is complex, and the specific trigger for the digital vessels alterations during laminitis is unknown. Its characteristics are vascular hypoperfusion, edema formation and ischemia that eventually lead to necrosis of the laminar tissues. The equine digital vessels, mainly the veins, are very reactive to endogenous vasoconstrictor mediators released at prodromal stages of laminitis. Gut-derived monoamines are suggested to induce digital vasoconstriction. Ifenprodil (IFE), an agent that blocks polyamine site of the NMDA receptor, has been shown to have neuroprotectant action in acute central ischemic insult.

**Study Objective:**
The objective of this study was to determine the effectiveness of IFE to inhibit the in vitro responses of equine palmar digital arterial and venous rings to graded concentration of NE and 5HT.

**Approach:**
The equine palmar digital (EPD) arteries and vein were collected from non laminitic horses immediately after euthanasia. Vessels rings were incubated with IFE to determined the contractile response to NE and 5-HT (10-10 to 10-4M) or precontracted with EC50 of NE and 5-HT and exposed to graded concentrations of IFE (10-8 to 10-4) to measure the vessels rings relaxation. In the first part the concentration-response relationships of the rings were determined separately without (control) and with incubation with three concentrations of IFE (10-8, 10-6 & 10-4M). The response produced by NE and 5-HT at 10-4M was taken as the maximal response, and the responses to other concentrations were taken as a percent of the maximum. Of the four arterial rings, one was used as a control and the remaining three were used for three concentrations of IFE. This same design was followed for venous rings. In the second part of the experiment, the tissues were pre contracted with EC50 of either NE or 5-HT. When the response reached a plateau, relaxation response was induced by graded concentration of IFE (10-8 to 10-4 M). The response of the tissues to reach the original baseline from the contracted state is considered as 100%, and the responses produced by different concentrations of IFE were considered as a percent of the maximum.

**Accomplishments/Results/Conclusions:**
Relaxation induced by IFE in the NE and 5-HT constriction response of EPD arteries and veins represented a significant right displacement of C-R curve, mainly at the 10-6 and 10-4M IFE concentrations with stronger results for venous rings. The total area under curve, EC50 values and the maximum contraction values to high concentrations of NE and 5-HT (10-4M) showed significantly dose-dependent reduction by IFE treatment. Similarly, IFE relaxed the EPD arterial and venous rings that were pre-constricted with a single dose (EC50) of NE or 5-HT. These effects were more evident in venous rings. The NMDA receptor polyamine site antagonist IFE blocked the contractile responses of EPD vessels rings to NE and 5-HT, in a dose-dependent manner when they were incubated with different concentrations of IFE. Graded concentrations of IFE were also able to relax the rings pre-constricted with NE or 5-HT. These findings indicate that IFE is effective as a relaxant in EPD vessels against NE and 5-HT.

**Benefits to/Impact on the Equine Industry:**
Since ifenprodil causes a substantial and sustained relaxation response in both EPD arteries and veins in this study, it may be useful for treatment or prevention of the vascular ischemic alterations induced by laminitis, in horses, causing vasodilatation and improving the laminar blood flow.

**Take Home Message:**
Several gut derived substances induce arterial and vein constriction that cause equine laminar ischemic disturbances at the prodromic stage of laminitis. Thus, this study suggests that equine laminar arteries and veins

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*Investigators monitor equine digital vessel relaxation using the in vitro tissue bath system. The primary aim of this study was to examine a potential link between primary conditions, such as colic, and the secondary development of laminitis.*
are responsible for relaxation induced by ifenprodil.

Acknowledgments:
This study was supported by funds from the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:

Evaluation of digital hemodynamics associated with black walnut-induced equine laminitis using colored isotopic-labeled microspheres

Authors/Investigators:
Ashley M. Stokes, DVM, PhD; Diane Savois; Mike Keowen; Susan C. Eades, DVM, PhD; and Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Laminitis is a common disease of horses involving the soft tissue structures that suspend the distal phalanx (P3) within the hoof capsule. Although the pathophysiology of this disease is not completely understood, there is substantial evidence suggesting local digital hemodynamic alterations occur and likely play a role in the initiation of this disease. Endothelin-1 (ET-1) is a peptide synthesized by the endothelium and is the most potent vasoconstrictor substance known. Because of the physiologic and pathophysiologic properties of ET-1, we believe that an increase in this substance could account for the initiation and propagation of many of the alterations known to occur with acute laminitis; therefore, the use of an ET antagonist may prevent some of these hemodynamic alterations. Our current lack of complete understanding of acute laminitis prohibits effective therapeutic and preventative measures from being developed and employed in the clinical management of this disease. Direct measurement of laminar capillary perfusion is limited due to the anatomic location between the hoof wall and bone. The use of colored microspheres (15 um in diameter) allows for differentiation between flow through the laminar capillaries (nutrient vasculature approximately 8 um in diameter) and flow bypassing the laminae via arteriovenous shunts (AVAs approximately 40 um diameter).

Fifteen μm diameter colored isotopic-labeled microspheres were injected into the digit of horses to measure the laminar perfusion and status of arteriovenous shunts in horses with experimentally-induced laminitis. Microspheres lodge in the capillaries and allow for the measurement of blood flow to tissues. A lack (or shunting) of blood flow would result in a reduction of microspheres present in the tissue sample. During shunting, microspheres are found in the venous circulation (note blood collection in Fig. 1).

Study Objective:
1) Laminar perfusion decreases and AVAs open during the developmental stages of acute laminitis induced by administration of black walnut extract (BWE); 2) Alterations in laminar perfusion and the opening of AVAs could be prevented by administration of an ET antagonist into the digital arterial circulation; 3) Onset and progression of the clinical signs of laminitis caused by BWE administration could be prevented or reduced by administration of the ET antagonist.

Approach:
Acute laminitis was induced in 13 clinically healthy horses utilizing BWE administration to study alterations in laminar blood flow, the presence of AVAs, and the potential role of ET-1 in the pathogenesis of these vascular derangements at the onset of clinical signs of acute laminitis. Before, and at specified times after BWE administration, colored microspheres were injected into one forelimb digital artery with subsequent withdrawal of digital venous blood for measurement of microspheres by-passing the digital capillary bed. Seven horses received local digital infusion of the ET antagonist PD145065 (10⁻⁵ M concentration; group 1) and six received an equivalent volume of saline (control; group 2) at specified times that correspond to documented alterations in digital blood flow. To determine the clinical efficacy of ET antagonist administration, hemodynamics, hematological parameters, and clinical signs were monitored for 72 hours in all horses. At the termination of the study, horses were euthanatized and laminar tissues collected for quantification of colored microsphere presence within laminar capillaries.

Accomplishments/Results/Conclusions:
A hyperemic period occurred eight hours post-BWE administration and palmar digital blood flow remained increased throughout the remainder of the study. Administration of the ET antagonist resulted in decreased alterations in blood flow to the digit and reduced digital arterial pressure compared with the saline group, possibly preventing laminar edema formation. White blood cell counts dropped by three hours post-BWE. Heart rate, respiratory rate, rectal temperature, and reaction to hoof testers increased following BWE administration with the greatest values from eight to 24 hours post-BWE. For horses in the treatment group, the foot receiving PD145065 responded less to hoof testers and ended with a lower Obel grade. The ET antagonist treated group had a substantially lower Obel score at the peak of clinical signs of laminitis, and the return to complete soundness was in half of the time compared with the saline treated horses. Microspheres present in the palmar digital venous circulation decreased with the development of BWE-induced laminitis and serve as an indication of AVAs within the digital circulation. In our study, microspheres in digital venous blood decreased with laminitis suggesting AVAs close during laminitis. In some horses, microspheres lodged within the laminar capillaries decreased below baseline values, suggesting decreased laminar perfusion. In summary, the alterations in microsphere values within the palmar digital blood and laminar tissues suggest altered digital circulation and perfusion during the developmental and early stages of BWE-induced laminitis.

**Benefits to/Impact on the Equine Industry:**
In addition to the extreme pain and debilitation of affected horses, there is substantial emotional distress and frustration for owners, trainers and veterinarians. Annual monetary losses related to laminitis have been estimated at greater than $13 million associated with its diagnosis, treatment and loss of horses subsequent to complications. Understanding the basic mechanisms of blood flow to the foot will enable better investigation of potential therapeutics for the prevention and treatment of numerous diseases of the equine digit.

**Take Home Message:**
These findings suggest laminar hemodynamic alterations occur during the developmental stages of laminitis and decreasing the vascular contractile effects of endogenous ET-1 (utilizing the ET antagonist) improves digital hemodynamics and clinical signs of acute laminitis. Using the BWE model and microspheres we have demonstrated the presence of hemodynamic alterations at the laminar level during the pathogenesis of acute laminitis; additionally, we have investigated the effectiveness of the ET antagonist, a potential therapeutic agent for the prevention and treatment of this disease. The information gained from these studies provides important information on the potential role of ET-1 in the pathophysiology of acute laminitis, and the potential therapeutic implications for ET antagonists in horses with naturally acquired disease.

**Acknowledgments:**
Supported by the Grayson Jockey-Club Research Foundation.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


Stokes AM, Savois D, Eades SC, Keowen M, Moore RM. Evaluation of Digital Hemodynamics Associated with BWE-

![Fig. 1 Injection of 15 um diameter colored isotopic-labeled microspheres into the digit of horses to measure the laminar perfusion and status of arteriovenous shunts in horses with experimentally-induced laminitis. Microspheres lodge in the capillaries and allow for the measurement of blood flow to tissues. A lack (or shunting) of blood flow would result in a reduction of microspheres present in the tissue sample. During shunting, microspheres are found in the venous circulation.](image-url)
Evaluation of the effect of repeated digital intra-arterial administration of 15 um colored microspheres on digital hemodynamics and laminar microscopic and ultrastructural integrity

Authors/Investigators:
Ashley M. Stokes, DVM, PhD; William G. Henk, M Ed, PhD; Daniel B. Paulsen, DVM, MS, PhD, Michael Keowen; Olga N. Borkhsenious; and Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Acute laminitis is a severely debilitating, excruciatingly painful, and life-threatening disease of the soft tissues (sensitive and insensitive laminae) of the equine digit. The prevalence of laminitis, combined with morbidity and mortality of horses, frustration, economic and emotional costs, and an incomplete understanding of the disease contribute to the explanation of why laminitis was considered the most important disease afflicting horses and the number one disease requiring further research by respondents to an American Association of Equine Practitioners-sponsored survey. Although the pathogenesis of laminitis is not fully understood, the fundamental mechanisms of tissue destruction that are currently under investigation include vascular dysfunction leading to laminar edema formation and tissue ischemia. Direct measurement of laminar capillary perfusion is limited due to the anatomic location between the hoof wall and bone. The use of colored microspheres (15 um in diameter) allows for differentiation between flow through the laminar capillaries (nutrient vasculature approximately 8 um in diameter) and flow bypassing the laminae via arteriovenous shunts (AVAs approximately 40 um diameter). The use of these microspheres needed to be validated in the laminar tissues in horses to fully utilize them in future studies.

Study Objective:
(1) The first objective is to determine if repeated administration of microspheres will result in altered digital hemodynamics since the very nature of the spheres is to lodge within the microvasculature as the arterioles transition into capillaries and decrease in diameter < 15 mm. This objective was accomplished by measurement of palmar digital blood flow, palmar digital venous and arterial pressures, and calculation of vascular resistance in conjunction with microsphere administration. (2) The second objective is to quantitatively evaluate lodging of the six colors of microspheres within the laminar microvasculature and passage through open AVAs using neutron activation techniques designed specifically for the microspheres utilized in the proposed study. This objective was accomplished by the collection of palmar digital venous blood in conjunction with microsphere administration to evaluate sphere passage through open AVAs, and upon termination of the study, laminar tissues were harvested for quantitative measurement of each of the six colors of microspheres within the laminar regions of interest. Since the size of the microspheres are such that they should not traverse the capillary bed, their presence in digital venous blood collected at designated times would indicate flow through open AVAs. (3) The third objective is to examine laminar tissues using light microscopy and transmission electron microscopy to determine if repeated administration of microspheres results in unintended, iatrogenic ischemia of the laminar tissues. This objective was accomplished by blinded evaluation of control laminar tissues collected from the contralateral un-instrumented forelimb and laminar tissues harvested from the instrumented

An image captured using electron microscopy of laminar tissues examined after repeated injections of microspheres to validate this technique in horses. The basement membrane between the dermal and epidermal laminae is fully intact and no signs of tissue damage are apparent. Note the red blood cells in the laminar vasculature found in this section.
Laminitis

forelimb receiving the microsphere injections (all regions were free of microspheres to maintain unbiased observation).

**Approach:**
Studies involved the use of eight healthy horses to study digital and laminar hemodynamics with repeated microsphere injections. One randomly selected forelimb was instrumented for hemodynamic measurements and microsphere administration. The contralateral limb served as a control. Palmar digital arterial blood flow was measured by use of an ultrasonic flow probe. At specified times, colored stable isotopic-labeled microspheres were injected into the digital artery with simultaneous withdrawal of digital venous blood for measurement of passage of microspheres through AVAs. Six different colored microspheres were injected at one hour intervals under constant thermal conditions (25°C) and normal load with equal load between forelimbs quantified using a digital scale. Pressures and blood flow were recorded 10 minutes before and 10 minutes after each microsphere injection. After 72 hours, laminar tissues were collected for quantification of microspheres within laminar capillaries. Laminar tissue samples collected from the uninstrumented, contralateral forelimb served as controls. Laminar samples were evaluated using transmission electron microscopy and light microscopy.

**Accomplishments/Results/Conclusions:**
Repeated administration of microspheres did not interfere with digital flow and laminar perfusion or laminar microscopic integrity, thus making this technique useful and reliable for serial evaluation in horses with experimentally-induced laminitis.

**Benefits to/Impact on the Equine Industry:**
In addition to the extreme pain and debilitation of affected horses, there is substantial emotional distress and frustration for owners, trainers and veterinarians. Annual monetary losses related to laminitis and associated with its diagnosis, treatment and the loss of horses subsequent to complications have been estimated at greater than $13 million. Understanding the basic mechanisms of blood flow to the foot will enable better investigation of potential therapeutics for the prevention and treatment of numerous diseases of the equine digit. The findings of this study will allow for new methods to determine blood flow alterations, thereby advancing the study of laminitis.

**Take Home Message:**
Repeated administration of microspheres does not interfere with digital flow and laminar perfusion or laminar microscopic integrity, thus making this technique useful and reliable for serial evaluation in horses with experimentally-induced laminitis.

**Acknowledgments:**
This study was supported by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

**Year Completed:** 2005

**Immunohistochemical staining and RT-PCR to determine and compare presence and location of matrix metalloproteinases-2 and -9 in laminar tissue of clinically healthy and laminitic horses**

**Authors/Investigators:**
Ashley M. Stokes, DVM, PhD; Erica Wallace, DVM; Daniel Paulsen, DVM, MS, PhD; Sharon Chirgwin, PhD; Giselle Hosgood, BVSc, MS, DACVS, FACVS; Susan C. Eades, DVM, PhD, DACVM; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Laminitis is an extremely painful, debilitating disease of the laminae of the equine hoof. This project explores the enzymatic theory of laminitis in relation to how over-stimulation of matrix metalloproteinases (MMPs)-2 and -9 leads to the degradation and failure of the laminar basement membrane. A combination of immunohistochemistry and real-time reverse transcription-polymerase chain reaction (RT-PCR) could aid in the understanding of the exact location and gene regulation of MMP-2 and -9 in the laminae.

**Study Objective:**
Our hypotheses are that there will be an increase in immunohistochemical staining of MMP-2 and -9 in the laminae of horses with experimentally-induced laminitis, compared with clinically healthy horses and naturally-acquired laminitis horses, and will be less in laminae treated with an endothelin (ET) antagonist. Similarly, gene expression will follow
the same patterns as the immunohistochemical staining with laminitic horses having upregulation of gene expression compared with normal horses. Our objectives are as follows: 1) Validate the immunohistochemical staining technique and RT-PCR protocol for use in equine laminar tissues to determine the expression, presence and location of MMP-2 and -9. 2) Determine the effect of a receptor antagonist of endothelin used in laminitis-induced horses on the presence and location of MMP-2 and -9 in the laminae. 3) Determine the difference in MMP regulation, synthesis and location between two experimentally-induced acute laminitis models, naturally-acquired laminitis horses, and clinically healthy horses.

**Approach:**
Immunohistochemistry and RT-PCR procedures for MMP-2 and MMP-9 were performed on laminar tissues from 57 horses (11 normal, 15 naturally-acquired laminitic, and 26 from experimentally-induced laminitis studies [black walnut extract [BWE; 11] and carbohydrate overload [CHO; 20]). In the experimentally-induced laminitis studies, a receptor antagonist of the potent vasoconstrictor ET was used locally in the digit in half of the horses. Validation of the immunohistochemistry staining techniques was performed for each of the antibodies and validation of RT-PCR was conducted. Using Image-Pro Plus 4.1.5 software, staining of laminae was analyzed using a macro that assigned color markers for intensities of staining and calculated statistical results for each assigned color. Data for RT-PCR finding was evaluated as a fold-change using a validated house-keeping gene.

**Accomplishments/Results/Conclusions:**
Validation of both techniques in equine laminae was achieved. MMP-2 staining for the naturally-acquired laminitic group was significantly greater than the laminitis negative group and the CHO group. MMP-2 staining for the BWE group was significantly greater than for the CHO group. In the BWE and CHO groups, MMP-9 staining was significantly less in the ET antagonist-treated groups compared with the control groups with no treatment, suggesting a protective effect of the antagonist. MMP-2 gene expression was increased in horses with laminitis compared to normal laminitis-free horses.

**Benefits to/Impact on the Equine Industry:**
This study contributes substantially to laminitis research and could possibly lead to development of more effective treatments. The ability to study the regulation and location of MMP-2 and -9 in the laminae will bring us a step closer to fully understanding the pathophysiology of laminitis and finding a method to prevent destruction of laminar attachments within the digit.

**Take Home Message:**
Laminitis is a complicated disease that has many contributing factors. This study has shown that the over-activation of enzymes (MMPs) in the hoof could play an important part in laminitis. Further studies should be done to explore the possible benefits of reducing the activation of MMP-2 and -9 and therefore preventing their destruction of the laminae.

**Acknowledgments:**
This study was supported by the Merck Merial Summer Scholars Program, the Morris Animal Foundation, the United States Department of Agriculture National Research Initiative, the Grayson Jockey Club Research Foundation, the LSU SVM Equine Health Studies Program, and the Louisiana State University School of Veterinary Medicine.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


Wallace, E, Stokes AM, Paulsen D, Hosgood G, Eades SC, Moore RM. Immunohistochemical Staining to Determine and Compare Presence and Location of Matrix Metalloproteinases-2 and -9 in Laminar Tissue of Clinically Healthy and
Altered laminar gene expression indicative of vascular, inflammatory and metabolic events in horses with acute laminitis

Author/Investigators:
Ashley M. Stokes, DVM, PhD; Sharon Chirgwin, PhD; Jeffrey P. Cardinale; Brenna K. Hanly; Jennifer Liford; Diane M. Savois; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Laminitis is a common disease of the equine digit characterized by cardiovascular alterations (ischemia/reperfusion) associated with other complex pathophysiologic cascades with many unidentified components. The complexity of the pathogenesis results in varied therapeutics utilized by veterinarians. Studies designed to examine multiple cascades would be very beneficial in our quest to unravel the events, and the timing of these events, which will lead to improved therapeutic regimens.

Diagram illustrating our hypothesis that multiple cascades (vascular, inflammatory, metabolic, and enzymatic) influence each other during the pathogenesis of acute laminitis. The eventual outcome is the clinical manifestation (or clinical signs) of laminitis. As we understand more about these cascades, we can aim our therapeutics more accurately during treatment and/or prevention of this devastating disease.

Study Objective:
The objective was to examine potential cardiovascular, metabolic and inflammatory mediators in naturally-acquired and experimentally-induced equine laminitis.

Approach:
Samples were collected from 54 horses from naturally-acquired (acute & chronic), 9-hours post-experimental induction (black walnut extract model), 72-hours post-experimental induction, and controls. TaqMan RT-PCR was completed for endothelin-1 (ET-1), interleukin (IL)-8, cyclooxygenase (COX)-1, COX-2 and glucose transporter (Glut)-1. When the equine gene sequence was not available, it was cloned, sequenced and validated.

Accomplishments/Results/Conclusions:
Significant differences were found for all genes. ET-1, IL-8, COX-1 and COX-2 gene expression increased in horses with acute disease. IL-8 and COX-2 returned to basal levels, but COX-1 and Glut-1 significantly decreased below basal levels in chronic disease. Horses with naturally-acquired disease had the most alterations, especially those with gross alterations of the distal phalanx.

Benefits to/Impact on the Equine Industry:
This study contributes substantially to laminitis research and could possibly lead to development of more effective treatments. Laminitis is a common disease in horses and carries tremendous emotional and financial burdens for owners, trainers, and treating veterinarians. The most important benefit of laminitis research will be for the welfare and health of the horse, which suffers greatly with this devastating disease.

Take Home Message:
Laminitis is a complicated disease that has many contributing factors. These findings demonstrate the roles of vascular alterations, inflammation and altered metabolism in the onset of this devastating disease.
Laminitis is a devastating disease. Our goal with this data is to form a timeline of events that occur during the developmental stages of laminitis in horses. This will ultimately allow for more targeted therapeutic intervention.

Acknowledgments:
This study was supported by the Merck Merial Summer Summer Scholars Program, the Louisiana Biomedical Research Network Summer Undergraduate Research Program, the Morris Animal Foundation, the United States Department of Agriculture National Research Initiative, the Grayson Jockey Club Research Foundation, the LSU SVM Equine Health Studies Program, and the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:


Effects of intramuscular administration of acepromazine on digital and systemic hemodynamic and clinical variables in clinically healthy, conscious horses

Authors/Investigators:
Britta S. Leise, MS, DVM; Lee Ann Fugler, DVM; Ashley M. Stokes, DVM, PhD; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Laminitis is a devastatingly painful disease of the equine digit that can affect any horse potentially ending their athletic career or even requiring euthanasia. Although the exact pathophysiology of laminitis remains to be elucidated, hemodynamic alterations associated with the onset and propagation of acute laminitis have been well documented. Alterations of both digital blood flow and laminar perfusion have also been reported during the developmental stages of laminitis. In a survey of 60 scientists and clinicians, over 93% reported that they use acepromazine routinely (58%) or occasionally (35%) in the prevention and treatment of acute laminitis. For convenience and because of the assumption that the vasodilatory effect on the digital vasculature would be prolonged, acepromazine is commonly administered intramuscularly to horses for the treatment and prevention of laminitis.

Study Objective:
We hypothesized that administration of acepromazine intramuscularly would decrease mean systemic and digital arterial blood pressure and increase mean digital arterial blood flow for a longer duration of time with less sedative and hypotensive effects than those reported for intravenous administration. The purpose of this study was to determine the magnitude and duration of the effects of acepromazine administered intramuscularly on systemic and digital hemodynamic and clinical variables in healthy, conscious horses.

Approach:
An ultrasonic Doppler flow probe was surgically implanted around the medial palmar digital artery prior to the study. Catheters were placed in the transverse facial artery, lateral palmar digital artery and jugular vein. A treatment group
Laminitis

(n=6) received 0.04 mg/kg of body weight of acepromazine intramuscularly. A control group (n=6) received an equivalent volume of saline intramuscularly. Palmar digital blood flow and digital and facial arterial pressures were measured at selected times 30 minutes before injection and for six hours after administration. Jugular venous blood was collected for measurement of packed cell volume and total plasma protein concentration. Horse instrumented for measurement of palmar digital blood flow, palmar digital arterial pressure, and transverse facial arterial pressure.

**Accomplishments/Results/Conclusions:**
Horses administered acepromazine had significantly lower transverse facial arterial pressure compared with control horses. Acepromazine-treated horses had significantly greater digital arterial blood flow post administration when compared with the respective baseline values for that group. Packed cell volume significantly decreased in horses administered acepromazine when compared to their baseline value.

**Benefits to/Impact on the Equine Industry:**
Cepromazine administered intramuscularly decreases systemic arterial pressure and packed cell volume and increases digital blood flow when compared with respective pre-treatment baseline values.

**Take Home Message:**
Intramuscular administration of acepromazine has a prolonged effect on systemic and digital hemodynamic variables in healthy horses with minimal sedation. Further evaluation of these variables is needed in horses during the developmental phase of acute laminitis to determine if similar effects occur.

**Acknowledgements:**
This study was supported by funds from the Louisiana State University School of Veterinary Medicine Department of Veterinary Clinical Science CORP grants and the Morris Animal Foundation.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**

**Expression of the GLUT-1 gene, an Ischemia-Related Glucose Transporter, In Laminar Tissue of Black Walnut-Induced Laminitic Horses**

**Authors/Investigators:**
Diane Savois; Ashley M. Stokes, DVM, PhD; Sharon Chirgwin, PhD; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Laminitis is a debilitating and excruciatingly painful disease of the equine laminae, and the pathogenesis is not completely understood. The three main theories of laminitis are the ischemic/vascular, the metabolic/enzymatic, and the mechanical theories. This project explored a possible link between the metabolic and vascular theories in relation to the expression of GLUT-1, an ischemia-related glucose transporter. Decreased expression of this transporter in laminar tissue, due to ischemia, may result in glucose deprivation that could lead to laminar death. Currently, gene expression of GLUT-1 has not been examined in horses with clinical signs of laminitis. Cloning, sequencing, and validation of equine GLUT-1 mRNA were performed in order to design primers and a probe for use with Taqman real time (RT)-PCR. RT-PCR was
performed on laminar tissue samples to determine the expression of GLUT-1 in normal laminae as compared to the laminae of horses with black walnut-induced laminitis at two different developmental stages of the disease. Knowledge of the expression of this gene may provide the missing link between the two most prominent theories regarding the pathogenesis of this disease. Discoveries made during this investigation may offer insight as to the steps leading up to necrosis of laminar tissue, stimulating future research aimed at the prevention and treatment of this devastating disease.

**Study Objective:**
The objectives of this study were 1) To clone and sequence the equine GLUT-1 gene and design primers and a probe suitable for use with TaqMan RT-PCR; 2) To validate the use of RT-PCR to determine the extent of gene expression of GLUT-1 in equine laminar tissue; 3) To determine the difference in GLUT-1 gene expression between BWE-induced laminitic horses (at nine hours post-BWE administration vs. 72 hours post-BWE administration), clinically laminitic horses, and clinically healthy horses.

**Approach:**
Tissue samples from normal horses and samples from two previously conducted experimentally induced laminitis studies were utilized to explore a gene influenced by ischemia that regulates glucose metabolism. Once we cloned and sequenced the equine GLUT-1 gene, we determined its expression in the various tissue samples by using RT-PCR and TaqMan analysis.

**Accomplishments/Results/Conclusions:**
There was a down regulation of GLUT-1 in laminae affected with laminitis as compared to laminae from normal horses. Also, the laminar tissues collected nine hours post-BWE administration showed more GLUT-1 gene expression than those collected 72 hours post-BWE administration. Laminae from horses with acute laminitis also had more GLUT-1 gene expression than laminar tissue from horses affected by chronic laminitis. As hypothesized, there was an increase in GLUT-1 gene expression in horse laminae affected by laminitis, strongly suggestive of ischemic conditions in equine laminitis.

**Benefits to/Impact on the Equine Industry:**
It is estimated that 15% of horses in the United States become afflicted with laminitis over their lifetime. Seventy-five percent of these horses do not return to athletic soundness or are ultimately euthanized due to severe pain associated with laminar separation. Understanding the events that take place in the pathogenesis of laminitis may help in stopping the progression of the disease.

**Take Home Message:**
Results of this study suggest a possible link between the ischemic/vascular and the metabolic/enzymatic theories of equine laminitis. Further research is needed to determine the extent of glucose deprivation and its connection with laminar basement membrane separation.

**Acknowledgments:**
This study was supported by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

**Year Completed:** 2005

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**Regulation of the Equine IL-8 Gene in Normal Horses Compared**
to Horses Acutely Affected by Laminitis

Authors/Investigators:
Jeffrey P. Cardinale; Ashley M. Stokes, DVM, PhD; Sharon R. Chirgwin, PhD; and Rustin M. Moore, DVM, PhD, ACVS.

Description of the Problem:
Laminitis is a common disease of horses that initiates the weakening of the soft tissues at the distal phalanx (P3) and hoof wall junctures. If allowed to persist, tissue separation, bone rotation and lameness can occur, frequently with a very low recovery rate. Currently, the pathophysiology of the disease is not completely understood. Local hemodynamic changes seem to play a primary role in the pathogenesis of this disease; however, inflammation has also been suspected as a player. Interleukin-8 (IL-8), an autocrine chemokine, plays a role in normal inflammation by attracting neutrophils to the site of inflammation. However, under high concentration levels of IL-8 and within the compressed area inside the hoof wall, the process continues to compound itself, resulting in increased swelling (with no room for the swelling to occur) causing blood vessel compression and depleted nutrient delivery to the cells. Due to stresses in the lamina connecting the P3 to the inner hoof wall, death and separation occurs quickly, resulting in laminitis. Using IL-8 as a marker gene of inflammation allows for verification of the role that inflammation plays in equine laminitis.

Study Objective:
The objective of this study was 1) to measure the gene expression differences of IL-8 in horses with laminitis compared to horses without laminitis; 2) to measure differences between horses with naturally acquired laminitis and horses experimentally induced with laminitis; and 3) to determine the role, if any, that inflammation plays in the pathophysiology of laminitis.

Approach:
Seventy samples from 54 horses were used for this study. Thirty-one were clinical cases, providing a broad set of data points, and the rest were from experimental groups, all presenting with laminitis to some degree. The experimental animals fell into two groups: those given Black Walnut Extract (BWE) and sacrificed after first presenting laminitis (titled BWE-9), and those sacrificed 72 hours post-BWE administration. Part of the 72 hour group (n=12) were given an endothelin receptor antagonist (n=5) to study the effect of this drug on laminar perfusion, and all were given oral phenylbutazone for the inflammation. The samples were homogenized and washed to yield total RNA, from which, following total RNA concentration measurements, cDNA was constructed. Using the cDNA and the already available Equus caballus IL-8 primer and probe sequences, the samples were run through RT-PCR in duplicates and along with â-Glucuronidase (the housekeeping gene) and the final levels were compared to the negative controls for statistical analysis.

Accomplishments/Results/Conclusions:
Comparisons across all groups showed a 25 fold difference in IL-8 levels between laminitis positive horses compared to laminitis negative horses. The clinical horses presenting chronic laminitis had levels comparable to the negative controls, but the acute animals showed the 25 fold increase from normal (p<0.0001). The BWE-9 group showed a 70 fold difference from the 72-hour group (p<0.0065), suggesting the possible correlation between the use of oral phenylbutazone and time with significant diminishing effects on IL-8 and overall inflammation. The Obel grade system, used to determine the stage of laminitis, also showed a significant correlation to the IL-8 levels, giving
credence to the evaluation system in connection to laminitis. IL-8 levels increased as the severity of laminitis increased, but showed significant drops as the duration increased and after oral phenylbutazone was administered to the horses. Overall, the study shows the intrinsic role inflammation plays in the development of acute laminitis, and the decreasing role of inflammation as the condition persists over time.

Benefits to/Impact on the Equine Industry:
Estimated annual monetary loss due to laminitis hovers greater than 13 million, due to veterinary visits, treatment and horse loss. However, this does not cover the pain endured by the horse or the families involved. With a greater than 15% occurrence in all breeds, no horse is exempt, thus it is imperative to establish a better understanding the pathogenesis of laminitis and to develop better therapeutic means to treat this crippling disease.

Take Home Message:
These findings suggest that inflammation does indeed play a major role in the progression of laminitis. Further, if caught early, treatments using anti-inflammatory drugs, including oral phenylbutazone, can help to curb, if not cure, the severity of the disease.

Acknowledgements:
This study was made possible by the Louisiana Biomedical Research Network

Year Completed: 2005

Use of a Digital Extracorporeal System to Study Laminitis in Horses

Authors/Investigators:
Aloisio C. D. Bueno, MV, MS; Rustin M. Moore, DVM, PhD, DACVS; Susan C. Eades, DVM, PhD, DACVIM; Jill R. Johnson, DVM, MS, DACVIM, DABVP; Ashley M. Stokes, DVM, PhD

Description of the Problem:
Equine laminitis is a severe disease involving the soft tissues of the foot. The pathophysiology of laminitis is poorly understood; therefore, methods to prevent and treat the disease are usually ineffective. Nonetheless, there is scientific evidence that ischemia and inflammation are involved. The purpose of this study was to develop an in vitro extracorporeal model of sustaining the isolated digit in a physiologic condition that could be used to study the pathophysiology of acute laminitis.

Study Purpose/Objectives:
We hypothesized that we could maintain the disarticulated equine digit physiologically for six hours with the extracorporeal perfusion system (MOX-100®), using heparinized autogenous whole blood as the perfusate. The objectives were 1) to monitor and establish guidelines for digital blood flow, digital arterial and venous pressures and the digital vascular resistance throughout a six-hour period of digital perfusion; 2) to measure & monitor metabolic, biochemical and hematological variables, as indicators of tissue viability; and 3) to evaluate microscopic alterations, including cell morphology and migration, and structural alterations that may occur during the perfusion period to assess laminar integrity.

Approach:
Adult horses (n=16) determined to be free of digital pathology were anesthetized and their feet disarticulated at the fetlock joint. Nine liters of whole blood were collected into heparinized sterile bags. Blood (750 mls) was used to prime the MOX-100®, and the remainder was stored and used to replace blood in the reservoir as needed. The selected digit was prepared for disarticulation, sodium heparin was administered, and the palmar digital vessels were cannulated. Perfusion begun immediately after disarticulation and the horse was euthanized. Arterial blood pressure was gradually increased to normal range (90-100 mm/Hg), venous blood pressure was maintained between 0-5 mm/Hg and venous flow was measured by timed collection. The digit was perfused with blood that was delivered from the arterial reservoir to the debubbler via pulsatile pump. The venous perfusate returned to the oxygenator to be oxygenated with a mixture of compressed air (1 L/ min) and CO₂ (90 ml/ min). Blood was collected from the arterial and venous ports of the circuit every 30 minutes for measuring blood gases, lactate, electrolytes, glucose, BUN, creatinine, and every hour for PCV,
plasma protein and WBC. The controlled flow of air and CO₂ into the oxygenator maintained the pH and PO2 within physiologic range. Abnormalities were corrected by adding glucose, electrolytes, bicarbonate, replacing perfusate with blood, altering CO₂ flow rate and/or altering perfusion parameters.

Accomplishments/Results/Conclusions:
We have modified and improved the perfusion system for the study needs. We initially used a membrane oxygenator with 100% O₂ and CO₂; however it was insufficient to deliver/maintain physiologic O₂ levels. We then began using a bubble oxygenator with 100% O₂. Because of the surface area of the bubble oxygenator, using 100% O₂ caused a hyperoxic state. The hyper-oxygenation problem was then solved with the use of compressed air and CO₂, which maintained the O₂ and pH within physiologic range. Moreover, we have added an in-line filter to prevent thromboemboli from occluding the microvasculature. The filter also helps to prevent microaggregates from entering the bubble oxygenator avoiding increases in resistance due to obstruction of its microfibers. The majority of the biochemical, and hemodynamic variables were not significantly different from the baseline throughout the study. The WBC was decreased at the baseline but gradually increased even though it remained below the normal values, indicating leukocyte adhesion/activation to the plastic materials and/or turbulent flow generate by the extracorporeal circuit, that has been reported by other investigators. Lactate gradually increased throughout the study (2.6-fold), suggesting tissue hypoperfusion. Additionally, venous blood flow gradually decreased and the arterial pressure gradually increased suggesting increased vascular resistance. The occurrence of these abnormalities is likely due to blood cellular activation and release of vasoactive mediators and/or erythrocyte settling. Glucose was consistently added to the perfusate, indicating tissue metabolism of glucose. Furthermore, blood had to be replaced in the reservoir owing to leakage of the small vessels on the disarticulated surface of the digit (sharp dissection, heparin use). Light microscopy revealed no visible differences between heparinized whole blood perfused and control digits. Overall, this extracorporeal circuit maintained the disarticulated digit well preserved for 6 hrs. For the near future we are planning to use a leukocyte depleting filter and a perfluorocarbon solution to test the possibility of preventing the unwanted side effects of this extracorporeal circuit.

Benefits to/Impact on the Equine Industry:
This model should lead to cost-effective and objective assessment for study of the role of ischemia, inflammation or toxic/metabolic factors in the pathophysiologic cascade and for discovery, development and safety/efficacy testing of potential therapeutic and preventative methods involved in the disease.

Take Home Message:
Laminitis (founder) is a painful, debilitating disease involving the soft tissues of the foot that afflict approximately 15% of horses in the US. The alterations that occur in the feet of affected horses are poorly understood, therefore, methods to prevent and treat the disease are often ineffective. Therefore, this study may help to elucidate the role of ischemia/inflammation in foundered horses.

Acknowledgements:
Funding was provided by the Louisiana State University Equine Health Studies Program (EHSP). The authors thank Dr. Britta Leise, Dr. Lee Ann Fugler, Frank Garza, Catherine Koch, and Mike Keowen for technical assistance.

Year Completed: 2004

Published Manuscripts/Abstracts:
Musculoskeletal

Ultrasonographic measurement of medial femoral condylar cartilage thickness adjacent to the medial meniscus in 60 racing thoroughbreds

Authors/Investigators:
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Description of the Problem:
Lameness evaluation of the equine hind limb can be a challenging task for the clinician, especially if the lesion involves the soft tissues of the stifle. The anatomy of the horse and the design of more sophisticated diagnostic imaging techniques intended for human medicine, such as CT and MRI, currently render these diagnostic tools useless for imaging the equine stifle. Hence, after localizing the source of lameness to the stifle by use of radiography, scintigraphy and intra-articular anesthesia, the clinician relies largely on ultrasonography to further evaluate soft tissue pathology. The medial aspect of the stifle is most commonly associated with soft tissue injuries, such as medial collateral ligament desmitis, meniscal injuries, as well as femorotibial joint effusion and synovitis. However, little is known about the variation of the medial femoral condylar cartilage thickness (MFCCT), which may indicate acute or chronic inflammatory and osteoarthritic changes in the joint in response to exercise or soft tissue trauma.

Study Objective:
The purpose of this study was to establish a reference data base for the cartilage thickness of the MFC adjacent to the medial meniscus in the standing, weight-bearing horse. The study objectives were (1) to assess the repeatability of ultrasonographic measurements of MFCCT adjacent to the medial meniscus in the weight-bearing horse, (2) to assess correlation of ultrasonographic MFCCT measurements with those of gross specimens and (3) to establish a database of ultrasonographic MFCCT measurements in a population of adult Thoroughbreds.

Approach:

Experiment 1 (US Variation): Repeat scans (n=3) and measurements of each scan (n=3) of the MFCCT adjacent to the medial meniscus were evaluated in 10 standing horses by two authors using a 7.5 MHz linear ultrasound transducer (Model 10L5, Terason™) connected to a computer to assess repeatability of the measurement and variation due to ultrasound (US) technique and measurement of the image. Experiment 2 (US Comparison vs. Gross Specimen): Caliper measurements of post-mortem gross cartilage specimens (n=6/joint) (Figure 3) from sixteen stifle joints from eight horses were used to determine the actual MFCCT and compared with ante-mortem ultrasonographic measurements (n=3 repeat measurements) using a 7.5 MHz linear ultrasound transducer (Model UST-5524-7.5, Aloka, Inc.) and an Aloka machine (SSD-900V) with a 5% display accuracy. Experiment 3 (Population Study): The 7.5 MHz linear ultrasound transducer (Model 10L5, Terason™) was used for ultrasonographic assessment of the MFC cartilage thickness on the medial aspect of both stifles (n=1 measurement/stifle) in a population of 60 Thoroughbred racehorses to establish the mean and standard deviation of the MFCCT to serve as a reference data base.

Accomplishments/Results/Conclusions:
Ultrasound measurements (Experiment 1 – US Variation) of two authors showed a strong correlation (R = 0.82) and the overall average coefficient of variation was 14.2 ± 5.4. Caliper measurements of gross sections (Experiment 2 – Gross Specimen vs. US Comparison) showed no correlation (R = 0.08) to the ultrasonographic measurements, which underestimated actual MFCCT by about 42%, precluding ultrasonographic predictions of actual MFCCT in the area.

Figure 1: Medial View of the stifle showing the ultrasound probe position (red oval) for imaging and measurement of MFCCT.
adjacent to the medial meniscus. The 60 Thoroughbreds used for this study (Experiment 3 – Population Study) were distributed in the following age groups: three-year-olds (n=26), four-year-olds (n=20), five-year-olds (n=10), six-year-olds (n=4). The mean ± standard deviation of MFCCT of all 120 stifle joints (60 horses) was 0.61±0.02 mm whereby MFCCT on the right side appeared to be thinner (P = 0.0015) compared to the left side, which was only apparent in horses > four years of age when individual ages were considered. The difference may be attributable to the left-handed racing direction on US race tracks. Comparative studies of lateral MFCCT and with race horses from other countries where races are run in both directions are required to attribute cartilage thickness and degeneration to racing direction. Further work is also required to assess the relationship between ultrasonographic cartilage assessment of the MFC and arthritic disease of the equine medial femorotibial joint.

Benefits to/Impact on the Equine Industry:
The medial aspect of the stifle joint and its associated soft tissue structures is the most common site of stifle injuries in the horse. Degenerative joint disease of the stifle due to injuries and the wear and tear during the career of an equine athlete affect the stifle joint cartilage composition and thickness. This study has established a reference data base of medial femoral condylar (MFC) cartilage thickness from 120 medial femorotibial joints of 60 young adult Thoroughbreds, which can be used to assess gross abnormalities of the MFC cartilage.

Take Home Message:
The results of this study show that ultrasonography is capable of repeatable MFC cartilage measurements but does not seem to correlate well with physical measurements of gross cartilage specimens. The data show that the mean ± standard deviation of MFC cartilage thickness (120 medial femorotibial joints) was 0.61±0.02 mm. Older Thoroughbreds (> four years of age) appear to have a thinner MFC cartilage thickness adjacent to the meniscus in the right stifle, which may be attributable to the left-handed racing direction on US race tracks.

Acknowledgments:
This study was supported by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine. We would like to extend our deepest gratitude to the trainers Alicia Cohn, Allen Milligan, Malcom Pierce, Jeff Thornberry and Bertrand at the former Fair Grounds Race Course in New Orleans for availing their horses, time and personnel for this study. We would also like to thank Dr. Vito Vinci and the technical staff of the LSU Equine Hospital for their help in facilitating this study.

Year Completed: 2005

Published Manuscripts/Abstracts:
Cell growth characteristics and differentiation frequency of adherent equine bone marrow–derived mesenchymal stromal cells: Adipogenic and osteogenic capacity

Authors/Investigators:
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Description of the Problem:
The stromal composition of postnatal bone marrow contains mesenchymal cells, which are now commonly referred to as adult stem cells. These cells are defined based on their multipotent characteristics and capability of *in vitro* differentiation along a number of lineage pathways. The majority of the published work documenting mesenchymal stromal cell differentiation potential, however, has been performed in species other than the horse, with the exception of the ability of equine mesenchymal cells to differentiate into chondrocytes. More recently cell-based approaches to tendon and ligament injury repair have employed supra-physiologic numbers of MSCs. However, the growth characteristics of equine MSCs as a basis for further tissue engineering approaches have not yet been established.

Study Objective:
The objectives of this study were to document and quantify the growth and differentiation characteristics of equine bone marrow derived MSCs using limit dilution assays for fibroblasts, adipocytes and osteoblasts. This data will assist future efforts to standardize the isolation, expansion, and transplantation of equine MSCs in clinical practice.

Approach:
Bone marrow (BM) was collected from the sternum of five young horses and three pony foals for the isolation and culture of primary bone marrow-derived mesenchymal stromal cells (MSCs). Cell doubling times and numbers for each passage were calculated. Limit dilution assays were used for MSCs of the adult horses to quantify colony forming units for fibroblasts (CFU-F), and cells capable of differentiation into adipocytes (CFU-Ad) and osteoblasts (CFU-Ob) from primary MSCs and those of passage 2 (P2) and 4 (P4). Day nine CFU-F cells were fixed (10% formalin) and stained with toluidine blue. CFU-Ad cells were exposed to an adipogenic induction medium for three days and maintained in adipocyte maintenance medium until day six when the cells were fixed with 10% formalin and stained for neutral lipid accumulation with Oil-Red-O. For CFU-Ob assays, cells were induced and maintained in osteogenic medium then fixed with 70% ethanol and stained with Alizarin Red.

Accomplishments/Results/Conclusions:
The cell doubling time (Figure 1-A) for primary cells was calculated at 5 ± 1.6 days/cell doubling, which was significantly (P < 0.0001) longer than the average DT of the subsequent passages (1.4 ± 0.26 days/CD). Cell differentiation of MSCs into bone and fat cells was consistently observed in all cultures under the appropriate conditions. Adipogenesis (Figure 2-B) experiments showed morphological changes of differentiating MSCs and obvious fat droplet formation within one to two days. Osteogenesis of the equine MSCs, characterized by bony nodule formation (Figure 2-C), occurred on average within 7.4 ± 2.6 days after induction. Limit dilution analysis revealed that approximately 0.026% of the total primary nucleated bone marrow (tnBM) cells appeared to be MSCs with a fibroblastic phenotype (Figure 2-A), and just as many of these tnBM cells differentiated into bone and fat cells. MSC
Musculoskeletal density was significantly enriched during subsequent passages.

Benefits to/Impact on the Equine Industry:
This work documents the frequency, growth characteristics and the potential of equine bone marrow-derived MSCs to differentiate into bone and fat cells. It was established that approximately 0.026% of bone marrow nucleated cells have the fibroblastic morphology of MSCs and most of these cells can be induced to differentiate into bone and fat cells. Therefore, from the average yield of 64 million nucleated cells per 10 ml of bone marrow aspirate, it is feasible to obtain 1.7 x 10^4 primary MSCs, which could be expanded to approximately 250 million progenitor cells during 23 days in culture, based on the established cell doubling times. Limited data are currently available concerning the number of cells required for the repair of tendon defects and strain injuries. However, a recently published mathematical model calculated that 70 million bone cells would be required to produce a cubic centimeter of bone.

Take Home Message:
Primary equine MSCs occur at approximately 0.026% of nucleated bone marrow cells and after an initial lag period can expand at a rate of 1.4 ± 0.26 days/cell doubling. Cell culture periods of a few weeks will produce sufficient cells for tissue engineering purposes. Primary and passaged MSCs are capable of differentiation into bone and fat cells.

Acknowledgments:
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Year Completed: 2005

Published Manuscripts/Abstracts:

An in vitro biomechanical comparison of a prototype equine metacarpal dynamic compression plate fixation with double dynamic compression plate fixation of osteotomized equine third metacarpal bones

Authors/Investigators:
Gary A. Sod, DVM, MA, PhD; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP

Description of the Problem:
Diaphyseal fractures of the third metacarpal (MC3) and metatarsal (MT3) bones constitute as many as 33% of all complete equine long bone fractures. Third metacarpal bone fractures in the horse challenge current treatment modalities. These fractures are often open due to overlying skin tension and lack of underlying soft tissue. In addition, MC3 fractures are typically sustained as the result of high energy injuries, and may be comminuted. Double plate fixation using two dynamic compression plates (DCP) placed 90° to each other currently represents the most stable treatment method. The goal of internal fixation is to have an athletically sound horse, yet only a 53% prognosis for return to athletic competition was reported for plate fixation of axially stable fractures in a recent retrospective study. Potential for return to athletic use is enhanced by fracture stability, to maintain a functional limb during healing. Implants that have an increased cyclic fatigue life are necessary. However, practical limits to increasing the size of fixation devices do exist. Larger plates occupy more space, which increases the difficulty of skin closure. Merely increasing plate or screw size is not entirely feasible. Equine specific orthopedic implants are necessary to facilitate equine fracture repair. To this end, the equine third metacarpal dynamic compression plate (EM-DCP) was specifically designed by the authors for equine metacarpal/metatarsal diaphyseal fractures (Fig. 1).

Study Objective:
The objective of this in vitro study was to compare the monotonic biomechanical properties of an EM-DCP fixation with a double broad DCP fixation to repair osteotomized equine third metacarpal (MC3) bones. It was hypothesized that the EM-DCP-MC3 construct would provide significantly better stability under both static loading (palmarodorsal four-point bending and torsion) and cyclic fatigue testing (palmarodorsal four-point bending) than the double broad DCP-MC3 construct.

Approach:
Twelve pairs of equine third metacarpal bones were collected from adult Thoroughbred horses. The right and left metacarpi were randomly chosen to receive the EM-DCP or the double broad DCP. An EM-DCP (10 hole, 4.5 mm) was applied to dorsal surface of one MC3 bone from each pair using standard AO/ASIF techniques (Fig. 2). A broad DCP (10 hole, 4.5 mm) was applied to the dorsal surface and a broad DCP (8 hole, 4.5 mm) was applied to the lateral surface of the contralateral bone from each pair (Fig. 3). All MC3 bones had mid-diaphyseal osteotomies. Four pairs of constructs were tested in single cycle to failure under palmarodorsal four-point bending. A load applied over a single cycle under displacement control at a constant rate of 15 mm/sec to failure using servo-hydraulic biaxial material testing system. Four pairs of constructs were tested for cyclic fatigue under palmarodorsal four-point bending. A cyclic load of 0 to 7.5 kN was applied at a rate of 6.0Hz. The number of cycles to failure was recorded. Four pairs of constructs were tested in single cycle to failure under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean values for each fixation method were compared using a paired t-test within each group with a statistical significance of P < 0.05.
Accomplishments/Results/Conclusions:
In single cycle to failure, four-point bending, the mean values for the yield bending moment and the failure bending moment were 3.0 times and 3.8 times greater, respectively, for the EM-DCP-MC3 composite compared to the double broad DCP-MC3 composite. In cyclic fatigue, four point bending, the mean number of cycles to failure was 4.5 times greater for the EM-DCP-MC3 composite compared to the double broad DCP-MC3 composite. In torsion, the mean stiffness was 8.4 times greater for the EM-DCP-MC3 composite compared to the double broad DCP-MC3 composite.

Both the EM-DCP and the double broad DCP fixations had greater failure bending moment in single cycle to failure under palmarodorsal four-point bending than that of intact MC3 diaphysis. Thus the EM-DCP-MC3 and the double broad DCP-MC3 constructs were stronger than intact bone in bending. The mean bending composite rigidity of the EM-DCP-MC3 construct exceeds the bending stiffness of intact equine MC3 bone while the mean bending composite rigidity of the double broad DCP-MC3 construct achieved 62% of the bending stiffness of intact equine MC3 bone. When both walking and weight shifting are considered, the mean activity of the forelimb over a 24-hour period has been reported as 190 + 184 steps/hour. At this loading rate, the EM-DCP should not undergo cyclic fatigue failure for approximately 4.7 months, whereas for the double broad DCP this would be approximately 32 days. The EM-DCP-MC3 construct exceeds the torsional stiffness of intact equine MC3 bone while the double broad DCP-MC3 construct achieved 44% of the torsional stiffness of intact equine MC3 bone.

While the EM-DCP construct has significantly greater rigidity than the double broad DCP construct, the 10-hole EM-DCP has 38.3% less surface area than double (10-hole and 8-hole) broad DCP. As such the EM-DCP had less plate to bone contact and provided less overall area of stress protection compared to that of the double broad DCP.

Benefits to/Impact on the Equine Industry:
Osteotomized MC3 bones with EM-DCP fixation were considerably stiffer in palmarodorsal bending and torsional composite rigidity compared with osteotomized MC3 bones with double broad DCP fixation. This demonstrates that the EM-DCP provided a more rigid overall construct and repair of osteotomized MC3 bones when compared with the double broad DCP. The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing. Fracture stability is achieved in part by a plate-MC3 construct that has greater rigidity.

Take Home Message:
The equine specific EM-DCP is biomechanically superior to the standard method of double broad DCPs for MC3 diaphyseal fracture fixation.

Year Completed: 2004

Published Manuscripts/Abstracts:


An *in vitro* biomechanical comparison between prototype tapered shaft cortical bone screws and AO cortical bone screws for an equine metacarpal dynamic compression plate fixation of osteotomized equine third metacarpal bones

**Authors/Investigators:**
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**Description of the Problem:**
Diaphyseal fractures of the third metacarpal (MC3) and metatarsal (MT3) bones constitute as many as 33% of all complete equine long bone fractures. These fractures challenge current treatment modalities. Double plate fixation using two dynamic compression plates (DCP) placed 90° to each other currently represents the most stable treatment method. The goal of internal fixation is to have an athletically sound horse, yet only a 53% prognosis for return to athletic competition was reported for plate fixation of axially stable fractures in a recent retrospective study. Potential for return to athletic use is enhanced by fracture stability, to maintain a functional limb during healing. Implants that have an increased cyclic fatigue life are necessary. Equine specific orthopedic implants are necessary to facilitate equine fracture repair. To this end, the equine third metacarpal dynamic compression plate (EM-DCP) was specifically designed by the authors for equine metacarpal/metatarsal diaphyseal fractures.

In an *in vitro* study the biomechanical properties of an EM-DCP fixation and a double broad DCP fixation to repair osteotomized equine third metacarpal (MC3) bones were compared. It was established that the EM-DCP-MC3 construct provided significantly better stability under both static loading (palmarodorsal four-point bending and torsional) and cyclic fatigue testing (palmarodorsal four-point bending) than the double broad DCP-MC3 construct. The mode of failure of the EM-DCP-MC3 constructs in the majority of the trials, in both single cycle to failure and cyclic fatigue testing, was due to screw breakage at the shaft/head junction through one of the four holes adjacent to the osteotomy. The weakest component of the EM-DCP-MC3 construct was the 5.5 mm AO cortical screw in the holes of the EM-DCP adjacent to the osteotomy. A prototype 5.5 mm tapered shaft cortical screw (TSS) was developed to overcome the stress riser created at the head/shaft interface (Fig. 1).

**Study Objective:**
The objective of this *in vitro* study was to compare the biomechanical properties between the prototype 5.5 mm TSS and the 5.5 mm AO cortical screw for an EM-DCP fixation to repair osteotomized equine third metacarpal (MC3) bones. It is hypothesized that the EM-DCP-MC3 construct with the prototype 5.5 mm TSS in the holes adjacent to the osteotomy would provide significantly better stability under static loading (palmarodorsal four-point bending and torsional) and in cyclic fatigue testing under palmarodorsal four-point bending than the EM-DCP-MC3 construct with the 5.5 mm AO cortical screw in the holes adjacent to the osteotomy.

**Approach:**
Twelve pairs of equine third metacarpal bones were collected from adult Thoroughbred horses. Right and left MC3 were randomly selected for EM-DCP with the prototype 5.5 mm tapered shaft cortical bones screws or 5.5 mm AO cortical screws in the holes adjacent to the osteotomy. The EM-DCP (10 hole, 4.5 mm) was...
applied to dorsal surface of the MC3 bone from each pair using standard AO/ASIF techniques. All MC3 bones had mid-diaphyseal osteotomies. Four pairs of constructs were tested in single cycle to failure under palmarodorsal four-point bending. A load applied over a single cycle under displacement control at a constant rate of 15 mm/sec to failure using servo-hydraulic biaxial material testing system. Four pairs of constructs were tested for cyclic fatigue under palmarodorsal four-point bending. A cyclic load of 0 to 7.5 kN was applied at a rate of 6.0Hz. The number of cycles to failure was recorded. Four pairs of constructs were tested in single cycle to failure under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean values for each fixation method were compared using a paired t-test within each group with a statistical significance of P < 0.05.

**Accomplishments/Results/Conclusions:**

In single cycle to failure, four-point bending, the mean values for the yield bending moment and the failure bending moment were 2.8 times and 3.1 times greater, respectively, for the TSS-EM-DCP-MC3 composite compared to the AO-EM-DCP-MC3 composite. In cyclic fatigue, four-point bending, the mean number of cycles to failure was 3.1 times greater for the TSS-EM-DCP-MC3 composite compared to the AO-EMDCP-MC3 composite. In torsion, the mean stiffness was 2.5 times greater for the TSS-EM-DCP-MC3 composite compared to the AO-EMDCP-MC3 composite.

The TSS-EM-DCP offers increased stability in cyclic fatigue testing, resulting in a significant increase in the number of cycles to failure. The tapered shaft of the 5.5 mm TSS and the corresponding tapered hole of the oval DCP and round holes adjacent to the proximal and distal aspects of the osteotomy site in the EM-DCP likely reduced the screw wobble and likely reduced micromovements between the EM-DCP and the bone during cycling (Fig. 2). Increased micromovements between the internal fixation and the bone leads to increased loads on the plate or screws resulting in high-strain cyclic fatigue of the implant. Both TSS and AO constructs exceeded the torsional rigidity of intact equine MC3 bone, the result of load sharing between the plate, screws and bone.

**Benefits to/Impact on the Equine Industry:**

Osteotomized MC3 bones with TSS-EM-DCP fixation were considerably stiffer in palmarodorsal bending and torsional composite rigidity compared with the AO-EM-DCP fixation. This demonstrates that the 5.5 mm tapered shaft cortical screw is biomechanically superior to the AO 5.5 mm cortical screw. The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing. Fracture stability is achieved in part by a plate-MC3 construct that has greater rigidity.

**Take Home Message:**

The equine specific 5.5 mm tapered shaft cortical screw is biomechanically superior to the standard AO 5.5 mm cortical screw. This combination with the equine specific EM-DCP offers superior stability for MC3 diaphyseal fracture fixation.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


An in vitro biomechanical comparison between prototype tapered shaft cortical bone screws and AO cortical bone screws for a limited contact dynamic compression plate fixation of osteotomized equine third metacarpal bones

Authors/Investigators:
Gary A. Sod, DVM, MA, PhD; Jeremy D. Hubert, BVSc, MRCVS, DACVS; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP

Description of the Problem:
The concept of biological plating has led to the development of the limited-contact dynamic compression plate (LCD-CP) (Fig. 1). The goal of the LC-DCP is to reduce trauma to bone, preserve the blood supply, and to avoid producing stress risers at implant removal. The evenly distributed undercuts of the LC-DCP reduces the contact between the bone and plate to a minimum. Compressive or bending forces in the plate and shear or bending forces in the screws may be magnified if there is any micromovement between the plate and bone. An in vitro biomechanical comparison of LC-DCP with dynamic compression plate (DCP) fixation of osteotomized equine third metacarpal (MC3) bones was performed. The conclusion of the study was that the LC-DCP offered increased stability in static overload testing (palmarodorsal 4-point bending and torsion); however, the LC-DCP offered significantly less stability (46% of that of the DCP) in cyclic fatigue testing (palmarodorsal 4-point bending). Screw breakage at the head/shaft interface was often the mode of implant failure. A 5.5 mm tapered shaft cortical screw (TSS) was developed to overcome the stress riser created at the head/shaft interface (Fig. 2). The tapered shaft portion of the screw has area moment of inertia at the head/shaft junction that is 6.4 times greater than the corresponding area moment of inertia of the 5.5 mm AO cortical screw.

Study Objective:
The objective of this study was to compare the biomechanical properties between the 5.5 mm TSS and 5.5 mm AO cortical screw for an LC-DCP fixation of osteotomized equine MC3 bones. It was hypothesized that osteotomized equine LC-DCP-MC3 constructs with 5.5 mm TSS in the proximal and distal holes adjacent to the osteotomy would provide significantly better stability under static loading (palmarodorsal 4-point bending and torsion) and cyclic fatigue testing (palmarodorsal 4-point bending) than the LC-DCP-MC3 construct with the 5.5 mm AO cortical screws in the proximal and distal holes adjacent to the osteotomy.

Approach:
Twelve pairs of equine MC3 bones were collected from adult Thoroughbred horses. A broad LC-DCP (8 hole, 4.5 mm) was applied to dorsal surface of a pair of MC3 bones. The LC-DCP was secured in place, on one MC3 bone chosen at random from each pair, with two 5.5 mm TSS (holes immediately adjacent to the osteotomies), two 5.5 mm AO cortical screws (proximal and distal holes in the plate), and four 4.5 mm AO cortical screws in the remaining holes using standard AO/ASIF techniques. The LC-DCP was secured in place on the contralateral MC3 bone from each pair, with four 5.5 mm AO (proximal and distal holes in the plate and the holes immediately adjacent to the osteotomies) and four 4.5 mm AO cortical screws in the remaining holes using standard AO/ASIF techniques. In all constructs, the screws were loosened, mid-diaphyseal osteotomies performed, and the screws tightened uniformly to a final torque of 4.2 N-m. Four matching pairs of constructs were tested in palmarodorsal 4-point bending in single cycle to failure. A load was applied at a constant displacement rate of 15 mm/sec to failure using servo-hydraulic material testing system. Four pairs of constructs were tested for cyclic fatigue under palmarodorsal 4-point bending. A cyclic load of 0 to 7.5 kN was applied at a rate of 2.0Hz. Four pairs of constructs were tested under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean test variable values for each method were compared using a paired t-test within each group with a statistical significance of P < 0.05.

Accomplishments/Results/Conclusions:
In single cycle to failure, four-point bending, the mean values for the yield bending moment and the failure bending moment were 3.1 times and 3.4 times greater, respectively, for the TSS-LC-DCP-MC3 composite compared to the AO-LC-DCP-MC3 composite. In cyclic fatigue, four-point bending, the mean number of cycles to failure was 2.9 times greater for the TSS-LC-DCP-MC3 composite compared to the AO-LC-DCP-MC3 composite. In torsion, the mean stiffness was 1.8 times greater for the TSS-LC-DCP-MC3 composite compared to the AO-LC-DCP-MC3 composite.

Fig 1. Prototype 5.5 mm tapered shaft cortical screw and 5.5 mm AO cortical screw.
The TSS-LC-DCP-MC3 construct had greater failure bending moment in single cycle to failure than that of intact MC3 diaphysis. The values of yield bending moment for both the TSS-LC-DCP and AO-LC-DCP fixations in palmarodorsal 4-point bending exceeded the greatest physiologic values of bending moments of MC3, such as recovery from anesthesia and trotting. The number of cycles to failure under palmarodorsal 4-point bending for the TSS-LC-DCP fixation was significantly greater (2.9 fold increase) compared with the AO-LC-DCP fixation. The mean bending composite rigidity of the TSS-LC-DCP-MC3 construct exceeded the bending stiffness of intact equine MC3 bone while the mean bending composite rigidity of AO-LC-DCP-MC3 construct achieved 39% of the bending stiffness of intact equine MC3 bone. The TSS-LC-DCP-MC3 and AO-LC-DCP-MC3 constructs achieved 74% and 40%, respectively, of the torsional stiffness of intact equine MC3 bone.

**Benefits to/Impact on the Equine Industry:**

Osteotomized MC3 bones with TSS-LC-DCP fixation were considerably stiffer in palmarodorsal bending and torsional composite rigidity compared with the AO-LC-DCP fixation. This demonstrates that the 5.5 mm tapered shaft cortical screw is biomechanically superior to the AO 5.5 mm cortical screw when used to secure an LC-DCP. This combination may be used in situations where a biological plate is desired. The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing.

**Take Home Message:**

The equine specific 5.5 mm tapered shaft cortical screw is biomechanically superior to the standard AO 5.5 mm cortical screw even in the case of its use to secure a LC-DCP. However, the 5.5 mm tapered shaft cortical screw used in combination with the equine specific EM-DCP still offers superior stability for MC3 diaphyseal fracture fixation.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


**An in vitro biomechanical comparison of a prototype equine spoon-plate with an axial dynamic compression plate in conjunction with two abaxial transarticular lag screws for equine proximal interphalangeal joint arthrodesis**

**Authors/Investigators:**

Gary A. Sod, DVM, MA, PhD; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP

**Description of the Problem:**

Arthrodesis of the equine proximal interphalangeal (PIP) joint was developed for the treatment of debilitating osteoarthritis, though it has also been used for the treatment of proximal and middle phalangeal fractures, and subluxation or luxation of the PIP joint (Fig. 1). Arthrodesis of the PIP joint in the horse challenges current treatment modalities. Six techniques have been used for the arthrodesis of the equine PIP joint. These include the use of dynamic compression plates, a T-plate, a Y-plate, three lag screws placed in parallel fashion, three lag screws placed in converging fashion, and two
lag screws placed in cruciate fashion. A major complication leading to failure of the arthrodesis is instability often due to implant failure or loosening due to the demands placed on them by the high-load, low-motion joint. A 46% prognosis in the fore limb, and 83% prognosis in the hind limb, for the horse performing its intended or previous activity was reported in a recent retrospective study. Potential for return to athletic use is enhanced by stability of the fixation, to maintain a functional limb during healing. Implants that have an increased cyclic fatigue life are necessary. However, practical limits to increasing the size of fixation devices do exist. Larger plates occupy more space, which increases the difficulty of skin closure and might impinge on the extensor process of the distal phalanx (P3) and distal interphalangeal (DIP) joint capsule attachments. Merely increasing plate or screw size is not entirely feasible. To this end, the equine spoon-plate (ESP) was specifically designed by the authors for the arthrodesis of the equine PIP joint.

The ESP was an 8-hole plate, with five holes over the proximal phalanx (P1) and three holes over the middle phalanx (P2) (Fig. 2). The proximal shaft was similar to that of the broad dynamic compression plate (DCP) (4.5 mm) with a staggered arrangement of three oval DCP holes. The ESP widened and thickened as it approached the distal two holes over P1. The distal two holes over P1 provided for 10° of angulation of a 5.5 mm screw in the same transverse plane. As the ESP crossed the PIP joint a central hole provided for up to 40° of angulation of a 5.5 mm screw in the midsaggittal plane, so that a cortical bone screw entered the proximodorsal aspect of P2 and ended at the distopalmar aspect of P2. The distal two holes provided for 30° angulation of a 5.5 mm screw in the same transverse plane. The three holes over P2 form a triangle with no two screws lying in the same plane. The distal end of the ESP was concave to prevent impingement of the extensor process of P3 and DIP joint capsule attachment.

**Study Objective:**
The objective of this in vitro study was to compare the monotonic biomechanical properties of the ESP with an axial narrow dynamic compression plate in conjunction with two abaxial transarticular lag screws (Lag-DCP) for the equine PIP joint arthrodesis. It was hypothesized that the ESP construct would provide significantly better stability under both static loading (axial compression and torsion) and cyclic fatigue testing (axial compression) than the DCP construct.

**Approach:**
Fifteen forelimb pairs intact from the mid-humorus distally were collected from adult Thoroughbred horses. Full limb preparations were used to more closely simulate the in vivo mechanical loading environment. It was thought that it would be more clinically useful to test constructs with a forelimb model that maintained the influence of the soft tissue structures supporting the PIP joint. An ESP (8 hole, 5.5 mm) was applied to dorsal surface of P1-P2 of one forelimb chosen at random from each pair using standard AO/ASIF techniques. A three hole narrow DCP was applied to the dorsal surface of P1-P2 with three 5.5 mm cortical screws where the screw in the central hole was applied in lag fashion across the PIP joint, and two 5.5 mm cortical screws were applied in lag fashion across the PIP joint abaxial to the DCP of the contralateral forelimb from each pair. Five pairs of constructs were tested in single cycle to failure under axial compression. A load applied over a single cycle under displacement control at a constant rate of 15 mm/sec to failure using servo-hydraulic biaxial material testing system. Five pairs of constructs were tested for cyclic fatigue under axial compression. A cyclic load of 0 to 7.5 kN was applied at a rate of 6.0Hz. The number of cycles to failure was recorded. Five pairs of constructs were tested in single cycle to failure under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean values for each fixation method were compared using a paired t-test within each group with a statistical significance of P < 0.05.

**Accomplishments/Results/Conclusions:**
In single cycle to failure under axial compression, the mean values for the yield load and the failure load were 9.0 times and 9.2 times greater, respectively for the ESP compared to the Lag-DCP for the arthrodesis of the PIP joint. In cyclic fatigue under axial compression, the mean number of cycles to failure was 7.5 times greater for the ESP compared to the Lag-DCP for the arthrodesis of the PIP joint. In torsion, the mean stiffness was 18.8 times greater for the ESP compared to the Lag-DCP for the arthrodesis of the PIP joint.

In the single cycle to failure test, loading rates for axial compression were chosen to represent high-rate/high-energy...
forces that might occur during recovery from anesthesia. The largest compressive force on the distal aspect of the forelimb occurs on MC3 during recovery from anesthesia. Both the ESP and Lag-DCP fixation techniques had mean failure loads greater than the compressive force exerted on the distal aspect of the forelimb during recovery from anesthesia. In cyclic fatigue testing, under axial compression, the loading rates were chosen to represent loads placed on MC3 during walking. When both walking and weight shifting are considered, the mean activity of the forelimb over a 24-hour period has been reported as 190 ± 184 steps/hour. At this loading rate, the ESP fixation should not undergo cyclic fatigue failure for approximately 4.7 months, whereas for the Lag-DCP fixation this would be approximately 21 days. The PIP joint with ESP fixation was considerably stiffer under axial compression and torsion compared with PIP joint with Lag-DCP fixation. The possibility of return to athletic use is enhanced by PIP joint stability, which keeps the limb functional during healing. PIP joint stability is achieved in part by an implant-P1-P2 construct that has greater rigidity.

Our results support the hypothesis that the ESP fixation was superior to the Lag-DCP fixation in resisting the static overload forces and in resisting cyclic fatigue. The results of this in vitro study support the conclusion that the prototype ESP is biomechanically superior to Lag-DCP for the equine PIP joint arthrodesis.

**Benefits to/ Impact on the Equine Industry:**
The specific design of the ESP implant may facilitate equine pastern joint arthrodesis and may obviate the complications encountered during convalescence due to cyclic instability, as the ESP implant is stable and resistant to cyclic fatigue.

**Take Home Message:**
The results of this study support the hypothesis that the ESP is superior to the Lag-DCP construct with regard to resisting the biomechanical forces that are most likely to cause failure with the fixation types used for equine pastern joint arthrodesis.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


An in vitro biomechanical comparison of an axial dynamic compression plate in conjunction with two abaxial transarticular lag screws with 3 parallel lag screws for equine proximal interphalangeal joint arthrodesis

Authors/Investigators:
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Description of the Problem:
Arthrodesis of the equine proximal interphalangeal (PIP) joint was developed for the treatment of debilitating osteoarthritis, though it has also been used for the treatment of proximal and middle phalangeal fractures, and subluxation or luxation of the PIP joint (Fig. 1). Arthrodesis of the PIP joint in the horse challenges current treatment modalities. Two of the most common techniques are an axial dynamic compression plate in conjunction with two abaxial transarticular lag screws and three parallel lag screws. A major complication leading to failure of the arthrodesis is instability often due to implant failure or loosening due the demands placed on them by the high-load, low-motion joint. A 46% prognosis, in the fore limb, and 83% prognosis, in the hind limb, for the horse performing its intended or previous activity was reported in a recent retrospective study. Potential for return to athletic use is enhanced by stability of the fixation, to maintain a functional limb during healing.

Study Objective:
The objective of this in vitro study was to compare the monotonic biomechanical properties of the an axial narrow dynamic compression plate in conjunction with two abaxial transarticular lag screws (Lag-DCP) with three parallel lag screws (3PLS) for the equine PIP joint arthrodesis. It was hypothesized that the Lag-DCP construct would provide significantly better stability under both static loading (axial compression and torsion) and cyclic fatigue testing (axial compresion) than the 3PLS construct.

Approach:
Fifteen forelimb pairs intact from the mid-humorus distally were collected from adult Thoroughbred horses. Full limb preparations were used to more closely simulate the in vivo mechanical loading environment. It was thought that it would be more clinically useful to test constructs with a forelimb model that maintained the influence of the soft tissue structures supporting the PIP joint. A three hole narrow dynamic compression plate was applied to the dorsal surface of P1-P2 with three 5.5 mm cortical screws where the screw in the central hole was applied in lag fashion across the PIP joint, and two 5.5 mm cortical screws were applied in lag fashion across the PIP joint abaxial to the DCP of the of one forelimb chosen at random from each pair (Fig. 2). Three 5.5 mm cortical screws were applied in lag fashion parallel to each other across the PIP joint of the contralateral forelimb from each pair (Fig. 2). Five pairs of constructs were tested in single cycle to failure under axial compression. A load applied over a single cycle under displacement control at a constant rate of 15 mm/sec to failure using servo-hydraulic biaxial material testing system. Five pairs of constructs were tested for cyclic fatigue under axial compression. A cyclic load of 0 to 7.5 kN was applied at a rate of 6.0Hz. The number of cycles to failure was recorded. Five pairs of constructs were tested in single cycle to failure under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean values for each fixation method were compared using a paired t-test within each group with a statistical significance of P < 0.05.

Accomplishments/Results/Conclusions:
In single cycle to failure under axial compression, the mean values for the yield load and the failure load were 1.18 times and 1.22 times greater, respectively, for the Lag-DCP compared to the 3PLS for the arthrodesis of the PIP joint. In cyclic fatigue under axial compression, the mean number of cycles to failure was 1.34 times greater for the Lag-DCP compared to the 3PLS for the arthrodesis of the PIP joint. In torsion, the mean stiffness was 1.28 times greater for the Lag-DCP compared to the 3PLS for the arthrodesis of the PIP joint.

Fig 1. Radiograph depicting osteoarthritis of the equine pastern joint.
In the single cycle to failure test, loading rates for axial compression were chosen to represent high-rate/high-energy forces that might occur during recovery from anesthesia. The largest compressive force on the distal aspect of the forelimb occurs on MC3 during recovery from anesthesia. Both the Lag-DCP and 3PLS fixation techniques had mean failure loads greater than the compressive force exerted on the distal aspect of the forelimb during recovery from anesthesia. In cyclic fatigue testing, under axial compression, the loading rates were chosen to represent loads placed on MC3 during walking. When both walking and weight shifting are considered, the mean activity of the forelimb over a 24-hour period has been reported as 190 + 184 steps/hour. At this loading rate, the Lag-DCP fixation should not undergo cyclic fatigue failure for approximately 21 days, whereas for the 3PLS fixation this would be approximately 15 days. The PIP joint with Lag-DCP fixation was stiffer under axial compression and torsion compared with PIP joint with 3PLS fixation.

Our results support the hypothesis that the Lag-DCP fixation was superior to the 3PLS fixation in resisting the static overload forces and in resisting cyclic fatigue. The results of this in vitro study support the conclusion that the Lag-DCP is biomechanically superior to the 3PLS for the equine PIP joint arthrod. 

**Benefits to/ Impact on the Equine Industry:**
These results support further the conclusion of the previous study that the specific design of the equine spoon plate implant may facilitate equine pastern joint arthrodesis and may obviate the complications encountered during convalescence due to cyclic instability, as the ESP implant is stable and resistant to cyclic fatigue.

**Take Home Message:**
The results of this study further support the hypothesis that the equine spoon plate is superior to the two most commonly used methods, Lag-DCP and 3 PLS, with regard to resisting the biomechanical forces that are most likely to cause failure with the fixation types used for equine pastern joint arthrodesis.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**
Reproduction

**Determination of the time of day of spontaneous ovulation in the mare**

**Authors/Investigators:**
E.A. Bradecamp, DVM, DACT; Sara K. Lyle, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Dale L. Paccamonti, DVM, MS, DACT

**Description of the Problem:**
The ability to accurately predict and control ovulation in the mare is essential for efficient breeding management. Knowing the time of day most mares ovulate would simplify the use of frozen semen in the mare. Some studies found that more mares ovulate at night, while others refuted this idea. It is perceived among equine veterinarians that a majority of mares ovulate in the early morning hours after midnight. Previous studies have examined mares two to four times a day when attempting to determine the time of day a mare was most likely to ovulate.

**Study Objective:**
The purpose of this study was to examine mares every two hours to determine if there was a most prevalent time of ovulation. Our hypothesis was that a significantly greater number of mares would ovulate between 8 p.m. and 6 a.m. with the peak number of ovulations occurring between 10 p.m. and 2 a.m.

**Approach:**
A herd of 50 mares was examined by transrectal ultrasound twice weekly to identify mares that were in estrus. Mares that were determined to be in estrus (follicle ^ 30 mm. uterine edema, and/or teasing to a stallion) were monitored daily until a 40 mm follicle was observed, at which time they were examined ultrasonographically every two hours until ovulation was observed. If a mare had two follicles, only the first ovulation was recorded to eliminate any effects that the first ovulation may have on the second follicle. Between June 22, 2004, and July 26, 2004, 25 mares were detected in estrus and were monitored via ultrasound every two hours until ovulation was observed. Statistical analysis was performed using a Chi-squared test.

**Accomplishments/Results/Conclusions:**
When the day was divided into three eight-hour segments (4 p.m.-12 a.m., 12 a.m.-8 a.m., and 8 a.m.-4 p.m.), a significantly greater number of mares (14/25) ovulated between 4 p.m. and 12 a.m. as compared to the other time segments (p = 0.0424). Furthermore, if the day was divided into two 12-hour segments (2 p.m.-2 a.m. and 2 a.m.-2 p.m.), a significantly greater number of mares (20/25) ovulated between 2 p.m. and 2 a.m. (p = -0.0027)

**Benefits to/Impact on the Equine Industry:**
Results of this study support the hypothesis that there is a trend for ovulations to occur in the late evening to early night hours. Further research is needed to explain the endocrinology behind this phenomenon.

**Take Home Message:**
Better understanding of the factors controlling ovulation in the mare will afford the practitioner better control when manipulating the estrous cycle.

**Acknowledgements:**
Funding for this study was provided by Pacific Equine, LLC.

**Year Completed:**
2005

**Published Manuscripts/Abstracts:**
Follicular aspiration or deslorelin treatment to initiate cyclicity in transitional mares

Authors/Investigators:
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Description of the Problem:
Advancing the onset of cyclicity has long been a goal in the equine industry. Follicular aspiration has resulted in luteinization in cycling mares, and an earlier study from our lab (Klump, et al., 2003) indicated it could advance the onset of cyclicity.

Study Objective:
The objective of this study was to more succinctly define selection criteria for follicular aspiration and to compare follicular aspiration to deslorelin treatment to initiate cyclicity in transitional mares.

Approach:
Thirty-six anestrous mares were assigned to one of three treatments (control, n=14; follicular aspiration, n=10; deslorelin, n=12). Control mares were monitored by palpation and ultrasonography twice weekly until ovulation was detected. The aspiration and deslorelin groups were monitored in the same manner twice weekly until a follicle >25 mm was identified, and then every other day until a follicle >35 mm and a uterine edema score ≥ 2 (on a scale of 0 to 3) was present. Transvaginal ultrasound guided follicular aspiration was performed in the aspiration group. In the deslorelin group, a deslorelin implant was placed in the vestibular mucosa for 48 hours. After treatment, the ovaries were monitored every other day for luteal tissue formation as indicated by the appearance of a hyperechoic structure at the site where the follicle had been located. If luteal tissue was not formed after treatment, monitoring continued in the same manner and mares were retreated when the aforementioned criteria were met, until luteal tissue formation was observed. Plasma was obtained from each mare at each examination to determine progesterone concentration.

Accomplishments/Results/Conclusions:
The time from January 1 to the first rise in plasma progesterone >1 ng/mL was compared among groups by survival analysis using the Kaplan-Meier method. No significant differences were detected among the groups (113.0 ± 4.9, 102.0 ± 6.7 and 110 ± 5.3 d [mean ± SE] for the control, aspiration and deslorelin groups, respectively, P=0.33). In our previous study (Klump, et al., 2003), follicular aspiration shortened transition by 22.8 days; however, the present study did not achieve similar results. Uterine edema was used as a criterion for aspiration in this study, and some of the mares developed >35 mm follicles without uterine edema, which either postponed or precluded re-aspiration. Of the 10 aspiration-treated mares, seven mares formed luteal tissue after aspiration, while three mares did not respond to the first aspiration and were not retreated because the uterine edema criterion was not met. In all, there were 17 missed aspiration opportunities due to lack of uterine edema. If the control mares were compared with the mares that responded to aspiration, there was a significant difference in the time of the first rise in progesterone (113.0 ± 4.9 vs. 93.9 ± 6.7, control and aspiration, respectively, P=0.045). Of the seven mares that responded to aspiration, four mares were treated twice and one mare three times, resulting in 1.6 ± 0.2 aspirations required per mare.

Benefits to/Impact on the Equine Industry:
Advancing the onset of cyclicity has long been a goal in the equine industry. Follicular aspiration has resulted in luteinization in cycling mares, and an earlier study from our lab (Klump, et al., 2003) indicated it could advance the onset of cyclicity.

Take Home Message:
Results of this and our previous study indicate that while follicular aspiration of a follicle >35 mm during late transition may be a means to shorten the transitional period, uterine edema should not be used as a criterion for the aspiration.

Year Completed: 2005

Published Manuscripts/Abstracts:
Post-mating endometritis after low dose hysteroscopic insemination in the mare

Authors/Investigators:
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Description of the Problem:
While some authors proposed that hysteroscopic insemination may reduce post-mating endometritis in mares with delayed uterine clearance (DUC), others maintained that the hysteroscopic procedure is inflammatory and should not be used in DUC mares.

Study Objective:
Our objective was to evaluate the inflammatory response after hysteroscopic insemination in reproductively normal and DUC mares. We hypothesized that low dose hysteroscopic insemination would result in less severe endometritis than routine uterine body insemination.

Approach:
Reproductively normal (n = 64) and DUC mares (n = 65) received each of four treatments during estrus during four consecutive estrous cycles: Uterine body insemination (1 x 109 spermatozoa, 20 mL) [UB], hysteroscopic insemination (5 x 106 spermatozoa, 0.5 mL) [HYST], sham hysteroscopic insemination (semen extender, 0.5 mL) [SHAM] and hysteroscopic infusion of seminal plasma (0.5 mL) [SP]. The presence of intrauterine fluid was evaluated by ultrasonography per rectum 24 hours and 48 hours after treatment. Uterine secretions were collected with an intrauterine tampon and a culture instrument 48 hours after each procedure to determine the concentration and percentage of leukocytes. Categorical data were analyzed with a Chi-square test. The response variables concentration and percentage of leukocytes were evaluated with a mixed effect linear model. Correlation and regression analysis were performed to evaluate the effect of duration of hysteroscopy on leukocyte numbers.

Accomplishments/Results/Conclusions:
More DUC than normal mares had intrauterine fluid 24 hours and 48 hours after inseminations (p < 0.05). There was no effect of treatment on fluid accumulation in normal mares (p > 0.05). There was a significant effect of treatment on fluid accumulation in DUC mares at 24 hours. More mares had fluid after HYST and SHAM than UB and SP (p < 0.05); however, at 48 hours, this difference was no longer significant (p > 0.05). The percentage of leukocytes was not different between mare groups or treatments (p > 0.05). The concentration of leukocytes after UB insemination in normal mares was greater than after any of the other treatments (normal-UB = 30.8 ± 14.5, normal-HYST = 0.0 ± 0.0, normal-SHAM = 2.4 ± 1.8, normal-SP = 7.6 ± 5.8, DUC-UB = 4.1 ± 1.2, DUC-HYST = 3.9 ± 3.1, DUC-SHAM = 3.2 ± 1.4, DUC-SP = 4.6 ± 1.9) (mean ± S.E.M., x 106 mL-1, p = 0.045). There was a strong positive correlation (R = 0.98) between the duration of the hysteroscopic procedure and the percentage and concentration of leukocytes in normal but not in DUC mares (p < 0.05) Regression analysis showed that if hysteroscopy extended beyond 7 minutes endometritis was likely to persist 48 hours after the hysteroscopic procedure.

Benefits to/Impact on the Equine Industry:
While some authors proposed that hysteroscopic insemination may reduce post-mating endometritis in mares with delayed uterine clearance (DUC), others maintained that the hysteroscopic procedure is inflammatory and should not be used in DUC mares. Our studies found that there is no contraindication to using hysteroscopic insemination in mares with delayed uterine clearance.

Take Home Message:
We conclude that although hysteroscopic insemination induces endometritis in reproductively normal and DUC mares, the inflammation is not greater than after standard insemination procedure. Although more DUC mares had fluid 24 hours after hysteroscopy, the number of leukocytes was not greater than after uterine body insemination.

Acknowledgements:
Funding was provided by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:
**Improvement of sperm recovery rates after centrifugation of stallion semen**

**Authors/Investigators:**
M.S. Ferrer, DVM, MS, DACT; Dale L. Paccamonti, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; A.H. Aljarrah, DVM, MS, DACT; R. Devireddy

**Description of the Problem:**
Maximum sperm recovery with minimal sperm damage is one of the goals when centrifuging stallion semen. While gravitational force and time are parameters of concern, the height of the fluid column may be an important aspect that is commonly overlooked. Reducing the volume (column height) contained in a tube should increase sedimentation, thereby minimizing sperm loss in the supernatant.

**Study Objective:**
Our objectives were to compare sperm yield and viability after centrifugation and after 24 hours of storage at 4°C. We hypothesized that an increase in gravitational force would increase sperm recovery rate without inducing further immediate or long-term damage to the cells and a reduction in the height of the column would increase recovery rate.

**Approach:**
Ejaculates were collected from eight stallions. Each ejaculate was extended to 25 X 106 cells/mL in a skim-milk extender and divided into nine aliquots. Aliquots were centrifuged in 50-mL conical tubes under the conditions in Table 1. After centrifugation, sperm concentration in the supernatant was determined and sperm recovery rate was calculated. Each aliquot was resuspended to 25 X 106 cells/mL and stored at 4°C for 24 hours. Motility patterns were analyzed with a computer assisted semen analyzer (Minitube of America, Inc. Verona, WI), and sperm viability was evaluated with SYBR14/Propidium Iodide (Garner, et al., Biol. Reprod. 1995; 53:276-284). These parameters were assessed before and after centrifugation, and after 24 hours of cooled storage. Means among treatments were compared using analysis of variance and paired comparisons were made between treatments using Tukey’s test.

**Table 1: Centrifugation conditions for the different treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
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<tr>
<td>Force (x g)</td>
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<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>900</td>
<td>900</td>
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<tr>
<td>Time (min)</td>
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<td>Volume (mL)</td>
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**Accomplishments/Results/Conclusions:**
There were no differences among treatments in total motility, progressive motility and percentage of live cells immediately after centrifugation and after 24 hours of storage (p>0.05). Sperm recovery rate was higher for treatments 1 (100%), 3 (95.3±5.6), 6 (91±4.6), 7 (99.1±0.8) and 9 (93.5±10) compared to treatments 2 (74.3±9.2), 5 (72±11.3) and 8 (72.4±11) (mean ± SD, p<0.05). Treatment 4 provided the lowest recovery rate (44.1±8) (p<0.05). The results supported our hypotheses. When using 400 x g for 10 min or 900 x g for 5 minutes, decreasing the height of the column of fluid increased the recovery rate.

**Benefits to/Impact on the Equine Industry:**
Increased recovery rate of sperm can be obtained by centrifuging semen at 900 x g for 10 minutes without any damage.

**Take Home Message:**
When dealing with a large volume of semen, recovery rate can be improved by centrifuging at 900 x g for 10 minutes without inducing immediate or long-term damage to spermatozoa.

**Year Completed:**
2004

**Published Manuscripts/Abstracts:**
Laparoscopic placement of an indwelling allantoic catheter in the mare: Development of the technique and preliminary findings

Authors/Investigators:
Sara K. Lyle, DVM, MS, DACT; Dale L. Paccamonti, DVM, MS, DACT; Jeremy D. Hubert, BVSc; D.H. Schlafer; R.C. Causey; Jill R. Johnson, DVM, MS, DACVIM, DABVP; Bruce E. Eltis, DVM, MS, DACT

Description of the Problem:
Invasiveness of catheterization of fetal vasculature, maternal vasculature, and allantoic and amniotic compartments has been detrimental to the study of normal physiology and pathologic conditions of pregnancy in many species. Side effects may include fetal demise, potential alteration of endocrine function of the dam and fetus, and limited ability to re-instrument the dam in future pregnancies. The mare has proven to be extremely problematic for these studies, especially with regards to allantoic catheterization.

Study Objective:
The objective of this study was to develop a technique for laparoscopic placement of an allantoic catheter the standing mare.

Approach:
Twenty-nine adult pony mares of various ages were used in this study over a 2.5 year period (2003-2005). Pony mares were pasture-bred with one of four different adult pony stallions. Two weeks after introduction of the stallions, mares were examined by transrectal ultrasonography for pregnancy every seven to 14 days to determine gestational age. Laparoscopic procedures were performed between days 234 and 285 of gestation.

Mares were not fasted prior to surgery. The hair of the left or right flank was clipped and the skin scrubbed with a povidone-iodine cleanser. Mares were restrained in stocks and sedated with 0.05 mg/kg detomidine i.v. and 0.1 mg/kg butorphenol i.v. After regional anesthesia with 2% lidocaine, the laparoscopic cannulas were inserted, the gravid uterus identified, the catheter introduced along a rigid guide to the uterine wall, and the catheter introduced into the allantoic space. The catheter was attached to a subcutaneous access port (PMIDA-SIL-C70 Custom, Instech Solomon, Plymouth Meeting, PA), which was connected to the catheter by silicon tubing and the entire system enclosed within the subcutaneous tissue of the flank wall. All incisions were closed routinely, and a sterile adherent dressing with an antibiotic ointment was applied. Post-operatively, physical examinations were performed twice daily for one week to monitor body temperature and vaginal discharge, and pain, heat or swelling at the incisional sites. Fluid was analyzed for creatinine, chloride, sodium, and calcium concentrations. Biochemical and cytologic analysis of each sample verified the nature of the fluid as allantoic, amniotic, or peritoneal. Fluid samples were obtained at surgery four, eight, 12, 16, 20, 24, 28, 36, 42, 50 hours post-operatively, and then daily until fetal delivery. The site of catheter entry into the chorioallantois was fixed in formalin, paraffin embedded, stained using hematoxylin and eosin, and evaluated.

Accomplishments/Results/Conclusions:
Catheters were indwelling for a mean of 9.6 days and were patent for 8.3 days. Biochemical and cytological analysis of each sample verified the fluid as allantoic, amniotic, or peritoneal. Out of a total of 198 collections for all mares, in only 16 instances could no fluid sample be obtained. The majority of the samples were easily withdrawn. Occasionally, fluid recovery was not immediate, presumably due to fetal position. In these instances, ballottement of the fetus resulted in sufficient fetal movement to allow sample retrieval.

These findings describe the use of laparoscopy to catheterize the allantoic space with a nephrostomy catheter in the pregnant mare. Patient discomfort was minimal. Mares were alert upon return to the stall and resumed eating immediately. Only slight tenderness was noted along the incision sites, and mares tolerated the bandaging well. Subcutaneous access ports, indwelling Huber needles and pre-operative skin preparation reduced bacterial tracking along the catheter system to a minimum. All of the mares used except for one were successfully re-bred and were used again. The ability to repeatedly use this technique from year to year greatly improves the welfare and economic aspects of gestational catheterization studies. Laparoscopic ultrasound produced a significant refinement in the procedure by localizing allantoic fluid.

Benefits to/Impact on the Equine Industry:
Results of this study illuminate the benefits of using an indwelling Huber needle and a locking pigtail catheter for collection of allantoic fluid. Further information in the pathology of late gestational disease in mares should be easier resulting in increased survival of late term abortion.
Take Home Message:
Ultrasound-guided laparoscopy can be successfully used to catheterize the allantoic space of the pregnant mare thereby providing a minimally invasive, reliable method of studying the pathophysiology of a variety of gestational diseases.

Acknowledgements:
Funding was provided by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005

Published Manuscripts/Abstracts:


Spermatozoal binding and phagocytosis by granulosa cells after intrafollicular injection in an estrual mare

Authors/Investigators:
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Description of the Problem:
Intrafollicular insemination (IFI) has been used to obtain pregnancies in humans and has potential applications for circumventing the uterine inflammation in mares with persistent mating induced endometritis, breeding mares with reduced numbers of sperm cells from oligospermic ejaculates or frozen semen, and research applications to further understand the fertilization process. Although attempted, there have been no documented cases of IFI resulting in pregnancy in the horse. Possible reasons include poor sperm cell survival in the follicular environment, inadequate sperm number per follicle, direct detrimental effects of the IFI procedure, and sperm binding to the granulosa cells in the follicle preventing adequate sperm numbers from encountering the oocyte.

Study Objective:
The objective of this study was to determine possible reasons that IFI has failed to attain pregnancies in the mare.

Approach:
Sperm cell survival in the follicular environment has been examined by this lab and unpublished data has shown sperm cells have 63, 49, 36, 16, 7, 2, and 1% motility after incubation in vitro for 0, 1, 2, 3, 4, 5, and 6 hours, respectively, in follicular fluid at 37°C. Despite these findings, our lab obtained no pregnancies after replacing the follicular fluid with embryo culture media while performing IFI.

Inadequate sperm number per follicle has not been fully investigated, but a maximum of 500 X 106 cells has been injected into follicles, and no pregnancies have resulted. Perhaps more cells may achieve pregnancy, but using more cells limits the usefulness of using IFI for frozen semen or oligospermic ejaculates. Direct detrimental effects of the IFI procedure or follicular puncture seem plausible. Previous work showed follicular puncture and sampling, or follicular puncture and total fluid removal, reduced pregnancy rates in mares compared to controls. Our laboratory was also able to attain only a single pregnancy out of ten mares bred after follicular fluid replacement and concomitant AI with 500 X 106 motile cells.

It has been hypothesized that after IFI sperm cells bind to the granulosa cells in the follicle, thus preventing adequate sperm numbers from encountering the oocyte. Cumulus cells, a mass of granulosa cells surrounding the oocyte have been shown to attract and guide sperm cells to the oocyte, and may increase fertilizing ability. A study in our lab showed that sperm recovery from the follicle four hours after IFI was only 0.53% ±0.38 (mean±SEM) when 50 million cells were injected and only 9.02% ±4.41 when 500 million cells were injected. This affirmed that sperm cells were difficult to recover but did not reveal why. The present study was done to determine if the poor recovery of the sperm cells from the follicle after IFI was caused by sperm cells binding to the granulosa cells.

The IFI procedure was performed as in a previous study. Briefly, a single estrual mare that had a follicle 35mm was administered hCG (Chorulon®, 2500 IU; Intervet Inc. Millsboro, DE 19966, USA) and 36 hours later 500 million motile spermatozoa in embryo culture medium were injected into the follicle using a transvaginal ultrasound guided approach. Four hours after the IFI, the mare was euthanized, and the ovary containing the follicle was excised. The follicle was
infused with a standard fixative while the follicular fluid was simultaneously drained. Pieces of follicle were prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Zeiss, 10). Fixed pieces were dehydrated in an ethanol series, critical point dried, coated with gold/palladium (Hummer V, Technics) and searched under SEM (Quanta 200, FEI Co). No attempt was made to quantify sperm cell binding.

**Accomplishments/Results/Conclusions:**
Electron microscopy demonstrated that sperm cells binded to the follicular granulosa cells and burrowed down in between them. Subsequent electron microscopy demonstrated phagocytosis of the sperm cell.

Human granulosa cells have been shown to be capable of phagocytizing latex beads; however, spermatozoa were phagocytized more extensively by the cumulus cells than by mural granulosa cells. Because of the fixation method used (an inflow of fixative concurrent with an outflow of follicular fluid) no data on percentage of non-recovered cells (bound or phagocytized) could be determined. This granulosa cell binding and subsequent phagocytosis probably diminishes the number of sperm cells penetrating the cumulus surrounding the oocyte and may explain the poor fertility after IFI but does not explain the poor fertility after IFI concomitant with vaginal AI.

**Benefits to/Impact on the Equine Industry:**
This study demonstrates that phagocytosis of sperm prevents pregnancy during intrafollicular insemination in mares.

**Take Home Message:**
Prevention of phagocytosis of sperm is necessary before intrafollicular insemination can be used successfully in mares.

**Acknowledgements:**
Funding was provided by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


**Inducing ovulation in mares using a nitric oxide donor**

**Authors/Investigators:**
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**Description of the Problem:**
Follicular aspiration results in apparent luteinization of the aspirated follicular structure when performed on cyclic mares during the breeding season and transitional period. Research has demonstrated the existence of a nitric oxide (NO) generating system in the equine preovulatory follicle that is upregulated following administration of hCG. Moreover, systemic administration of a NO inhibitor to mares in estrus delayed ovulation up to five days after hCG-stimulation. We hypothesized that introducing a NO donor into a preovulatory follicle in mares will result in ovulation.

**Study Objective:**
Our objective was to induce ovulation in mares by injecting a NO donor (DETA NONOate) into the dominant follicle during estrus.

**Approach:**
Normally cycling light horse breed mares in good body condition, aged five to 17 years, were used in this study. Palpation per rectum and ultrasonography were performed daily to document follicular development, presence of a corpus luteum, endometrial edema, and cervical tone. When in estrus with a follicle of 33 to 38 mm, mares were randomly assigned to one of three treatment groups: Control (n=7), Saline (n=4) or NO (n=4).
In the control group, the interval from time of appearance of a follicle 33 to 38 mm to ovulation was recorded. In the saline and NO groups, when a follicle of 33 to 38 mm was found, the follicle was injected with 1 ml of either saline or the NO donor DETA NONOate (Cayman Chemical Company, Ann Arbor, MI, USA) (7.27 mg) by a transvaginal ultrasound guided technique. Mares with two developing preovulatory follicles were excluded from the study.

The dose of NO was calculated based on prior studies examining the concentration of NO in the preovulatory follicle after hCG stimulation. The NO was prepared by dissolving 100 mg DETA NONOate in 1.225 ml sterile 0.01M NaOH (final pH 11.8). DETA NONOate was chosen as the NO donor based on its relatively long half-life of 20 hours at pH 7.4, 37°C. Most other available NO donors have half-lives measured in minutes. Aliquots of NO (7.27 mg/ 95 μl) were prepared to achieve a concentration of 2 mmol NO in a 35 mm follicle. The aliquots were stored at -80°C and thawed at ambient temperature just prior to injection.

For the treatment groups, follicular injection of saline or NO was performed with the mare in stocks, under sedation (detomidine, 20 μg/kg, iv [Orion Corp., Espoo, Finland] and butorphenol, 0.1 mg/kg, iv [Fort Dodge Animal Health, Fort Dodge, IA, USA]). Real time ultrasound (Sonovet 600, Universal Ultrasound, Bedford Hills, NY, USA) with a transvaginal, curvilinear, 6.5 MHz transducer equipped with a 60 cm, 18 g needle, was used for follicular injection.

The time of apparent ovulation and progesterone concentration > 1 ng/ml were recorded. To document luteal formation, palpation and ultrasonography were performed on the day of treatment, at four hours post treatment and then every 12 hours until apparent ovulation, and at two, four and eight days post ovulation. Control mares were palpated every 12 hours (n=3) or 24 hours (n=4). Blood samples were obtained via jugular venipuncture on the same days to determine the concentration of circulating progesterone. Plasma was removed after centrifugation and stored at -20°C until progesterone analysis.

Concentrations of progesterone were determined with commercially available reagents (Diagnostic Systems Laboratory, Webster TX, USA) that were previously validated for horse plasma in the Louisiana State University Department of Animal Sciences. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 8% and 0.1 ng/ml.

Accomplishments/Results/Conclusions:
Of the eight injections (NO, n=4; Saline, n=4) the follicles collapsed within 16 hours after the procedure on five occasions (NO, n=3; Saline, n=2). Care was taken during ultrasonographic examination to ensure that pressure on the ovaries/ follicles was not the cause of the collapse. In one saline and three NO mares a hyperechoic structure appeared within 48 hours at the site of the previous follicle. In one mare (NO) this hyperechoic structure was transient, disappearing within 24 hours, otherwise it remained for at least eight days.

In the Control group, circulating progesterone concentrations reached >1 ng/ml in 7.00 ± 0.72 d (mean ± SE) after appearance of a follicle 33 to 38 mm. Of the mares injected with saline, 3/4 responded by luteinization of the injected follicle and had circulating progesterone concentrations of > 1 ng/ml 6.50 ± 0.87 d after injection. In the remaining mare injected with saline, the injected follicle was no longer visible within 24 hours and a new follicle grew and subsequently ovulated within the following eight to 12 days. After intrafollicular injection of NO, mares had circulating progesterone concentrations of > 1 ng/ml in 5.75 ± 0.85 d (mean ± SE).

Nitric oxide plays a role in many reproductive processes, including ovulation and luteinization. In mares, inhibition of NO synthase during estrus delayed ovulation and resulted in a higher concentration of estrogen and lower concentration of progesterone in follicular fluid. Furthermore, administration of hCG to estrous mares increased the concentration of NO in follicular fluid. As the pre-ovulatory follicle in the mare matures, the concentration of progesterone in follicular fluid increases while estrogen decreases. In vitro, NO synthase inhibitors decreased the progesterone:estrogen ratio in granulosa cell cultures, while an NO donor increased the ratio.

Although it appeared clinically that injection of NO into a pre-ovulatory follicle hastened luteinization, no statistical difference was observed between groups. A hyperechoic structure appearing at the site of the follicle that had been injected was not always associated with a rise in circulating progesterone. It is possible that the transient hyperechoic structure may have been associated with hemorrhage due to the follicular puncture procedure. It appeared that when follicular collapse occurred soon after injection that luteinization was delayed. For example, in the mare injected with NO that had apparent follicular collapse within four hours, a rise in circulating progesterone was not observed until eight days post treatment. The other mares treated with NO exhibited a rise in circulating progesterone within four to six days after treatment. A similar effect was noted in mares injected with saline. The two mares injected with saline that did not exhibit follicular collapse had a rise in circulating progesterone by five days after treatment, whereas those that did have follicular collapse within four hours did not have elevated circulating progesterone until eight days post treatment. It is possible that early follicular collapse was associated with the leaking of the follicular contents into the peritoneal cavity thereby delaying the normal process of luteinization. This does not explain the apparent luteinization seen after follicular aspiration in other reports, where the follicular contents were removed, follicular puncture may result in release of sufficient endogenous NO, either due to inflammation or hemorrhage to initiate the process of luteinization.
**Benefits to/Impact to Equine Industry:**
The results of this study provide more evidence that NO in initiation of luteinization in the mare.

**Take Home Message:**
Further studies are needed to determine whether NO donors and NO synthase antagonists can be used to control cyclicity in mares.

**Year Completed:** 2005

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**The use of cefquinome in equine semen extender**

**Authors/Investigators:**
J.M. Parlevliet; J.W. Lynn; Dale L. Paccamonti, DVM, MS, DACT

**Description of the Problem:**
Cefquinome, an aminothiazolyl cephalosporin and a member of the fourth generation of cephalosporins that have been developed especially for use in animals, has a very broad spectrum of activity and is a potent agent against many bacteria. In cattle and swine, cefquinome has successfully been used for the treatment of endometritis and post partum metritis. Recently in horses it was shown that intrauterine cefquinome concentrations were higher than the MIC50 and MIC90 for the most common bacteria reported to cause endometritis, for up to 36 hours after a single treatment. Antibiotics are commonly used as a component of semen extender to eliminate pathogenic or potentially pathogenic bacteria from semen and reduce the risk of post mating endometritis. Because of the broad spectrum of activity that cefquinome possesses, it may be ideally suited for use in semen extender. However, no data are available on the use of cefquinome in semen extender and its effect on spermatozoa.

**Study Objective:**
The purpose of this study was to evaluate the use of cefquinome in equine semen extender and its effect on semen parameters compared to a commonly used semen extender containing gentamicin.

**Approach:**
Semen was collected from reproductively sound and healthy light horse stallions (age four to 20 years, n=9). Ejaculates were diluted to a concentration of 50 million spermatozoa/ml with either a skim milk extender without antibiotics (Equi-Pro, Minitube, Verona, WI, USA) to which cefquinome (2.2 ml of a 4.5 g/100 ml solution /100 ml extender) (pH= 7.05) (Intervet International, Boxmeer, NL) was added or skim milk extender with gentamicin (pH= 7.30) (Equi-Pro with gentamicin, Minitube, Verona, WI, USA). Extended semen was stored for 48 hours at 5°C in an Equitainer IITM (Hamilton Research, South Hamilton, MA, USA). At 0, 24 and 48 hours, aliquots (20 µl) of the stored semen were evaluated using a computer assisted motility evaluation system (Spermvision 3.01; Minitube, Verona, WI 53593, USA). Membrane integrity of the spermatozoa was assessed using a live/dead stain (Live/Dead Sperm Viability Kit, Molecular Probes, Eugene, OR 97402, USA) and acrosomal staining (FITC-PNA) (Molecular Probes, Eugene, OR 97402, USA) was also evaluated.

**Accomplishments/Results/Conclusions:**
Statistical analysis was performed using a Student’s t-test. No significant differences were found in total motility, progressive motility and acrosomal status of spermatozoa between extenders with cefquinome or gentamicin at any time point. After 24 hours storage at 5°C, curvilinear velocity (VCL) and average path velocity (VAP) were lower (P<0.05) for spermatozoa stored in the cefquinome extender. The amplitude of the lateral head displacement (ALHD) was lower (P<0.05) at 24 and 48 hours for spermatozoa stored in the extender containing cefquinome than in the extender containing gentamicin. The extender with cefquinome had significantly more intact cells at 48 hours after storage (P<0.05).

**Benefits to/Impact on the Equine Industry:**
Using nonfat dried skim milk may be a superior to extending semen because of the wider spectrum of antibacterial effect.

**Take Home Message:**
The current study shows that semen parameters after storage in nonfat dried skim milk semen extender containing cefquinome are comparable to those after storage in semen extender containing gentamicin. The wider spectrum of bactericidal activity possessed by cefquinome may prove to be beneficial in some cases.

**Year Completed:** 2005
Effect of post-ovulatory PGF2α or cloprostenol on plasma progesterone concentration in mares

Authors/Investigators:
C.M. Mocklin; Dale L. Paccamonti, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; E.G.H. Wouters; L.R. Gentry; Robert A. Godke, PhD

Description of the Problem:
Impaired uterine clearance of fluid and inflammatory products after mating is a major cause of infertility in the mare. This inflammation, if it persists, can affect embryo viability after fertilization and may cause early regression of the corpus luteum. Exogenous oxytocin administration to the mare causes myometrial contraction and the expulsion of post-breeding debris. However, oxytocin has a relatively short duration of action and its use is often impractical for many horse owners. Cloprostenol, a PGF2α analogue, has also been used clinically to aid in the removal of fluid, in part because prostaglandins have a much longer duration of action than oxytocin. However, recent reports have indicated that cloprostenol administered during the early post-ovulatory period may reduce circulating progesterone levels during diestrus. The effect of administering cloprostenol during the post-ovulatory period on pregnancy rates is unclear. In one report pregnancy rates were unaffected. Dinoprost tromethamine, a natural PGF2α, did not reduce progesterone concentrations during diestrus.

Study Objective:
The objective of this study was to compare the effects of PGF2α and cloprostenol administered in the early post-ovulatory period on circulating progesterone concentrations during diestrus in the mare.

Approach:
Light horse mares (n=36) exhibiting normal-length estrous cycles were used in this study. Mares were monitored by palpation per rectum and transrectal ultrasonography to detect the onset of estrus. During estrus, the mares were evaluated daily to detect ovulation (day 0).

On the day of ovulation, mares were randomly assigned by age (< 14 yr, > 14 yr) to one of three treatment groups. Group 1 (Control, n=12) received no prostaglandin treatment, Group 2 (PGF, n=13) received PGF2α (5 mg, IM, Lutalyse®, Pharmacia & Upjohn, Kalamazoo, MI, USA) and Group 3 (CLO, n=11) received cloprostenol (250 μg, IM, Estrumate®, Schering-Plough Animal Health Corp., Union, NJ, USA). Treatments were administered once daily for three days, beginning on the day ovulation was detected (days 0, 1 and 2).

On days 0, 1, 2, 4, 6, 8, 10, 12, and 14 after ovulation, blood samples were collected via jugular venipuncture into heparinized tubes, with the plasma stored at -20°C until assayed for plasma progesterone. Progesterone concentrations were determined with commercially available reagents (Diagnostic Systems Laboratory, Webster, TX, USA) that were previously validated for horse plasma at the Louisiana State University Department of Animal Sciences. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 8% and 0.1 ng/ml. An ANOVA using repeated measures was used to compare progesterone concentrations among treatments, and one-way ANOVA was used to locate mean differences between groups within days.

Accomplishments/Results/Conclusions:
Differences between the groups were detected on days 4, 6, 8 and 10 after ovulation but not on days 1, 2, 12 and 14. On day 4, the concentration of progesterone in Control group mares was greater than that in either of the prostaglandin treatment groups (P< 0.001). On days 6, 8 and 10, the progesterone concentration in the Control group was greater than in the Cloprostenol group (P< 0.05) but was not different than the PGF2α group (P>0.05).

Both the concentration of progesterone during diestrus and the length of diestrus are of concern in regards to mare fertility. Cloprostenol treatment in the early post-ovulatory period was associated with decreased progesterone concentrations during early – mid diestrus, which is in general agreement with previous studies. However, concentrations on days 12 and 14 post-ovulation, when maternal recognition of pregnancy would be occurring, were not different from Controls. In a previous report from our laboratory, PGF2α (5 mg, IM) given on the same days as in the current study did not affect progesterone concentrations through day 8 after ovulation. In the present study circulating levels of progesterone were significantly lower than Controls on day 4 post-ovulation.

While it is often stated that a corpus luteum should be at least five days post ovulation in order to respond to prostaglandin administration, an early study reported that two of five mares that received prostaglandin (10 mg, SC) on day 3 post ovulation responded by returning to estrus. In the current study, progesterone concentrations at day 14 were not different between controls and either treatment group. Although mares were not teased to detect onset of behavioral estrus, based on palpation, ultrasonography and progesterone concentrations, no difference was observed in length of diestrus between groups.
Benefits to/Impact on the Equine Industry:
Both PGF2α and cloprostenol decreased plasma progesterone concentration at least temporarily during diestrus.

Take Home Message:
Even though mares in this study were not mated and thus pregnancy rates are not available, a decrease in progesterone concentrations during diestrus may be of concern, especially with a developmentally retarded embryo. Oxytocin may be a safer alternative for the treatment of delayed uterine clearance in the post-ovulatory period.

Year completed: 2005

Acknowledgements:
This study was funded by the Merck Merial Summer Scholars Program at the Louisiana State University School of Veterinary Medicine.

Published Manuscripts/Abstracts:

The use of cefquinome (Cobactan 4.5%®) in the mare’s uterus

Authors/Investigators:
Joyce M. Parlevliet, DVM, PhD, MSc; Dale L. Paccamonti, DVM, MS, DACT; Steven A. Barker, MS, PhD

Description of the Problem:
Cefquinome is an effective drug against pathogens commonly found in cases of equine endometritis, such as Streptococcus zooepidemicus, E.Coli and other Enterobacteriaceae. Intrauterine infusion of cefquinome resulted in concentrations of cefquinome in the endometrium that exceeded the reported minimal inhibitory concentrations (MIC50 or MIC90) of most important bacterial species involved in equine endometritis. Furthermore, infusion of cefquinome did not cause significant inflammation in the endometrium.

Cefquinome, an aminothiazolyl cephalosporin, is a member of the fourth generation of cephalosporins which have been developed especially for use in animals. Cefquinome has a very broad spectrum of activity and is a potent agent against various bacteria such as Streptococcus spp., Staphylococcus spp., Pseudomonas spp., Moraxella spp., Haemophilus spp., Corynebacteriae, enterococce, E.Coli isolates and gram-positive anaerobes tested in vitro. Cefquinome has successfully been used for the prevention and treatment of bovine endometritis.

Endometritis is an important cause of pregnancy failure in the mare. The use of an appropriate antibiotic is an important aspect of treatment. No data are available regarding the intrauterine use of cefquinome in the mare.

Study Objective:
The purpose of this study was to measure the concentration of cefquinome1 in the endometrium of the mare after intrauterine treatment and to evaluate any treatment related inflammation.

Approach:
Mares used in this experiment had palpably and ultrasonographically normal reproductive tracts without significant inflammation detected in endometrial biopsies. The mares were healthy light horse mares aged four to 10 years (n=14). At least one week after the control biopsies were taken, the experiment was performed. The reproductive tract was palpated per rectum and scanned ultrasonographically daily using a 5.0 MHz linear probe for follicle size and the presence of fluid in the uterus throughout estrus until 48 hours after treatment and at least until ovulation. When mares were in estrus with a follicle > 30 mm, uterine edema and cervical relaxation, they were randomly assigned to one of the following four groups: Control mares (n=4) were either not treated (n=2) or treated (n=2) with an intra-uterine infusion of 33 ml Lactated Ringer’s solution for one or three consecutive days; Treated mares (n=10) received an intra-uterine infusion of 1.5 g cefquinome (in 33 ml solvent) for one day (n=5) or at 24 hour intervals for three consecutive days (n=5) during two estrous cycles. A minimum of 10 days after treatment (and ovulation), PGF2α (5 mg, IM) was administered. During the second cycle, mares received an alternate treatment within their Control or Treatment grouping. The cefquinome powder was mixed with the diluent just prior to use and any unused portion was stored at 5°C for a maximum of 24 hours. Endometrial biopsies were obtained at 2, 8, 24, and 48 hours after the last infusion of cefquinome during half of the cycles and at 4, 12, and 36 hours after the last infusion of cefquinome during the other half of the cycles using a Pillig uterine biopsy forceps. Biopsies were taken from untreated control mares (n=2) and saline treated control mares (n=2) at the same time points as treated mares. Multiple samples were taken at random sites in the uterus. Approximately 0.5 g of endometrial tissue was stored in liquid nitrogen (-196°C) until assayed for the concentration of cefquinome. A portion of
the endometrial tissue was fixed in Bouin’s solution and hematoxylin and eosin (H&E) stained sections were evaluated to assess the infiltration of leucocytes into the endometrial tissue. Inflammatory response at the sampling time points were scored 0 (no) to 3 (severe). Only after scoring the histological samples for inflammation was the treatment or the ID of the mare revealed.

Cefquinome concentrations in the endometrial tissue samples were quantified using a high-performance liquid chromatography (HPLC) assay per time point and days of treatment. The proven level of quantification was 10 ng cefquinome/g of tissue.

Concentrations of cefquinome in the endometrium and inflammatory response were evaluated using Friedman Repeated Measures ANOVA on Ranks5.

Accomplishments/Results/Conclusions:
Age of the mares and uterine biopsy score at start of the work did not differ between treatment groups. In a few cases in all treatment groups, a trace to moderate amount of fluid was observed in the uterus during the ultrasound examination between biopsy sessions. In all cases, the fluid disappeared after the last biopsy was taken or after ovulation. Mares ovulated within 2.6 ± 0.3 days after treatment and 1.1 ± 0.3 days after obtaining the last endometrial biopsy. Variation in concentration of cefquinome between and within mares at different time points was found. Very high concentrations of cefquinome were detected during the first 12 hours after treatment in both one-day and three-day treatment groups (Table 1). Concentrations of cefquinome were greater during the first 12 hours after treatment in the 1-d group (P<0.05) and for 48 hours after treatment in the three-day group (P<0.05) than in either control group. Concentrations of cefquinome were similar between treatment groups at two and four hours after treatment (P>0.05). At eight hours, as well as at 24 and 48 hours, concentrations were greater in the three-day group (P<0.05). In all cases, until 36 hours post treatment, cefquinome concentrations were higher than the MIC50 and MIC90 for the most common bacteria reported to cause endometritis. No significant leucocyte infiltration was found in any group. No difference was found in the inflammatory response of the uterus at the time points sampled after infusion of saline, one or three days treatment with cefquinome, or no treatment (P>0.05)

Cefquinome has been tested in vitro and in vivo in several species without any side effects. Cefquinome is effective in the prevention and treatment of endometritis in cattle. This study demonstrated very high concentrations of cefquinome in the endometrial tissue of mares after one- or three-day treatments. In healthy horses, intrauterine administration of 1.5 g cefquinome (in 33 ml solvent) resulted in concentrations of cefquinome in the endometrium that exceed the reported minimal inhibitory concentrations (MIC50 or MIC90) of most important bacterial species involved in equine endometritis. After a single treatment, the concentration of cefquinome at eight hours was significantly lower than after three-day of treatment. However, at other time points until 24 hours after the last treatment and at 36 hours there was no difference between one- and three-day treatment. The difference at eight hours cannot be explained by the results from one individual mare. However, the sampling site of the uterus or the amount of fluid or blood contamination of the samples might have played a role. Another explanation could be that each subgroup of mares was not sampled at consecutive time points but at alternate time points. Furthermore, the low number of mares could have influenced the outcome. General treatment volume recommendations vary in the literature from 10 to 250 ml for intrauterine treatment of endometritis. Therefore, the volume used in the present study should have been sufficient for infiltration of the endometrium.

Fourth generation cephalosporins, including cefquinome, generally have an extended spectrum of activity for gram positive, including beta lactamase organisms, and gram negative organisms, including those with multiple drug resistance patterns, and Pseudomonas sp. In vitro studies have shown that the spectrum of activity of cefquinome is very broad and that it should be an effective antibiotic against bacterial pathogens commonly found in animal diseases. The wider spectrum of bactericidal activity possessed by cefquinome may prove to be beneficial in some cases of endometritis where the specific pathogen is not known. The three-day treatment appeared to result in more sustained antimicrobial concentrations, and therefore repeated daily infusion may be more beneficial for treating endometritis. The one-day treatment regimen could be used to treat post-mating endometritis, which regularly occurs in the horse.

Benefits to/Impact on Equine Industry:
Cefquinome, a new fourth generation cephalosporin may be very useful in treating endometritis and also resistant endometritis in mares.

Take Home Message:
The lack of significant inflammatory response indicates cefquinome can be safely used for intrauterine therapy. Cefquinome has also been used as an antibiotic in semen extender and did not negatively affect semen parameters. Therefore, cefquinome, the new fourth generation cephalosporin, may be useful in the treatment of endometritis and in resistant endometritis in mares in particular.
Acknowledgements:
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Year Completed: 2005
Respiratory

Characterization of equine airway endothelin receptors in health and disease

Authors/Investigators:
Changaram S. Venugopal, BVSc, MSc, MS, PhD; Sumanth Polikepahad, BVSc & AH, PhD; Rustin M. Moore, DVM, PhD, DAVCS

Description of the Problem:
Recurrent airway obstruction (RAO) is a common asthma-like pulmonary disease affecting the lower respiratory system in horses worldwide and is characterized by frequent bronchoconstriction, pulmonary hypersecretion, edema and increased resistance to airflow in lungs leading to morbidity and loss of performance in horses. During periods of airway obstruction, horses develop airway hyperreactivity (hyperresponsiveness and hypersensitivity) to inflammatory mediators, released locally into the airway lumen and across bronchial smooth muscle. These mediators are responsible for bronchial smooth muscle contraction, increased vascular permeability, increased mucus secretion and damage to the airway epithelium.

Recently, attention has been given to endothelin-1(ET-1), an endothelium-derived factor, which is a 21 amino acid peptide that causes profound bronchoconstriction. Endothelin is produced by various cells that exert numerous biologic and pathophysiologic effects by binding to ETA and ETB receptors. Our pilot studies showed that when equal concentrations of ET-1 were applied to bronchial rings from healthy and SPAOPD-affected horses, the contractile responsiveness of the rings from the affected horses was significantly increased.

Study Objective:
We hypothesized that the increased responsiveness to ET-1 of bronchial rings of horses affected with RAO is due to alteration in ET receptors. Therefore, the purpose of this study was to examine alterations in ET receptors of equine airways in health and disease using pharmacological, immunohistochemical and molecular methods.

Approach:
Pharmacological studies were conducted using bronchial rings from diaphragmatic lung lobe of all horses. The rings were placed in organ baths for response studies. Concentration response curves were constructed for endothelin (10⁻⁸.₅ to 10⁻⁶M). Separate rings were used for each CR relationship. The same procedure was repeated in both groups, in the presence of either ETA or ETB blocker. Carbachol 10⁻³M was used to get the maximal contraction of the rings. From each CR curve with and without the antagonists, EC₅₀ values were determined, which were used for statistical comparisons. From these EC₅₀ values, pA₂ values were determined using Schild plot. The pA₂ value is a measure of drug antagonism reflecting the affinity of receptors. In general, the greater the pA₂ value the greater the affinity of the antagonist for the receptor. Three concentrations of each antagonist were used.

Immunohistochemical Studies were conducted using polyclonal primary antibodies against ETA or ETB receptors, at a dilution of 1:200. Biotinylated IgG secondary antibodies were applied to tissue sections, followed by the addition of an avidin-biotin immunoperoxidase complex. Photographs of the stained slides were digitally recorded and analyzed, using image analysis software to determine the intensity of staining. Two-way ANOVA was used for statistical analysis.

Molecular studies were conducted using appropriate primers for ETA, ETB, and the housekeeping gene B-actin. cDNA synthesis was performed from pulmonary tissue samples. Then PCR was conducted, and the products were loaded for electrophoresis. Western Blots were performed in five independent replications per horse to validate our immunohistochemical techniques.

Accomplishments/Results/Conclusions:
Results showed that bronchial rings of RAO horses contracted significantly greater than those of healthy horses to equimolar concentrations of ET-1, suggesting alterations in ET receptors. In addition, ETB receptor affinity was significantly increased in RAO, whereas that of ETA remained the same. The IHC studies showed that ETA receptor staining increased only in one lobe whereas all other lobes showed significantly increased staining for ETB receptors. Both ET receptors in the epithelium remained unaltered. Western blotting and IHC techniques showed that expression of ETA and ETB receptors was significantly greater in RAO horses. However, RT-PCR demonstrated greater mRNA expression only for ETB receptors in RAO horses.

Overall, the study showed that the response of bronchial rings from RAO-affected horses was significantly greater than the response of rings from clinically healthy horses. Both ETA and ETB receptors mediate contraction in equine airways. The ETB receptor affinity in clinically healthy animals is very low, suggesting no important role in physiological response.
whereas ETA receptors play a role in the normal physiological airway tone. ETB receptors are up-regulated in RAO, which was indicated by increased mRNA expression. Western blot confirmed increased ETB receptor protein expression, thus indicating an important role for ETB receptors in bronchoconstriction observed in RAO-affected horses.

**Benefits to/Impact on the Equine Industry:**
Recurrent airway obstruction (RAO) is a common asthma-like pulmonary disease affecting the lower respiratory system in horses worldwide and is characterized by frequent bronchoconstriction, pulmonary hypersecretion, edema and increased resistance to airflow in lungs leading to morbidity and loss of performance in horses. Endothelin and its receptors, particularly ET-B receptors play an important role in RAO.

**Take Home Message:**
Endothelin and its receptors, particularly ET-B receptors play an important role in RAO. The antagonists of ET-B receptors have potential role as therapeutic agents for the treatment of airway hyperreactivity.

**Acknowledgments:**
The study was supported by grants from NRI-USDA (#2001-35204-10809) and the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

**Year Completed:** 2005

**Published Manuscripts/Abstracts:**


**Characterization of pulmonary endothelin receptors in clinically healthy and RAO affected horses**

**Authors/Investigators:**
Changaram S. Venugopal, BVSc, MSc, MS, PhD; Sumanth Polikepahad, BVSc & A.H., PhD; Rustin M. Moore, DVM, PhD, DACVS.

**Description of the Problem:**
Recurrent airway obstruction (RAO) in horses is a common pulmonary disease of horses characterized by chronic cough, exercise intolerance, labored expiratory effort and nasal discharges. Frequent bronchoconstriction and pulmonary hypersecretion, edema and loss of performance are observed in horses with this disease. The inflammatory response in this disease is characterized by airway inflammation, particularly that of the lower respiratory tract. Bronchoalveolar lavage fluid (BALF) neutrophilia, and elevated levels of inflammatory mediators. Endothelin-1 (ET-1) is a potent smooth muscle constrictor and an inflammatory mediator. It elicits its actions by acting through two receptors namely endothelin-A (ET-A) and endothelin-B (ET-B). By directly acting through its receptors, ET-1 can cause potent contraction of vascular and non-vascular smooth muscles *in vivo* and *in vitro*. It is demonstrated that ET-1 plays an important role in the pathogenesis of allergic airway diseases of humans and other species. Similarly, bronchoconstrictor action of ET-1 has been demonstrated in equine airways.

**Study Objective:**
Our objective was to determine and compare the expression of ET-A and ET-B receptors in the lungs of healthy and RAO horses. Based on the findings of our preliminary pharmacological studies and previous reports, we hypothesized that the expression of ET-A and ET-B receptors is altered in the lungs of RAO-affected horses. Hence the objective of the study was to determine and compare the expression of ET-A and ET-B receptors in the lungs of healthy and RAO-affected horses using RT-PCR, Western blotting and immunohistochemical techniques.

**Approach:**
Eight clinically healthy (or control) horses, 10 to 20 years old (mean ± SD, 15.8 ± 4.1 years) and eight RAO-affected horses, 10 to 20 years old (mean ± SD, 16.5 ± 3.2 years), were included. Clinical scores were determined by use of the following algorithm: 

\[ CS = (\text{Medial nostril flare} + \text{Lateral nostril flare})/2 + \text{Abdominal lift} \]

Each of the variables in the above algorithm was scored from 0 to 4. A score of “0” indicates that the nostril had little movement and the ventral flank showed little or no movement. A score of “4” indicates that the nostril remained maximally flared throughout the respiratory cycle and the abdominal lift resulted in a “heave line” that extended cranially to the fifth intercostal space. Thus, the maximum
clinical score is “8”. The change in transpleural pressure (ΔPpl) was measured indirectly by using an esophageal balloon secured over the end of a catheter connected to a pressure transducer interfaced with a polygraph. To be included in the RAO-affected group, in addition to the history of frequent airway obstruction, the horses must have had a CS > 5.0 and a ΔPpl > 15.0 cm of H₂O. To be included in the healthy group, the horses must have had a CS < 4.0 and a ΔPpl < 10 cm of H₂O.

For the immunohistochemistry, five samples, one from each lung lobe were collected. For the Western blotting and RT-PCR studies, five samples per horse were collected.

RT-PCR was performed by using appropriate primers for ETA receptors, ETB receptors, and the housekeeping gene B-actin.

Total RNA was extracted and reverse transcription was performed to synthesize cDNA. Then PCR was performed. The products were loaded into PCR gel and electrophoresis was performed. Five independent replications per each horse (n=8) were performed for Western blot studies to confirm antibody binding. The data was collected in terms of average intensity of bands of ETA or ETB receptors per average intensity of bands of B-actin. For immunohistochemical staining, five independent replications were performed (n=14). The automated Dako autostainerq was used. Sites of immunostaining were visualized by developing sections in Nova red. The slides were counterstained with Mayer’s hematoxylin for five minutes. The slides were independently evaluated by two authors for staining intensity. The results were interpreted as absence of staining (0), weak staining (1+), moderate staining (2+) and strong staining (3+). The slides were evaluated for the presence of pathological changes in the bronchioles, blood vessels and alveoli. The pathological changes evaluated included Goblet cell metaplasia, epithelial hyperplasia, mucus plugs in the bronchioles, neutrophils in the bronchiolar lumen, peribronchial eosinophils, alveolar macrophages, peribronchiolar inflammation, perivascular inflammation and alveolar inflammation.

Accomplishments/Results/Conclusions:
The most consistent finding in the lungs of RAO-affected horses was the presence of mucus plugs in the bronchioles. Other consistent and significant findings in the RAO-lungs were the presence of goblet cell metaplasia, bronchiolar epithelial hyperplasia, infiltration of neutrophils into the bronchiolar lumens and peribronchial inflammation. In RT-PCR studies, there was no difference in the mean intensity of ETA receptor bands between healthy and RAO-affected horses, whereas the mean intensity of ETB receptor bands was significantly greater in RAO-affected horses compared with healthy horses. In healthy horses, there was no difference between the mean intensities of ETA and ETB receptors; however, in RAO-affected horses, the mean intensity of ETB receptors was significantly greater than that of ETA receptors. The mean band intensities of ETA and ETB receptors were significantly greater in lungs of RAO-affected horses compared with values from healthy horses, respectively. In healthy horses, there was no difference between the mean intensities of ETA and ETB receptors; however, in RAO-affected horses, the mean intensity of ETB receptors was significantly greater than that of ETA receptors. The mean scores for ETA and ETB receptor immunostaining was significantly greater in lungs of RAO-affected horses, compared with values in healthy horses. In healthy horses, the mean scores for ETA and ETB receptor immunostaining were similar; however, in RAO-affected horses, the mean score of ETB receptor immunostaining was significantly greater than that of ETA receptors.

Benefits to/Impact on the Equine Industry:
Recurrent airway obstruction (RAO) in horses is a common pulmonary disease of horses characterized by chronic cough, exercise intolerance, labored expiratory effort and nasal discharges. Frequent bronchoconstriction and pulmonary hypersecretion, edema and loss of performance are observed in horses with this disease. The study shows that ETA and ETB receptor expression is up-regulated in the lungs of RAO-affected horses suggesting a pivotal role for ET-1 and its receptors in the pathogenesis of RAO in horses.

Take Home Message:
ETA and ETB receptors are expressed in the lungs of both, healthy and RAO-affected horses. In RAO-affected horses, the expression of ETB receptors is significantly greater than that of ETA receptors. The expression of ETA receptors is significantly greater in the lungs of RAO-affected horses than that of normal horses. The expression of ETB receptors is significantly greater in the lungs of RAO-affected horses than that of normal horses. This increase is greater than that of ETA receptor expression.

Acknowledgments:
The study was supported by grants from NRI-USDA (#2001-35204-10809) and the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine.

Year Completed: 2005
Published Manuscripts/Abstracts:


**Endothelin and nitric oxide production by equine bronchial epithelial cells cultured under air-liquid interface conditions**

**Authors/Investigators:**
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**Description of the Problem:**
Several mediators have been implicated in the pathogenesis of equine airway diseases. Amongst them, nitric oxide (NO) derived from inducible nitric oxide synthase and endothelin (ET) were shown to be increased in airway epithelium of asthmatic horses affected with summer pasture-associated recurrent airway obstruction (SPARAO). Moreover, a number of stimuli, especially cytokines, have been incriminated in the induction of ET and NO synthesis.

**Study Objective:**
The overall goal of this study was to stimulate cultures of differentiated equine bronchial epithelial cells with LPS, TNF-alpha and IL-4 and measure the synthesis of ET and NO.

**Approach:**
Fresh post-mortem specimens of lung tissue were obtained from two adult horses affected with SPAOPD while horses were in clinical remission (i.e., without signs of respiratory disease, intrapleural pressure difference less than 10 cm of water, and neutrophil in bronchoalveolar lavage less than 15%). The bronchial epithelium was dissected, subjected to cold trypsinization and cultured on Transwells with Dulbecco’s modified Eagle’s medium:Ham’s F12 (1:1 v/v) containing fetal bovine serum and epithelial growth factor (EGF) as previously described. Once cultures were established, they were placed in air-liquid interface (ALI) and maintained in a serum-free media containing low concentration of EGF. After nine days, the cells were stimulated basolaterally with either LPS (10 ng/ml), human recombinant TNF-alpha (5 and 20 ng/ml) or equine recombinant IL-4 (1%, 10% and 50% v/v). Cell-free supernatants from the bottom of the wells were harvested at 24, 48 and 72 hours and stored at −70°C until assayed for ET and NO. ET concentrations were determined using a commercially available sandwich enzyme-linked immunosorbent assay (Biomedica). NO determination was performed using an electrochemical detection system, ISO-NO Mark II. Morphologic differentiation of the cell cultures after 14 to 28 days in ALI was evaluated using light microscopy (thin-sections were stained with Toluene Blue), confocal microscopy (stained for cytokeratin and actin) and transmission electron microscopy.

**Accomplishments/Results/Conclusions:**
Stimulation with hrTNF-alpha, LPS or eqrIL-4 for 24 and 48 hours, and eqrIL-4 for 72 hours resulted in increased production of ET (ranging from 1.5 to 4 fold). Stimulation with hrTNF-alpha, LPS and eqrIL-4 for 48 hours and eqrIL-4 for 72 hours induced 1.5 to 2.5 fold increases in NO production by primary bronchial epithelial cell cultures. Our results suggest that bronchial epithelial cells represent a potentially important source of ET and NO in response to cytokine (TNF-alpha and IL-4) stimulation.

**Benefits to /Impact on the Equine Industry:**
Because these two inflammatory mediators, ET and NO, appear to be...
involved in the pathogenesis of recurrent airway obstruction, it is likely that epithelial cells contribute significantly as a source of these mediators. The interactions of these mediators may play a role in the pathogenesis of SPARAO.

**Acknowledgments:**
This study was supported by funds from the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine. The authors acknowledge the skilled technical help from Olga Borkhsenious at the LSU SVM Microscopy Center, Dr. D. Paulsen, Cheryl Crowder and the Histology Laboratory, and Catherine Koch and the LSU SVM Equine Health Studies Program. The equine recombinant IL-4 was a kind gift from Dr. D. Horohov. Finally, the authors thank the insights from Dr. Kenneth Adler’s Laboratory, especially Nancy Akley and Dr. Linda Martin from the School of Veterinary Medicine, North Carolina State University.

**Year Completed:** 2004

**Published Manuscripts/Abstracts:**
2004 Veterinary Comparative Respiratory Society Symposium, Montreal, Quebec, CA.

2005 American College of Veterinary Internal Medicine, Baltimore, MD.
Surgery

Evaluation of a vessel-sealing device for laparoscopic granulosa cell tumor removal in standing mares

Authors/Investigators:
Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; Daniel J. Burba, DVM, DACVS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
A novel method of removing ovarian tumors.

Study Objective:
The objective of this study was to describe a surgical technique using minimally invasive techniques and specific equipment to remove ovarian tumors.

Approach:
This is a retrospective study using eight adult client owned mares. Before surgery, ovarian size and adjacent body wall thickness were determined by ultrasonography. Mares were sedated and after local anesthesia (inverted L and local infiltration), laparoscopic cannulation was performed without insufflation. The mesovarium was anesthetized and the LigaSure instrument applied to the mesovarium for hemostasis and resection to remove the affected ovary. Mares were hospitalized for 24 hours before discharge.

Accomplishments/Results/Conclusions:
Median ovarian diameter was 10.5 cm (range, 6 – 14 cm). Median surgery time was 75 minutes (range, 40 - 180 minutes). Hemostasis was achieved using the LigaSure device in all mares. Median length of the abdominal wall incision made to remove the ovary was 13 cm (range, 5 - 17 cm); no incisional complications occurred.

Concerns of ligature placement can be alleviated by use of the LigaSure device and standing laparoscopic technique provides excellent observation of the surgical field ensuring hemostasis.

Benefits to/Impact on the Equine Industry:
This provides a safe economically viable method of treating these mares and enabling them to return to breeding soundness.

Take Home Message:
The LigaSure vessel sealing device provided adequate hemostasis for removal of larger neoplastic ovaries in standing mares.

Year Completed: 2005

Published Manuscripts/Abstracts: