

**NUTRITION & ENVIRONMENTAL MANAGEMENT RESEARCH UNIT****Influence of heifer diet on growth of preantral follicles in bovine ovarian cortical cultures**

Scientist: R. A. Cushman, Ph.D.

**Background:** Reducing caloric intake is a proven method to increase lifespan and alter the ovarian reserve in mammals. Rats raised on 65% of maintenance during the peripubertal period have an increased number of primordial follicles in their ovaries. In preliminary data at USMARC, heifers developed on reduced caloric intake after weaning have an increased number of primordial follicles in their ovaries at breeding, indicating that the mechanisms controlling initiation of follicle growth have been altered by reducing intake.

**Project Description:** Ovaries will be collected at 9-, 12-, or 14-months of age from heifers (n = 6/diet) raised on one of two diets during the peripubertal period. Pieces of ovarian cortex will be placed in bovine ovarian cortical culture for 0, 2, 4, or 7 days to allow for activation and growth of the primordial follicles. Culture medium will be changed every other day. At the end of cultures, cortical pieces will be fixed and embedded in paraffin for evaluation of follicle growth and development. Primordial, primary, and secondary follicles will be counted and measured in a minimum of 5 sections per animal per day of culture. Histological data will be analyzed using the MIXED Procedure of SAS with diet, age, day, and the interactions as fixed effects, and animal as a random effect.

**Duties and Responsibilities:** The incumbent will be responsible for performing sectioning, staining, histological evaluation of bovine ovarian tissue from cortical cultures, counting follicle populations, and analyzing data. Further duties will include real-time RT-PCR to examine gene expression in ovarian tissue collected from these heifers.

**GENETICS, BREEDING, AND ANIMAL HEALTH RESEARCH UNIT****Evaluating Microbial Community Variation Associated With Bovine Respiratory Disease Complex in Cattle**

Scientist: T. G. McDanel, Ph.D.

Bovine respiratory disease complex (BRDC) is the most expensive disease in U.S. beef cattle costing the cattle industry over 1 billion dollars annually. Past efforts to reduce the incidence and severity of BRDC have been frustrated by complexity of the disease. However, recent advances in genomics (high density genotyping arrays and whole genome sequencing) have improved capabilities for identifying factors (variation in bacterial community) associated with complex diseases such as BRDC. The objective of this research proposal is to identify the bacterial species that may predispose cattle to becoming susceptible to BRDC.

The student selected for this project will identify bacterial species present in the U.S. Meat Animal Research Center disease population of cattle by learning and implementing a variety of laboratory methods. The student will accomplish this by first learning and using laboratory techniques that include basic microbiology techniques for working with bacteria, DNA and RNA extraction, polymerase chain reaction (PCR), and basic sequencing protocols. In the first two weeks, the student will become familiar with sequence analysis software by assisting a scientist to evaluate 16S sequence data collected from the 2012 Disease Resistant population last summer. In the remaining six weeks, the student will (1) extract DNA from the nasal samples collected in 2013 from the Disease Resistant population at USMARC (2) identify bacterial species present in the nasal samples through initial 16S sequencing of the DNA and (3) help in collection of lung tissue from abattoir to evaluate variation in the cattle genome associated with BRDC. In cooperation with other scientists at US Meat Animal Research Center, we have developed methods to sequence and analyze DNA sequence from bacterial samples. Therefore, we believe that the intern will be able to complete this proposed project in the eight-week time frame.

Applicants for this position should be interested in and have taken coursework that encompasses biology, microbiology, and genetics. Applicants should also be willing to learn laboratory techniques and how to use DNA information to improve cattle genetics.

**Host Immune Response associated with Bovine Respiratory Disease Complex (BRDC)**

Scientist: C. G. Chitko-McKown, Ph.D.

Despite advances in antibiotic treatments and vaccine development, bovine respiratory disease complex (BRDC) continues to cause significant losses to the cattle industry. Cattle are genetically diverse, and this variation appears to affect disease susceptibility. Our group seeks to exploit this variation with the following goals: 1) identify genetic and biological determinants of respiratory disease susceptibility, and 2) identify genetic-based measures to prevent and control cattle respiratory diseases. These goals are challenging, as respiratory diseases manifest through diverse pathogens, both bacterial and viral, environmental factors and host genetics. Understanding this complexity requires a multidisciplinary approach that addresses host genetic disease resistance and susceptibility

at the pathogen, cellular, and animal levels. This level of understanding is mandatory for devising genetic-based intervention strategies for controlling infectious respiratory diseases in cattle and other ruminants.

Our laboratory studies the innate immune response in food animals. We are currently analyzing plasma samples obtained from a population of cattle that were purchased at sale barns throughout the southeast and transferred to a feeding facility in Kansas. Blood was obtained from these animals over the course of a month allowing us to follow the cytokine profiles in these animals as they showed signs of BRDC. We hypothesize that we will be able to identify animals suffering from BRDC based upon increasing/decreasing concentrations of inflammatory mediators. The student selected for this project will have the opportunity to learn immunological techniques ranging from blood collection to immune cell isolation, tissue culture, immunological assay procedures, as well as molecular techniques such as RNA isolation and RT-PCR assay development and performance. The student will also have the opportunity to interact with other laboratories studying the genetic diversity of pathogens and of the host.

## MEAT SAFETY & QUALITY RESEARCH UNIT

### **Influence of Cattle Production Environment on Prevalence of Antibiotic Resistant Bacteria and Levels of Antibiotic Resistance Genes**

Scientist: J. W. Schmidt, Ph.D.

It is frequently assumed, without strong scientific evidence, that “antibiotic free” animal production practices result in lower levels of antibiotic resistant bacterial contamination of the environment and final meat products than “conventional” animal production practices that use antibiotics. While some studies have detected lower prevalence of antibiotic resistant bacteria in antibiotic free environments and products than conventional environments and products, other studies have found no significant differences. Regardless, the scientific conclusions drawn from most of these studies were limited since they frequently examined small sample sets and relied upon culture methods that excluded antibiotic resistance genes from uncultured bacteria.

This project will compare the levels of important antibiotic resistant bacteria (*Escherichia coli*, *Salmonella* sp., and *Enterococcus* sp.) and antibiotic resistance genes present in the feces of cattle raised under conventional production practices or those raised as antibiotic free. This project also will compare the levels of antibiotic resistant bacteria and antibiotic resistance genes present in the ground beef labeled as “antibiotic-free” or “USDA organic” and ground beef with no label claim.

Intern responsibilities may include: use of microbiological techniques for the enumeration and isolation of antibiotic resistant *E. coli*, *Salmonella*, and *Enterococcus* from fecal and ground beef samples; preparation of genomic DNA from fecal samples, ground beef samples, and bacterial isolates; performance of PCR assays for bacterial species confirmation and identification of antibiotic resistance genes; performance of biochemical tests for the species identification of bacterial isolates; and performance of antibiotic susceptibility testing.

### **Developing an Image-Based System for Sorting Feedlot Cattle into Optimum Marketing Groups**

Scientist: S. D. Shackelford, Ph.D.

There is generally a lot of variation in weight, size, muscling and fatness of cattle when they come into the feedlot. This results from the variation in breeds, genetic composition, age, backgrounding/feeding history, etc. Some companies have demonstrated the benefits of automated system for sorting cattle into more uniform groups but these have not gained widespread use due to cost and ease of implementation. USMARC scientists are testing a live cattle evaluation imaging system to facilitate sorting feedlot cattle into optimal marketing groups. This system will be installed at the USMARC feedlot and used to collect data to determine its usefulness for increasing feeding profitability by optimizing how long cattle are fed. An intern is needed to monitor operation of the system when cattle are processed through the USMARC feedlot working facility. The intern would be responsible for insuring data integrity and monitoring links between the imaging system and the EID reader. The intern will assist in collection of carcass data, at large-scale commercial beef packing plants, to evaluate efficacy of the system. Additionally, the intern will have a

variety of opportunities to participate in meat safety and quality research activities including instrument grading research projects, measuring meat tenderness with slice shear force, measuring retail meat color stability, and sampling and testing for microbial pathogens.

**REPRODUCTION RESEARCH UNIT****Evaluating the effects of neonatal environment on developmental programming of the reproductive tract in the female pig**

Scientist: C. A. Lents, Ph.D.

Lifetime productivity of swine is limited by the number of pigs a sow produces. The number of pigs that a sow can carry during gestation and that are born alive is a function of the development of her uterus. During critical periods of development, external factors can alter an animal's phenotype through changes in gene expression, which can permanently alter the animal's long-term production potential. There is an increasing body of evidence that a female's long-term development can be programmed by events that occur in the first weeks of life. Previous studies indicate that uterine glands, which are responsible for the secretion of uterine proteins that support the fetus during pregnancy, begin development within the first week of life. Other studies have shown that female pigs (gilts) nursed in small litters give birth, when they become mothers, to more piglets than gilts that were nursed in large litters. Our hypothesis is that development of the uterine glands is affected by the size of the litter in which gilts are nursed. To test this hypothesis, we conducted a study in which gilts were raised in either small or large litters. The uterus was collected from these gilts at 4 critical time points during development; weaning, 60, 100, and 140 d of age. The student selected for this project will quantify uterine development in these samples. The student will learn how to (1) prepare tissue specimens for histology, (2) use the microscope to perform computer assisted morphometry in order to quantify various aspects of uterine development. This project will also provide the student with the opportunity to assist in animal surgery (i.e., ovariectomy and catheterization of veins) and to collect blood samples and tissues from pigs to quantify reproductive function. The student may have the opportunity to learn additional molecular biology techniques (e.g. quantifying gene expression). The student should be willing to assist with collection of tissues at necropsy.

**mRNA target expression associated with differentially expressed miRNA in the endometrium adjacent to necrotic tip and normal placental tissue from 22 to 42 days of gestation.**

Scientist: J. Miles, Ph.D. & E. Wright, Ph.D.

Embryo development and placentation have a significant impact on reproductive performance and sow productivity. A unique characteristic of the placenta of the pig is the formation of necrotic tips on the distal ends of the allantochorion between days 22 to 42 of gestation. Necrotic tips of the pig placenta lack vascular support, whereas angiogenesis and placental development occur throughout the remainder of the placenta. To explore the physiology of necrotic tips and how it compares to normal placental tissue, necrotic tip, normal placental tissue and adjacent uterine tissue were taken from gilts at 22, 27, 32, 37 and 42 days of gestation. Initially, we wish to explore the contribution of miRNA to necrotic tip development. MicroRNAs are small non-coding RNA that regulate many biological processes including angiogenesis, cell cycle, tissue differentiation and the immune response. They do this by modifying the activity of the mRNA of various genes, so an exploration of their function must also examine predicted mRNA targets and the abundance of proteins

that result from their translation. To determine the biological processes being regulated by miRNA via post-transcriptional regulation of their target mRNA, we will evaluate miRNA, mRNA and protein abundance within the placenta and uterine tissues at each time point collected.

We discovered 17 miRNA that were differentially expressed in the uterine endometrium adjacent to normal placenta compared to endometrium adjacent to necrotic tip. A majority of these miRNAs were increased in the endometrium adjacent to the necrotic tip compared to endometrium adjacent to normal placental tissue. Selected miRNA(s) with known mRNA targets will be used to identify potential pathways and evaluate mRNA targets and post-transcriptional regulation via protein abundance within endometrium between days of gestation. We will also use in situ hybridization and immunohistochemistry to localize miRNA, mRNA and protein abundance within the fetal maternal interface.

The student will perform PCR with miRNA and mRNA, in situ hybridization, immunohistochemistry and immunoblotting. The experiment will contribute information regarding the role of miRNAs and the regulation of biological pathways during necrotic tip development in the gilt during early gestation.