

Animal Reproduction and Biotechnology Laboratory

Colorado State University

COLLEGE OF VETERINARY MEDICINE
AND BIOMEDICAL SCIENCES



2011 Edition

DEPARTMENT OF BIOMEDICAL SCIENCES

Advancing the Reproductive Sciences

In 1990, ARBL became the first University program to be designated a Program of Research and Scholarly Excellence by the Colorado Commission on Higher Education.

Elk in Rocky Mountain National Park are being studied by Terry Nett, Ph.D., to determine if fertility can be controlled using nonsurgical approaches.

The Animal Reproduction and Biotechnology Laboratory at Colorado State University is dedicated to advancing the reproductive sciences through research, education, clinical service, and outreach. The world-renowned center investigates basic reproductive biology to better understand normal function, as well as examining factors that challenge reproductive success, and developing products and procedures to enhance reproductive health for both humans and animals.

Set in the foothills of the Rocky Mountains, ARBL brings together a diverse group of researchers with international reputations. Attracting visiting scientists from around the world, the laboratory is a vibrant center of collaborative research, clinical services to an international clientele, and comprehensive academic programs that engage undergraduate, graduate, professional veterinary medical, and postdoctoral students. Popular one- to four-day continuing education programs bring to the campus assorted groups of participants, from breeding-farm managers to horse owners to practicing veterinarians.

ARBL was established in 1949 and in 1990 became the first University program to be designated a Program of Research and Scholarly Excellence by the Colorado Commission on Higher Education. The designation recognizes ARBL's legacy of research excellence, interdisciplinary faculty, international prestige, and continual grant funding since its inception, including a National Institutes of Health training grant that has been funded for more than 35 years. More recently, a United States Department of Agriculture National Needs Fellowship grant was awarded to the ARBL to train underrepresented minority Ph.D. students. State-of-the-art facilities and an international reputation of excellence have kept ARBL at the forefront of its

field, attracting high-caliber faculty and graduate students to further advance its mission.

Primary Areas of Research

Specialized areas of research at ARBL include fertility, sterility, and contraception; reproductive endocrinology, pregnancy, embryo-fetal development and stem cell biology; environmental reproductive toxicology; reproductive cancers; assisted reproductive technologies; and infectious disease. Currently, ARBL has 17 faculty members in the Department of Biomedical Sciences directly affiliated with its programs, three faculty members from the Department of Animal Sciences, and one from the Department of Clinical Sciences; 22 additional CSU faculty members collaborate with ARBL. Research programs reflect the areas of expertise and specialty of each of these individuals, as well as interdisciplinary investigations that bring together unique sets of skills to pursue new fronts in reproductive science and infectious disease.

Academic Programs

ARBL faculty members are actively engaged in the education of undergraduate, graduate, postdoctoral, and professional veterinary students at Colorado State University. While in-classroom

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Dear Friends and Colleagues,

The key to success for any living species on Earth is the ability to reproduce, whether from a simple cell division or from a complex biological dance between members of the opposite sex. Though fundamental to survival, reproduction often can present challenges. Environmental toxins weaken spermatozoa; aging compromises the health of the egg; illness and accidents threaten the preservation

of valuable genetic material; and inefficiencies in reproduction result in wastage in agricultural animals. A different set of problems arises when reproduction is too successful and overpopulation results in any one species, upsetting nature's delicate balance.

The Animal Reproduction and Biotechnology Laboratory is an interdisciplinary program that includes faculty members from three departments: Biomedical Sciences, Clinical Sciences, and Animal Sciences. The Equine Reproduction Laboratory is a program within ARBL. Two ARBL faculty members are University Distinguished Professors and one is a member of the National Academy of Sciences. The Hill Professorship, Traubert Professorship, Alexander Chair, and Iron Rose Ranch Chair are held by ARBL and ERL faculty. More than 100 other faculty members and scientists collaborate with ARBL at local, regional, national, and international levels.

Several research and graduate training focal groups exist within ARBL including: Assisted Reproductive Technologies; Fertility, Sterility, and Contraception; Reproductive Endocrinology; Reproductive Toxicology; Pregnancy, Development, and Stem Cell Biology; Reproductive Cancers; and Infectious Disease. These groups were developed largely based on discoveries in reproductive endocrinology, genomics, proteomics, and animal disease.

Even with the recent decline in the U.S. economy and federal funding sources, total funding and revenue for ARBL has stabilized to approximately \$8.3 million per year over the last five years.

ARBL has an excellent history of developing relationships with industry and completing research that translates into applications in the private sector. For example, sexing sperm led to development of XY Inc., which recently was sold to another company. This transfer of technology resulted in a long-term royalty-revenue stream for CSU and ARBL. A more recent example is development of nonsurgical animal sterilization

and the formation of an ARBL startup company called Gonex. ARBL will continue to seek extramural funds and private donations for research and clinical activities, while creating a broader public awareness and greater appreciation of Colorado State University.

These activities broaden the perspective, research, and educational approach and collaborative nature of our students, faculty, and staff members. We ensure that technologies developed at ARBL and ERL serve the citizens of Colorado, the United States, and the world.

In addition to commercial services offered in equine theriogenology through ERL, ARBL houses other active service centers that include Electron Microscopy and Morphological Services, Large and Small Laboratory Animal Facilities, Endocrinology and Gene Expression Services, and Cryopreservation of Animal Germplasm Services.

The ARBL at Colorado State University is at the cutting edge of research into reproductive health, assisted reproductive technology, and developing new methodologies to improve reproductive success while seeking to preserve all the threads in the planet's web of life. We embrace the One Health Initiative, which strives to develop collaborations between physicians, veterinarians, and other scientific-health related disciplines. Our research extends beyond the animal kingdom and often has direct impact on human medicine, where our researchers are having a profound impact not only in reproductive medicine but also in applying their knowledge to advance research in cancer, infectious diseases, and zoonotic diseases. We also are training the next generation of reproductive specialists to continue this important work.

You're invited to explore what we do and get to know our distinguished faculty members and programs. As a program, we have an extensive international presence and work with collaborators worldwide. Our faculty members are the finest in the world in their areas of expertise. Our work is an integral part of the future sustainability of basic, translational, clinical, and outreach programs in the College of Veterinary Medicine and Biomedical Sciences at Colorado State University. We welcome your visit to our website at www.cvmb.colostate.edu/bms/arbl to learn more, or you may plan a visit to our laboratory facilities located on Colorado State University's Foothills Research Campus.

Best regards,

Thomas (Tod) Hansen

Director, Animal Reproduction and Biotechnology Laboratory

Animal Reproduction and Biotechnology Laboratory



ARBL uses small-animal models to study the genetic regulation of reproduction. These models are particularly valuable because researchers can manipulate the genome to study abnormal reproductive phenotypes.

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The Animal Reproduction and Biotechnology Laboratory newsletter is produced by the Department of Communications and Creative Services. The Animal Reproduction and Biotechnology Laboratory is housed in the Department of Biomedical Sciences within the College of Veterinary Medicine and Biomedical Sciences at Colorado State University.

The mission of ARBL is to excel through discovery, translation, and dissemination of knowledge in reproductive sciences and biotechnologies at local, national, and international levels. We approach this mission through ensuring the health and welfare of animal and human populations and setting high standards and values, such as demonstrating inclusiveness and diversity, encouraging and rewarding innovation, acting with integrity and mutual respect, and supporting excellence in teaching, research, service, and outreach.

Our Research Programs

Investigative programs in the Animal Reproduction and Biotechnology Laboratory emanate out of one or more research focal groups. Collaboration is key to the continuing success of these programs, and ARBL faculty members work with researchers from across departments, colleges, and universities nationwide, and even worldwide. Research today demands an integrated approach, with each member of each research team bringing their own strengths to create a unified program greater than the sum of its parts. In addition, ARBL works closely with the Colorado State University Research Foundation to move innovative ideas, products, and technologies off the lab bench and into the marketplace.

Research at ARBL is supported by internal and external funding from the National Institutes of Health, National Institute of Environmental Health Sciences, United States Department of Agriculture, and National Institute of Food and Agriculture, as well as private industry, other academic institutions, and through programs at Colorado State University. We also collaborate internationally and have an extensive visiting scientist program, helping to train researchers from around the world. Areas of research focus at ARBL are:

Assisted Reproductive Technologies

Fifteen former ARBL graduate and postdoctoral students direct human in vitro fertilization clinics, and many others hold academic appointments or are leaders in equine, bovine, porcine, and ovine biotechnology industries. Spinoff companies (e.g., XY Inc., Gonex) evolved from ARBL research in ART and related technologies. A dozen ARBL patents have been filed, and licensing agreements continue to be developed with industry and biotechnology companies. In addition to being well known for research associated with ART applications in humans, cattle, and horses, and approaches toward controlling or inhibiting reproduction in wildlife and pet animals, ARBL faculty have research programs that are directly connected to state, national, and international concerns regarding environmental toxicology and the impacts of pollutants on mammalian development and reproduction. Assisted reproductive technologies have been used extensively for humans and domestic animals to advance our knowledge of reproductive processes, promote reproductive efficiency, and preserve valuable genetics. Recent advances include the continued development of sex-sorting technologies and the development of advanced

procedures, such as oocyte transfer and intracytoplasmic sperm injection. Equine assisted reproduction is being used to produce offspring from mares and stallions that would otherwise be considered infertile and to preserve valuable bloodlines. Substantial developments have been made in oocyte transfer, embryo cryopreservation, intracytoplasmic sperm injection, and harvesting of gametes from valuable mares or stallions after death.

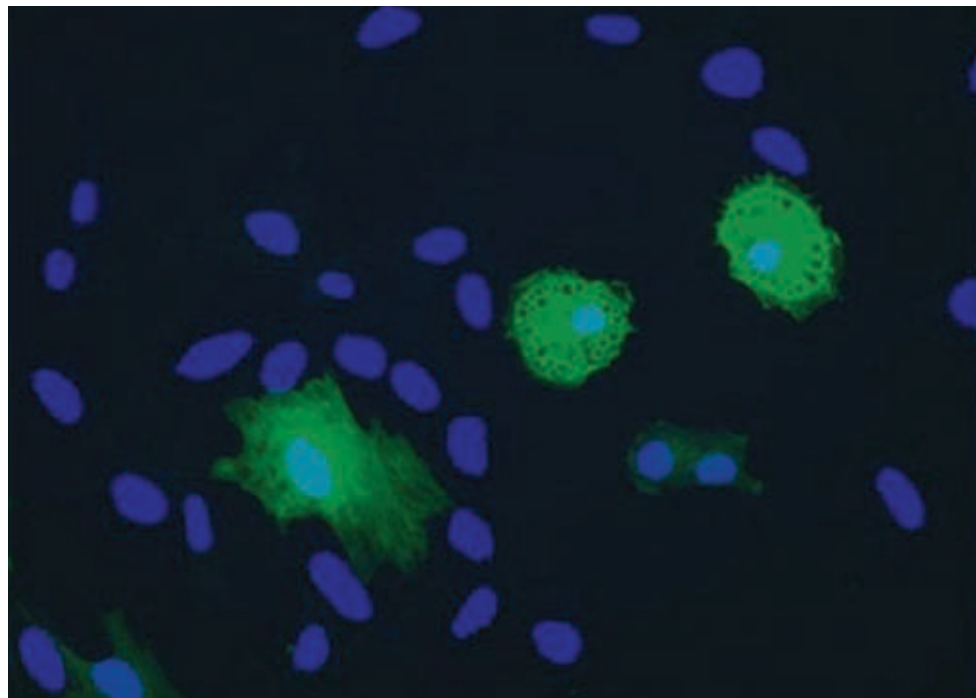
Fertility, Sterility, and Contraception

ARBL focuses on understanding the most basic reproductive mechanisms (e.g., hypothalamic-pituitary, ovarian, uterine, testicular, immune, etc.) and then applies this information to manipulating reproductive endocrinology and processes. The exploding human population is putting extreme burdens on all aspects of life and the environment. Some species are becoming endangered, while valuable resources are being consumed by excessive populations of other species. In addition, we will have to produce as much food in the next 40 years as we have in the entire history of mankind to avoid major famines and starvation in large areas of the world. *The common solution to these apparently conflicting issues is to develop better methods for regulating*

reproductive processes. Management of animal populations is a critical global challenge for the welfare of the planet, but one that scientists at CSU and in the greater CSU community are well-positioned to address. These challenges can be broadly grouped into three categories, each of which is impacted by growth of the human population: 1) Numerous *animal species are becoming endangered* for a variety of reasons, primarily as the result of loss of habitat stemming from the burgeoning human population or from environmental decisions that impact multiple species; 2) several *species have become overpopulated* due to restrictions that protect them or due to the fact that they live in areas where accepted management practices cannot be used to control their numbers; and 3) world *populations of domestic livestock* must be managed to maximize their efficient conversion of forage resources and byproducts (that are otherwise unsuitable for human consumption) into high-quality protein in meat, milk, and eggs.

Reproductive Toxicology

ARBL faculty members also have been investigating the developmental and long-term reproductive consequences of perinatal exposure to ubiquitous pollutants



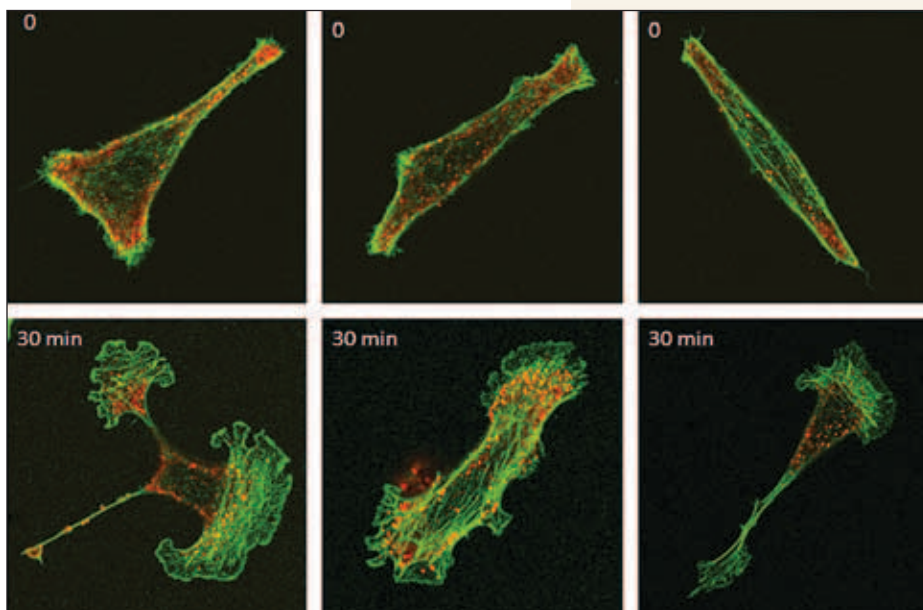
Isolated sheep trophoblast cells transfected with a lentivirus containing a cytomegalovirus promoter – green fluorescent protein reporter construct – are shown in this photo. Russ Anthony, Ph.D., and his research group used lentivirus-mediated stable transfection of sheep embryos to effectively “knock down” periattachment factor and determine its function in development of the conceptus and maintenance of early pregnancy.

using mammalian and amphibian models. Several recent reports indicate deteriorating trends in optimal reproduction in the human male, particularly in the industrialized world. Common pollutants having hormone-mimicking capabilities have been implicated in these phenomena. The compounds being studied include anti-androgenic pesticides (DDT and its metabolites; vinclozolin), water disinfection byproducts (dibromoacetic acid, bromochloroacetic acid), detergents (alkyl phenols), and plasticizers (phthalates). Two aspects of male reproductive function have been studied – sexual function, and efficiency and quality of spermatogenesis.

Pregnancy, Development, and Stem Cell Biology

The successful delivery of a healthy newborn is the ultimate goal of the mammalian reproductive system. A number of critical developmental windows must be negotiated for successful establishment and maintenance of pregnancy, as well as for optimal fetal development and delivery. Pregnancy is the physiological state where convergence of developmental biology, metabolism, stem cell biology, and reproductive biology occurs. It has been estimated that while only one percent of life is spent in utero, 80 percent of the lifetime cell divisions occur in utero. Consequently, complications in negotiating the “developmental windows” of pregnancy can result in recurrent miscarriage, preeclampsia, intrauterine growth restriction, congenital malformations, preterm delivery, and fetal demise.

Furthermore, it is now clear that the progression of pregnancy impacts the health and well-being of the offspring throughout life. This is supported by the plethora of data demonstrating the link between suboptimal in utero development and the predisposition for cardiovascular disease, diabetes, hypertension, and stroke (metabolic syndrome) as well as a growing list of mental disorders including autism, schizophrenia, and depression. Faculty research efforts associated with this focal group span initial embryonic development through parturition, and include projects on maternal-to-zygotic genome transition; maternal recognition of pregnancy; the regulation of trophoblast stem cell differentiation, implantation, and placentation; primordial germ cell and gonad development; gonad and cardiac development in normal and compromised pregnancies; the impact of environmental stressors; toxins and viral infections on fetal and postnatal development and well-being; and the regulation of uterine contractions with the onset of labor.



Reproductive Cancers

The study of cancer, another national concern, was recently formalized as a CSU Supercluster program. About 83,000 women in the United States are diagnosed annually with cancers affecting the reproductive organs (American Cancer Society). In males, there are an estimated 218,000 new cases and 27,000 deaths each year due to prostate cancer. Cancers of the reproductive system, such as prostate, breast, and testicular cancer are being studied by ARBL faculty. In 2010 alone, 207,000 new cases of breast cancer will be diagnosed in women and nearly the same number of prostate cancers in men. Colorado State University is home to the Cancer Research and Treatment Supercluster, a collaborative network of public and private partnerships, including ARBL researchers, to improve basic cancer research, diagnostics, treatments, and cures.

Infectious Disease

The impact of viral diseases on fetal growth restriction, the fetal and maternal immune system, and postnatal health and viability is a focus of ARBL faculty members. Research in this area includes transplacental viral infection of the fetus and mechanisms of action of bovine viral diarrhea virus on maternal and fetal immune cells and intrauterine growth restriction of the developing fetus. BVDV infections result in a loss of \$400 million annually to beef and dairy producers in the United States. The Animal Disease Laboratory, a major component of CSU’s first supercluster (Infectious Disease), is adjacent to ARBL and managed by an ARBL faculty member, Dr. Richard Bowen. Dr. Bowen contributes to research and teaching in reproductive biology, serves as the primary ARBL veterinarian, and is an international leader in the study of pathogenesis and transmission of West Nile virus, rabies, Venezuelan encephalitis, and avian influenza viruses. His laboratory also develops and provides animal models for development of vaccines and therapeutic agents.

The release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland in response to gonadotropin releasing hormone (GnRH) is essential for normal reproductive physiology in the male and female. Understanding how gonadotrope cells in the pituitary gland are able to respond to GnRH and release these hormones may provide insight into mechanisms of infertility as well as novel forms of contraceptives. Colin Clay’s laboratory recently described that GnRH elicits reorganization of the actin cytoskeleton in gonadotrope cells and mobilization of LH secretory granules. Primary cultures of ovine pituitary cells were either untreated (0 min) or treated for 30 min with 2 nM GnRH. Cells were then fixed and stained with phalloidin (green) which stains actin filaments and immunostained for LH (red). Infertility as a result of insufficient LH or FSH release may be due to inappropriate cytoskeletal reorganization.

In addition to on-site learning programs, ARBL features distance learning and continuing education courses. A complete course listing and schedule is available at www.cvmbs.colostate.edu/bms/arbl/.



Dr. Elaine Carnevale (right) shows students an ultrasound at the Equine Reproduction Laboratory.

Academics at ARBL Takes Experiential Learning to New Levels

Tomorrow's scientists must be innovative, critical thinkers who are able to understand unrelated disciplines to address questions of concern. They must be skilled in many research areas and able to communicate with diverse audiences. We train students to be considerate of animal welfare and scientific ethics. These strategies are used to refine the leadership, management, critical thinking, and problem-solving skills of the trainees. Our students become teachers through serving as mentors for newer students, and through structured teaching requirements.

In addition to the NIH training grant that has been active for the past 35 years, ARBL faculty members also manage the Merck Foundation training grant which provides research experience for up to 12 professional DVM students per year. ARBL has developed new relationships with the Bridges to Doctorate Program and recently received funding to support three underrepresented Ph.D. students through the USDA NIFA National Needs Fellowship program. Teaching and learning do not take a back seat to research at the Animal Reproduction and Biotechnology Laboratory, but are intertwined with the investigative process. Undergraduate, graduate, and professional veterinary medical students; interns, residents, and postdoctoral fellows; and practicing professionals and producers – all share an experiential learning environment at ARBL.

Undergraduate, graduate, PVM, and postdoctoral student research experiences are distinctive and enhanced because of outstanding

faculty teachers and mentors and state-of-the-art facilities and research instrumentation at ARBL. In addition to classroom coursework at the undergraduate, graduate, and professional veterinary level, ARBL faculty serve as mentors to part-time hourly, work study, and honors undergraduates who work directly with faculty members and their staffs. Graduate students and postdoctoral students assist in training undergraduate students, including teaching them to care for hundreds of horses, sheep, cattle, and small laboratory animals, as well as completing highly sophisticated tasks in the laboratory. These tasks may include research technology based in histology, biochemistry, molecular biology, and physiological studies. Because the ARBL is involved with basic, applied-translational, and clinical research, and has strong relationships with biotechnology companies and industry, our students are trained to think in broad, global terms, rather than with narrow disciplinary perspectives.

Graduate students work closely with their advisers to devise programs of study and participate in cutting-edge research while being trained on the latest technologies and encouraged to advance their own novel approaches to unique research investigations. Graduate students also work closely with clients, gaining practical experience in clinical service while completing coursework that meets the needs of today's professionals in reproductive physiology and equine reproduction.

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Clinical Services and Service Laboratories

The Animal Reproduction and Biotechnology Laboratory, and its Equine Reproduction Laboratory, house numerous laboratories and clinical services for both internal and external clients.

Reproductive Endocrinology Laboratory

The Reproductive Endocrinology Laboratory conducts various assays to study reproductive function of many animal species. Radioimmunoassay techniques are used to quantify hormones to evaluate pituitary function and target organ response. The lab offers tests to determine spay/neuter/cryptorchid status, pregnancy status, occurrence of ovulation, and presence of tumors of the reproductive system, as well as other more specialized analytical technologies.

Sperm Morphology Services

Sperm Morphology Services provides clinical and research support services to a broad-based clientele that includes veterinarians, physicians, research scientists, and clinical clients. The laboratory evaluates male reproductive tissues from a variety of species including laboratory, domestic, and wild animals. Client services include evaluation of semen for sperm morphology and ultrastructural cytopathology.

Equine Reproduction Laboratory

Approximately \$1.6 to \$2.2 million annually was earned in revenue during the last five years because of the equine commercial theriogenology clinical services through ERL. The success in this ARBL program illustrates how basic science can be translated to clinical practice. ERL develops new technologies and approaches that are often, but not always, based on similar

approaches that were developed in cattle and sheep. ERL also has the best professional veterinary medicine equine theriogenology training program in the nation because of the unique access to horses and the outstanding ERL faculty and staff.

ERL Mare Services

The Mare Clinical Services provides horse owners with the most current technology available for the reproductive management of their mares. Services include breeding by live-cover and artificial insemination with fresh, shipped-cooled, or frozen-thawed semen. Depending on the needs of the owners, breeding services can be provided on an outpatient (mares trailer-in) or inpatient basis. Embryo transfer services are also provided for mares that might not be capable of carrying their own foals to term or if more than one foal is desired from a mare in a given season.

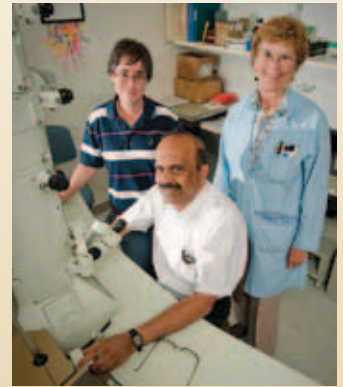
ERL Stallion Service

The ERL offers a full range of commercial stallion services including breeding soundness evaluation of the stallion; semen collection, evaluation, and processing for fresh or cooled-transported use; and infectious disease diagnostic testing. A related area is the Equine Semen Freezing Service, which provides equine semen freezing and storage approved by the USDA for international export.

ERL Advanced Assisted Reproductive Technologies

Assisted reproductive technologies available include oocyte transfer and intracytoplasmic sperm injection. Owners of mares that are not capable of becoming pregnant or producing

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Dr. Rao Veeramachaneni (center), Jennifer Palmer (left), and Carol Moeller (right) manage ARBL's Sperm Morphology Services and the transmission electron microscope.

Visit our website for additional information about our services:

www.cvmbms.colostate.edu/bms/arbl/

Newborn foal at the ERL being attended by clinical staff members (left to right) Drs. Jason Bruemmer, Erica Gee, Pat McCue, and Christianne Magee.



Services

Faculty

As a program within the College of Veterinary Medicine and Biomedical Sciences, ARBL enjoys unparalleled access to experts throughout the health spectrum.

Our faculty includes leading authorities in infertility, conception, gamete biology and cryopreservation, trophoblast stem cell and developmental biology, embryo and fetal mortality, epigenetics, intrauterine growth restriction, fetal basis of adult disease, parturition and preterm labor, prostate and testicular cancer, and assisted reproductive technologies. ARBL also hosts international experts in infectious disease, particularly zoonotic disease.



Russ Anthony

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Research Focus

- Early conceptus development and implantation, including the regulation and function of proline-rich 15 in sheep, human, and mouse implantation.
- Placental and fetal development in intrauterine growth-restricted pregnancies, including chronic in vivo catheterization and instrumentation to investigate altered placental and cardiac function in IUGR pregnancies.

Impact

- A better understanding of implantation, placental function, and fetal development in normal and compromised pregnancies will not only enhance neonatal care, but may improve health and wellness throughout life.



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Research Focus

- Understand the molecular regulation of gene expression and function during fetal gonad development and differentiation in mouse and sheep models.
- Study the molecular changes that are associated with abnormal transformation of gonadal somatic cells and germ cells following perinatal exposure to environmental chemicals.
- Investigate the genetic network controlling pluripotency in stem cells of reproductive tissues.
- Stem cell factors and noncoding RNAs in ovarian cancer.

Impact

- A better understanding regarding the normal processes controlling gonad development, and the effects of exogenous exposures on gene expression and function during fetal gonadal programming, can provide much needed insight into adult onset of reproductive disorders.



Richard Bowen

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Research Focus

- Pathogenesis of zoonotic pathogens in their natural hosts including West Nile virus in horses and birds, avian influenza virus in birds, and rabies in bats.
- Development of animal models for investigations into vaccine and therapeutic agents.

Impact

- Enhance the understanding of major zoonotic infectious diseases, which is necessary to implement control strategies.



Jason Bruemmer

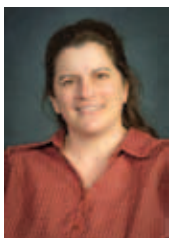
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Research Focus

- Semen preservation of the stallion, including new ways to preserve semen such as cryopreservation and freezing of semen harvested from the epididymis.
- Cell differentiation with regards to maternal recognition and maintenance of pregnancy in the mare, including the use of equine genetics to better understand the genetic changes that occur when a mare becomes pregnant.

Impact

- A better understanding of the more unique aspects of early pregnancy in the horse may help to better prevent early embryonic loss and enhance reproductive outcomes for at-risk mares.
- Improved semen preservation will enhance the viability of stallion genetic material, particularly in cases of catastrophic injury or diminished reproductive performance.



Elaine M. Carnevale

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Research Focus

- Understanding of changes within the follicle and oocyte that occur with maternal aging and their effect on fertility and health of the offspring.
- Optimization of advanced assisted reproductive technologies, such as oocyte transfer and intracytoplasmic sperm injection, for the production of offspring from subfertile mares and stallions.

Impact

- Study of reproductive aging and assisted reproductive techniques in the mare will help in understanding of reproductive aging in other species, including humans.
- Long-term goal is to develop methods to improve reproductive success in the older female.



Colin M. Clay

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Research Focus

- Hypothalamic and ovarian regulation of the anterior pituitary cells that produce luteinizing hormone and follicle-stimulating hormone.
- Molecular mechanisms underlying transcriptional regulation of the GnRH receptor gene during the period surrounding ovulation.
- Intracellular signaling cascades elicited by GnRH binding to the pituitary GnRH receptor.
- Role of estrogen receptor in regulating GnRH receptor and gonadotropin production in the anterior pituitary gland.

Impact

- Improved understanding of the molecular mechanisms underlying the effects of GnRH and estrogen on gonadotropin production by the anterior pituitary gland will help to elucidate fertility regulation and reproductive function.
- Unraveling the mysteries of GnRH, estrogen, and their cognate receptors may help pave the way to improved treatments for a host of hormone-dependent diseases, including fibroid tumors, endometriosis, and cancers of the breast, prostate, testes, and pituitary.



David Denniston

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Research Focus

- Cryopreservation in equine embryos.
- Superovulation in mares.
- Several endocrinology-based projects.
- Identification of characteristics of equine sperm cells that may contribute to differences in an individual stallion's sperm to survive cryopreservation and thawing.
- Development of a progesterone assay to quickly and easily measure progesterone levels in a mare.

Impact

- Enhance reproductive viability of stallions and mares in the equine industry, particularly with regard to improved outcomes for assisted reproductive technologies.
- Progesterone assay would be useful in determining if it is necessary to supplement progesterone to a mare during early pregnancy.



James K. Graham

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Research Focus

- Cryopreservation of cells and improving cryosurvival rates of cells.
- Gamete physiology and cryobiology, emphasis on membrane biochemistry and function.
- Changes in the membranes and cytoplasm induced by cryoprotectants and low temperatures, and the interactions between frozen/thawed spermatozoa and the epithelium of the female tract and/or egg.
- Development of oral contraceptives to control populations of feral pest animals, including pigs and geese.

Impact

- Improving the cryosurvival of sperm, oocytes, and embryos would impact the movement of genetics around the world to improve animal agriculture, as well as preserve the genetics of exotic and endangered species.
- Effective oral contraceptives can help implement control strategies for species that currently destroy agricultural fields and the environment.



Hyungchul Han

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Research Focus

- Maternal nutritional status and its impact on fetal/neonatal growth and development, including how suboptimal fetal development affects postnatal growth and increases the incidence of adult diseases later in life.
- Nutrient restriction during early stages of fetal development, or through late gestation, and its effects on offspring growth.
- Understanding basic mechanisms and applying this knowledge to nutrient utilization during fetal/postnatal growth.

Impact

- Proper management of nutritional status during early gestation will greatly improve offspring health. Developing feeding strategies during early pregnancy will help manage the health and productivity of ruminant livestock.

Faculty



Thomas R. (Tod) Hansen

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Research Focus

- Maternal uterine and ovarian responses to early pregnancy.
- Mechanisms associated with implantation of the embryo and placentation.
- Maternal and fetal responses to fetal persistent infection with bovine viral diarrhea virus.

Impact

- Greater understanding of early pregnancy and embryo-maternal signaling may help to reduce reproductive losses.
- Develop strategies designed to prevent preeclampsia and intrauterine growth restriction for improved fetal health and development.
- Ongoing studies of persistent fetal BVDV infection will contribute to establishing methods of control for the most devastating bovine viral disease in the United States, with losses of more than \$400 million per year.



Patrick M. McCue

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Research Focus

- Understand the pathophysiology of reproduction in the mare, including identification of biomarkers for ovarian tumors and uterine infections as well as applied aspects of hormone therapy and endocrinology.
- Determine optimal techniques for collection, storage, and transfer of equine embryos.

Impact

- New techniques or diagnostic tests can be directly applied in the equine breeding industry to enhance reproductive success.



Terry M. Nett

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Research Focus

- Obtain a better understanding of factors that regulate synthesis and secretion of hormones that control reproduction, particularly the gonadotropin follicle-stimulating hormone and luteinizing hormone.
- Develop methods to sterilize both male and female mammals by injection of cytotoxic chemicals.

Impact

- A treatment that can permanently eliminate the function of gonadotropes without affecting other cells in the anterior pituitary gland would have a variety of uses ranging from nonlethal control of wild animal populations to treatment of hormone-dependent cancers in humans.



Gordon D. Niswender

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Research Focus

- Understand the mechanisms that control luteal secretion of progesterone to help reduce early embryonic wastage in food-producing animals.

Impact

- Understanding the mechanisms by which luteolytic and lutolytic hormones regulate the function of the corpus luteum and the secretion of progesterone is central to controlling reproductive processes. A greater understanding of these processes will help reduce embryonic wastage and ultimately increase and optimize food production.



Deborah A. Roess

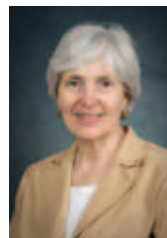
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Research Focus

- Molecular motions of luteinizing hormone receptors on luteal and Leydig cells as relates to regulating functions of reproductive organs.
- Evaluation of effects of LH receptor density on receptor function and identification of other cell membrane proteins that interact with LH receptors on single cells.
- LH receptor interactions that regulate the receptor's response to hormone under physiological conditions.

Impact

- A greater understanding of how hormones regulate functions of the reproductive organs can lead to new discoveries in addressing reproductive concerns in both humans and animals.



Barbara M. Sanborn

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Research Focus

- Define the molecular signal transduction pathways of hormone action relating to the control of differentiation and intracellular calcium dynamics in the myometrium (uterine smooth muscle).
- Understand the molecular aspects of hormone action and signal transduction, the mechanism of action of relaxin in the uterus, and the biochemical aspects of androgen action in Sertoli cells and testicular germ cells.

Impact

- A greater understanding of reproductive hormone actions and how hormones work at the molecular level will help to advance reproductive medicine, particularly as these affect women's health issues.



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Research Focus

- Improve methods of cryopreserving bovine and equine oocytes and embryos.
- Optimize procedures for sexing sperm without compromising viability.
- Understand roles of micro-RNAs during early development of bovine embryos.
- Develop procedures for pre-implantation genetic diagnosis of equine embryos.

Impact

- Commercial availability of bovine sexed semen in many countries.
- Cryopreservation of embryos has led to a number of practical applications.
- Improved procedures for producing embryos in vitro. Studies also may eventually lead to improved outcomes with conventional reproduction, for example, fewer birth defects.



Cynthia A. Smeraski

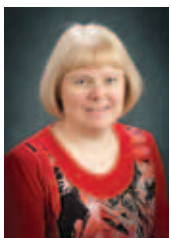
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Research Focus

- Viral diseases that affect the nervous system, including West Nile virus infection in birds and mammals.
- Pathogenesis of WNV with an emphasis on delineating the mechanisms of neural invasion using a combination of neuroanatomical and immunohistochemical techniques along with virologic assays and behavioral measurements.
- Determine any neurologic manifestations that may lead to lasting anatomical, behavioral, or neurophysiological deficits in species that survive the disease.

Impact

- An improved understanding of the neuropathology of WNV and other viral diseases that affect the nervous system may lead to better treatments and improved health outcomes.



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Research Focus

- Mechanisms of the immune response to viral infections, focusing on the fetal persistent infections and their consequences for the health of the mother and fetus in bovine viral diarrhea virus infected cows.
- Search for biomarkers distinguishing cows with fetuses persistently infected with bovine viral diarrhea virus.

Impact

- Understanding of the mechanisms of persistent viral infections can lead to finding ways to prevent the deleterious consequences for maternal and fetal health in both animal and human infectious diseases.
- Diagnostic and timely elimination of cows persistently infected with BVDV fetuses will help prevent the spread of BVDV in cattle herds.



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Research Focus

- Cellular and molecular mechanisms of brain sexual differentiation.
- Cellular and molecular bases of the development of neurons that synthesize gonadotropin releasing hormone and related neurons that contribute to their development.
- The formation of nuclear structures using the ventromedial and paraventricular nuclei of the hypothalamus as model systems.
- Development of methods and technologies that image molecular signaling in vitro.

Impact

- Develop an understanding of sex differences in function and susceptibility to sex-selective health issues.
- Advance study of reproductive function, and how fertility is regulated.
- Greater understanding of how key cell groups in the nervous system develop, and how fetal antecedent events program adult function or disorder.



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Research Focus

- Delineate if deteriorating reproductive health in males is mostly idiopathic, as male infertility often is labeled, or a consequence of exposure to ubiquitous environmental pollutants.
- Using appropriate animal models, identify vulnerable windows and critical targets in the development and differentiation of male reproductive system.

Impact

- A better understanding of the causality and the pathogenesis of male reproductive anomalies such as cryptorchidism and testicular cancer will help mitigate the increasing incidence of male reproductive problems worldwide.



Quinton Winger

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Research Focus

- Study the regulation of proliferation, differentiation, and pluripotency of trophoblast stem cells, and the role of stem cells in healthy and abnormal placentas.
- Identify the genes and their pathways necessary for specification, migration, and proliferation of primordial germ cells.

Impact

- Investigating the genes required to produce a healthy placenta is important for understanding the causes of various reproductive diseases and disorders.
- A better understanding of trophoblast cell growth and differentiation in early pregnancy will improve the diagnosis and treatment of placental insufficiency due to inadequate trophoblast growth and will reduce the number of premature births.

Advancing the Reproductive Sciences *(continued from Page 1)*

learning is significant, students at ARBL also have the advantage of teaching and research experiences ranging from the care of approximately 600 horses, and sheep, cattle, and small laboratory animals to field trials with cattle, elk, deer, wild horses, and bats. Experiential learning also includes working with highly sophisticated equipment in histology, biochemistry, endocrinology, molecular biology, and physiology. ARBL offers special academic programs for high school and community college students as well as outreach education through short courses designed for producers and veterinarians.

Clinical Services

Clinical services at ARBL evolved from bovine theriogenology services in the 1970s that were focused on bovine gamete preservation and embryo transfer. These bovine services have been largely replaced with equine theriogenology services managed through the Equine Reproduction Laboratory, including stallion breeding, mare breeding, foaling, and Assisted Reproductive Technologies Services. Through ongoing research in collaboration with ARBL and clinical programs, ERL enhances and expands clinical services to best serve its clients and to help preserve important equine genetics.

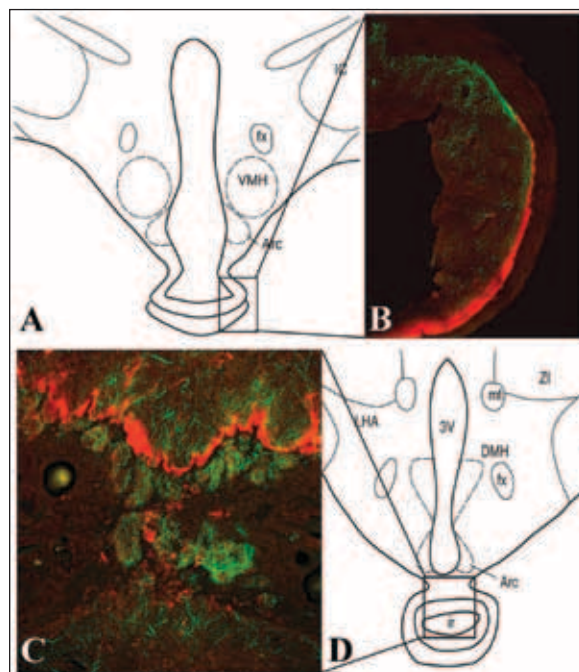
Our mission is to excel in discovery, translation, and dissemination of knowledge in reproductive sciences and biotechnologies at the local, national, and international levels.

Continuing Education

Early in its history, the Animal Reproduction and Biotechnology Laboratory delivered a full suite of short courses to livestock interests and veterinarians. Courses initially focused on artificial insemination in cattle and have since expanded to include a diverse set of offerings such as foaling and care of the newborn foal, techniques for handling and using frozen semen, reproductive management and artificial insemination, equine embryo transfer, diagnostic imaging in reproduction, and more. ARBL continually strives to meet demands in the marketplace through its continuing education program, as well as to develop new programs as reproductive advances move from the laboratory bench to the ranch and farm.

The Animal Reproduction and Biotechnology Laboratory's mission is to excel in discovery, translation, and dissemination of knowledge in reproductive sciences and biotechnologies at the local, national, and international levels. From its agricultural beginnings, ARBL has grown to become a world-class center of innovative research in animal and human reproductive health, as well as an important contributor to the body of knowledge of infectious disease.

Kisspeptin is a small peptide that may be the signal controlling seasonal reproduction in the horse as well as possibly communicating factors for fetal-placental well-being. This work was initiated by Dr. Christianne Magee, in collaboration with members of the ARBL and ERL. The group provided the first anatomical and biological evidence for a role for kisspeptin in the mare. Current research objectives include developing a radioimmunoassay for kisspeptin and using non-terminal methods to study the ability of kisspeptin to release gonadotropin releasing hormone from the hypothalamus (part of the brain). GnRH causes the secretion of luteinizing and follicle stimulating hormone from the pituitary gland. Representative drawings of the equine medial basal hypothalamus (MBH; A and D), specifically confocal images focusing on the median eminence (B) and infundibular stalk (C; acts as the connection between the hypothalamus and the pituitary gland). Immunoreactive neurons expressing kisspeptin (red) and GnRH (green) are highly associated with each other in the horse brain, suggesting that they communicate with one another. X10 magnification; Arc, Arcuate nucleus; DMH, dorsomedial nucleus; fx, fornix; ir, infundibular recess; LHA, lateral hypothalamic area; mt, mammothalamic tract; ME, median eminence; 3V, third ventricle; VMH, ventromedial nucleus; ZI, zona incerta.





2010 D.V.M.s and D.V.M. students in graduate studies at the ARBL. Shown from left to right are: Dr. Brett Webb (D.V.M. Anatomic Pathology Resident/Ph.D. student), Dr. Christianne Magee (D.V.M.-ERL Resident/M.S. and ARBL Ph.D., Postdoctoral student), Dr. KC McBroom (D.V.M./Ph.D. student), Dr. Alfredo Antoniazzi (D.V.M./Ph.D./Postdoctoral student), Dr. Cristina Weiner (D.V.M. Comparative Animal Resident, M.S. student), Dr. Christian Urias (D.V.M., Ph.D. student). Not shown are Drs. Ryan Ferris, Alicia Lindholm, and David Scofield (D.V.M.-ERL Residents and M.S. students).

Academics at ARBL (continued from Page 6)

Active and experiential learning is encouraged at all levels, as is assimilation of research results and preparing reports, such as poster and platform presentations at local, regional, and national meetings. The ARBL student research experience attracts and trains a diverse array of students who are prepared, motivated, well-rounded, and exceptionally educated. This rigorous and demanding training actively engages students, while ensuring their well-being.

Examples of support for these formal research training experiences include the Hughes Undergraduate Research Program, NIH-sponsored Science Motivation Program for undergraduate students, and the NIH and Merck-Merial programs for beginning students of veterinary medicine. We also have provided hands-on training for large numbers of Front Range Community College students. ARBL faculty members have served as principal investigators and/or training mentors for an NIH training grant that has supported four Ph.D. graduate students and two postdoctoral fellows per year for the past 35 years, as well as a Merck Foundation training grant that provides research experience for up to 12 DVM students per year. Preparation and administration of these education grants represents a major commitment by ARBL faculty members. We also have a stellar record in placing our graduate students in careers with opportunities for advancement and fulfillment. Finally, we have several students supported by scholarships and who have received local and national academic awards. Many of our students also network with other scientists

through extensive collaborative research completed by the ARBL faculty members

In addition to students at Colorado State University, ARBL's outreach programs and short courses reach veterinary professionals, producers and owners, visiting scientists, and human-health professionals. A variety of short courses are offered through ARBL, including courses in Assisted Reproductive Technologies, foaling, reproduction management, and embryo transfer.

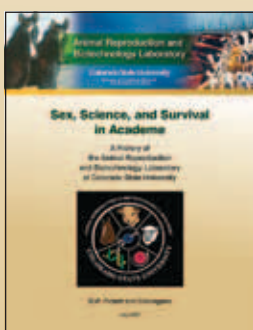
ARBL Clinical Services

(continued from Page 7)

embryos for transfer have the option of oocyte transfer. ICSI involves microinjection of a single sperm cell into the mature donor egg, resulting in a fertilized egg. An important application of ICSI is to circumvent certain kinds of stallion infertility. In the case of catastrophic illness or sudden death of a mare, the ERL has developed techniques to harvest and ship the mare's ovaries and generate pregnancy once the eggs are grown to maturation and fertilized.

For owners interested in foaling out their pregnant mares, the ERL accepts a limited number of mares into its Foaling Service. The service includes 24-hour monitoring, foaling stalls, screening to predict foaling, a veterinarian on call for each foaling, and medical services including umbilical cord care, evaluation of passive immunity transfer, deworming the mare to reduce the risk of parasite infestation, and postpartum examinations of both the mare and the foal.

News



Coming Soon

The Animal Reproduction and Biotechnology Laboratory at Colorado State University has been around, in one form or another, since the early 1940s. In that time, ARBL has made many groundbreaking discoveries and seen many momentous events. After years of combing the archives, we're proud to offer *Sex, Science, and Survival in Academe*, the complete ARBL history book. The book was written by longtime CSU professor, researcher, and administrator, B.W. Pickett, along with several colleagues to document the history of an important phase of animal reproductive research, teaching, outreach, philosophy, and service; and the benefit of those activities to the livestock industries and public that we serve.

Please call (970) 491-3456 or visit www.cvmb.colostate.edu/bms/arbl for information on ordering your copy of this book.



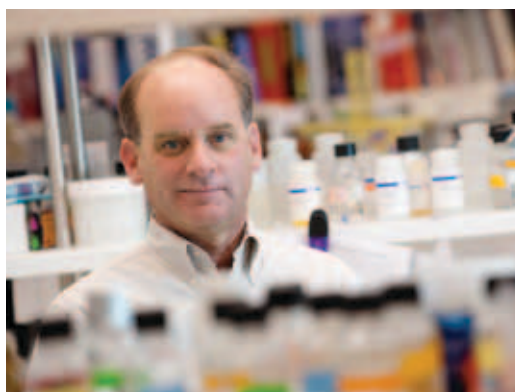
ARBL office staff, left to right: Brenda Martin, Sarah Legare, Kay Gallatin, and Sallie Varner.



Shown at left is Dr. Gordon Niswender (former ARBL faculty member) and above, Dr. Joanne Richards from Baylor College of Medicine. Dr. Richards delivered the 2010 Niswender Lecture in Reproductive Endocrinology.



Niswender Minisymposium. Former Head of Biomedical Sciences, Dr. Barbara Sanborn, presents a chair to Dr. Niswender in honor of his many years of service as a University Distinguished Professor. On this occasion, the ARBL hosted several of Dr. Niswender's former students who presented research completed while working in Dr. Niswender's laboratory. Former students in attendance who delivered lectures were: Dr. Char Farin, Dr. Dean Hawkins, Dr. Milo Wiltbank, Dr. Bill Silvia, Dr. Ric Silva, and Dr. Pat Hoyer. Dr. Keith Inskeep delivered the closing lecture, which highlighted Dr. Niswender's career at CSU.



Dr. Colin Clay, an ARBL faculty member, was recently named Head of CSU's Department of Biomedical Sciences.



Dr. Pat McCue, second from left, a Professor in ARBL's Equine Reproduction Laboratory, was named the Iron Rose Ranch Chair in Equine Reproduction. This chair will enhance our work in equine reproduction. "This program is a core part of our overall program in veterinary medicine, which consistently ranks among the best in the world and contributes in significant ways to the advancement of human and animal medicine," said former Colorado State University President Larry E. Penley. The ARBL and the ERL sincerely thank the Iron Rose Ranch for their contribution to our program. Shown left to right: Dr. Thomas R. Hansen (ARBL Director), Dr. Patrick McCue (Iron Rose Ranch Chair in Equine Reproduction), Dr. Jerry B. Black (Director, Equine Sciences Undergraduate Program), Dr. Colin M. Clay (Head, Department of Biomedical Sciences).



Yes, I'd like to support the work of the ARBL and the ERL!

Gifts to the Animal Reproduction and Biotechnology Laboratory and Equine Reproduction Laboratory are used to fund scholarships, support research programs, and provide discretionary funds that are used where most needed. We appreciate all gifts made

to support our needs and goals. If you have questions, please contact Paul Maffey in the College of Veterinary Medicine and Biomedical Sciences Office of Development at (970) 491-3932 or at paul.maffey@colostate.edu.

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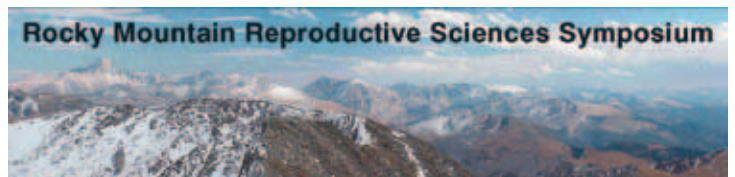
The Campaign for Colorado State University

ARBL Seminars

The ARBL hosts seminars in Physiology each Monday at 4 p.m. during the fall semester for the Division of Physiology in the Department of Biomedical Sciences. Seminars are held during the spring semester for the ARBL Graduate Student Training Program. The ARBL also hosts an annual lecture series to honor Dr. Gordon Niswender (Niswender Lecture in Reproductive Endocrinology) and an annual lecture series to honor former faculty member, Dr. Edward Squires (Squires Lecture in Equine Reproduction). Please visit our website for a listing of these seminars and other events: http://www.cvmbms.colostate.edu/bms/arbl/arbl_events.htm.

Short Courses

The Equine Reproduction Laboratory offers short courses ranging from foaling care to problem mares to artificial insemination. These one- and two-day seminars are geared toward horse owners and breeding managers who want to learn more about equine reproductive health. Please visit www.cvmbms.colostate.edu/bms/erl/erl_sc.htm for additional information and a complete course schedule.



The Rocky Mountain Reproductive Sciences Symposium is held each spring and is open to the public. Please visit our website for details, www.cvmbms.colostate.edu/bms/arbl/arbl_invitedlecture.htm.

The symposium was launched in Spring 2008 to allow CSU faculty to meet and discuss shared regional and local interests in research and clinical applications in reproductive sciences. Undergraduate, graduate, PVM, and postdoctoral student research experiences are greatly enhanced because of this collaboration with outstanding faculty teachers and mentors and ARBL's state-of-the-art facilities and research instrumentation.



Animal Reproduction and Biotechnology Laboratory
College of Veterinary Medicine and Biomedical Sciences
Department of Biomedical Sciences
1683 Campus Delivery
Fort Collins, CO 80523-1683



NNF Grant to Fund Fellowships

The National Needs Graduate and Postgraduate Fellowship Grants Program is designated for graduate degree programs and postgraduate training of the next generation of policymakers, researchers, and educators in the food and agricultural sciences. The grant will fund fellowships for students interested in pursuing doctoral degrees in food and agricultural sciences, specifically in reproductive sciences and biotechnology.

Working collaboratively with eligible higher education institutions, the NNF competitive grants program develops the intellectual resources necessary to preserve and advance food and agricultural systems in the United States as well as internationally.

"This grant will enhance our recruitment and retention of graduate students in the reproductive sciences and biotechnology, particularly underrepresented minority students," said Thomas Hansen, director of the ARBL, as well as a co-principal director of the three-year, ARBL-NNF grant.

The ARBL-NNF fellowship program is specifically designed for pre-doctoral students. Research focus areas include:

- assisted reproductive technology
- environmental reproductive toxicology
- fertility, sterility, and contraception
- pregnancy, development, and stem cell biology
- reproductive cancers and disease

The research training of ARBL pre-doctoral students is designed to develop a comprehensive understanding of philosophy of research, and mastery of diverse research techniques, and to encourage the development of the student as an independent investigator as well as a team researcher. Multidisciplinary doctoral training will expose students to CSU's research core in genomics and proteomics as well as to technology transfer and biotechnology companies.

ARBL-NNF applicants must be U.S. citizens or nationals and underrepresented minority students as specified in the NNF program. Applicants also must complete a master's or doctor of veterinary medicine degree prior to entry in the ARBL-NNF training program.