**Additional Accomplishments and Impact Statements**

**W-1171 Regional Research Project**

**January 1, 2005 - December 31, 2008**

***Objective 1. Understand the biology and underlying mechanisms of gamete development, fertilization, and embryogenesis.***

**Accomplishment**: A low sodium medium was found to be a viable alternative to synthetic caudal epididymal fluid medium for maintaining sperm viability during storage (AR).

**Impact:** Improvement in semen storage procedures could enhance the efficiency and use of artificial insemination, especially in species where the semen cannot be successfully cryopreserved (AR).

**Accomplishment**: Grazing bulls on endophyte infected fescue was shown to reduce sperm motility and morphology, especially during elevated ambient temperatures (AR).

**Impact:** Identification of causes of infertility due to endophyte infected fescue could lead to better management practices and improved cow herd productivity and profitability (AR).

**Accomplishment:** We have been studying various ways of making in vitro-produced embryos more like in vivo embryos, particularly with respect to lipid content. Forskolin-treated embryos had much less lipid than controls. Caffeine and epinephrine were not effective at doses used (CO).

**Impact:** In vitro-produced embryos have excess lipid content, and developing a method to decrease lipids will aid in improving systems of producing embryos in vitro (CO).

**Accomplishment:** We attempted to apply IVP procedures in a practical way by ET to lactating dairy cows. Success rates were considerably lower than with AI using commercially available oocytes and semen. Sexed semen worked reasonably well with AI (CO).

**Impact:**  Our research in applying commercially available oocytes and sperm indicates that this technology is not yet ready for on-farm application (CO).

**Accomplishment:** A fairly efficacious method of freezing small equine embryos was developed that appeared superior to a standard vitrification procedure, which performed more poorly in this experiment than in previous experiments for unknown reasons (CO).

**Impact:** The promising technique for freezing equine embryos may also work well with ruminant embryos, but this requires further study (CO).

**Accomplishment:** Investigated strategies to improve oocyte freezing survival (CT).

 **Impact:** The knowledge gained in oocyte preservation improvement will be important in preservations of elite livestock, endangered species as well as human eggs (CT).

**Accomplishment:** Utilizing a novel embryonic stem cell differentiation culture system, BMP and KIT ligand signaling were demonstrated to have a significant effect on the derivation of germ cells from ESCs. This provided further insight into important signaling events in germ cell development and highlights the potential use of this system to study developmental problems hindering livestock production (GA).

**Impact:** Understanding of cell signaling has helped elucidate reproductive problems, a major hurdle in livestock production (GA).

**Accomplishment:** The development of miniaturization technologies toward microfluidics and small mechanical systems, microelectromechanical systems (MEMS), has created means for dynamic culture on a volumetric scale reportedly more consistent with the needs of the embryo (IL).

**Impact:** The development of efficient IVP systems using microscale technologies will enable the automation of such techniques for production of large numbers of embryos in vitro, which will speed the genetic improvement and selection of superior livestock (IL).

**Accomplishment:** The presence of *SPP1* mRNA in porcine oocytes and cumulus cells and the larger mRNA abundance before maturation may suggest a role of this protein prior maturation of oocytes (IL).

**Impact:** Identification of specific mRNAs regulating meiotic maturation will allow the development of systems to improve the developmental competence of in vitro matured mammalian oocytes by specifically arresting nuclear maturation while facilitating cytoplasmic maturation during continued culture (IL).

**Accomplishment:** Fusion using 2 pulses, 0.9 Kv/cm or 1.2 Kv/cm, for 30µs was more efficient for embryonic structure reconstruction in the hand-made cloning technique, when comparated to 0.6 Kv/cm for 30µs (IL).

**Impact:** The use of hand-made cloning technology will allow the more wide-spread use of this technology as it simplifies the nuclear transfer procedure to the point where it can be used by more labs and individuals (IL).

**Accomplishment:** Gene expression is altered in oocytes matured in vitro compared to those in vivo. Dysregulation of gene expression in the oocyte resulting in altered DNA methylation and an up regulation in cell death pathways are potential developmental mechanisms influenced by in vitro culture conditions that correlate to reduced embryonic developmental potential (IL).

**Impact:** Manipulation of specific molecular mechanisms critical to oocyte competence, including DNA methylation, cell death pathways, cellular metabolism and mRNA processing, will allow scientists and clinicians to positively alter the developmental potential of oocytes (IL).

**Accomplishment:** Aberrant metabolism, incorrect mRNA post-transcriptional processing and increased apoptosis are some of the mechanisms contributing to reduced developmental potential in gilt oocytes (IL).

**Impact:** Identification of specific mRNAs regulating the resumption of meiosis in mammalian oocytes will provide novel opportunities for development of gene-targeted contraceptive methods useful for the manipulation of reproductive cycles in livestock and other mammalian species (IL).

**Accomplishment:** Abnormal fetal and placental development and differential expression of imprinted genes in the ovary and brain of aged mice suggests that epigenetic regulation of oocyte and fetal development is impaired with advanced maternal age (IL).

**Impact:** Understanding the regulation of fetal development will lead to methods for identifying normal and abnormal growth patterns resulting from ARTs, and improved systems for producing embryos in vitro (IL).

**Accomplishment:** Methods have been established which allow propagation of the TE model CT1-cells to be grown in a feeder-free system. This allows cleaner determination of gene expression without issues of feeder cell contamination. CT-1 cell line expresses TE lineage associated transcription factors: Cdx2, Ets2, Errβ, Id2, Sox15, Elf5, Hand1 and Gata6, along with the pluripotency-associated transcription factor, Oct4 (POU5F1) (MD).

**Impact:** Identification of regulatory mechanisms controlling ICM and trophectoderm lineage differentiation will provide a better understanding of fetal and placental development and insights into the etiology of early embryonic loss and implantation failure. Furthermore, knowledge gained will aid in the improvement of in vitro procedures and will ultimately result in improved fertility especially in procedures involving advanced biotechnological approaches (e.g. cloning and transgenics) (MD).

**Accomplishment:** Provided functional genomics blue prints (transcripts) of bovine spermatozoa (MS).

**Impact:** Identified spermatozoal transcripts in bull spermatozoa can be used to understand biology of spermatozoa and improve bull fertility. In addition, identification of comparative functional genomics of chromatin remodeling proteins, HMGN3A and SMARCAL1, can lead to better understanding of epigenetic control of mammalian embryonic development (MS).

**Accomplishment:** Established an in vitro follicle culture system to identify the effect of excess insulin and IGF exposure on oocyte gene expression (NE).

**Impact:** The effect of different in vitro conditions, including different media and protein supplementation, on expression of key developmental genes has been determined (NE).

**Accomplishment:** Chronic in vitro exposure of follicles to insulin changed the expression of NIMA-related kinase 2 (Nek2), Nek4 and TACC1. Similarly, insulin treatment of granulosal cell primary cultures induced alterations in Nek2, Nek4 and TCAA1 mRNA abundance (NE).

**Impact:** Defining genes that are important for establishing an oocyte of high quality will identify new targets for reversing the deleteriouseffects of a persistent follicle and increase the fertility of dairy and beef cattle (NE).

**Accomplishment:** We have determined an important role for GnRH during embryogenesis, having a receptor-mediated effect on 1-cell embryos that occurs within the first 36 hours of embryo development (NE).

**Impact:** Manipulation of the interaction between GnRH and its receptor in preimplantation embryos could culminate in novel contraceptive procedures (NE).

**Accomplishment:** A specific antagonist of GnRH completely blocked embryonic development and acted via alteration of the cell cycle rather than apoptotic pathways (NE).

**Impact:** A better understanding of the biological mechanisms underlying GnRH regulation of early embryonic development could lead to new methodologies that reduce early embryonic loss in livestock species and improve in vitro development rates of human embryos (NE).

**Accomplishment:** We have accomplished the following: (i) established biotin effects on oocyte maturation in mice; (ii) identified genes that are up- or down-regulated in oocytes from biotin-deficient compared to biotin-normal mice; and (iii) examined intracellular signaling cascades and molecular mechanisms underlying biotin effects on oogenesis and subsequent development (NE).

**Impact:** Determination of biotin effects on oocyte development could lead to establishment of appropriate levels of biotin supplementation for livestock and women (NE).

**Accomplishment:** Found production of bovine embryos in vitro using a serum based system was associated with increased body weight, normal liver and kidney morphology, and increased concentrations of blood urea nitrogen of fetuses during late gestation (NC).

**Impact:** Improving methods for manipulating the timing of meiotic maturation will improve the efficiency and effectiveness of in vitro embryo production (NC).

**Accomplishment:** Updated an annotation that is freely available that increases the usefulness of the porcine Affymetrix microarrays (NC).

**Impact:** Platform comparison and annotation of Affymetrix arrays will help other investigators make a better choice and get more information when planning gene expression profiling studies on swine (NC).

**Accomplishment:** Research in cell migration events occurring in the early embryo may provide valuable insights into mechanisms that predispose the embryo to pregnancy loss due to abnormal development and/or implantation failure (OR).

**Impact:** Improvements in livestock reproductive efficiency will provide consumers food and fiber products at reduced cost (OR).

**Accomplishment:** Increasing the proportion of female offspring (embryos or calves) by manipulating semen (via incubation, etc.) or the female (frequent ultrasound, or hormonally) will be beneficial to the beef or dairy producer without the added cost and potential negative effects (reduced pregnancy rates, bis-benzimidazole dye, etc.) of sexed semen protocols (SC).

**Impact:** Enhanced reproductive efficiency of beef and dairy cattle to produce sex-selected offspring (SC).

**Accomplishment:** Recombinant FSH produced from non-mammalian expression systems will provide a safer, less expensive, and perhaps more effective method of producing embryos from hyperstimulated cattle. Currently the superovulation and embryo transfer industry is dependent upon FSH products purified from mammalian pituitaries and are of non-domestic origin (SC).

**Impact:** Safer, more effective, and less expensive alternatives to facilitate cattle hyperstimulation and embryo transfer (SC).

**Accomplishment:** Oocytes microinjected with FAK siRNA had significantly lower cleavage rates compared to control oocytes. Oocytes pre-incubated with the function blocking antibodies for the αV and β1 subunits had significantly lower cleavage rates (P < .05) compared to all other treatment groups (UT).

**Impact:** Investigation of integrins within the oocyte will enhance our understanding of how integrin heterodimers are involved in sperm-oocyte interaction (UT).

**Accomplishment:** We have identified and characterized a new member of the importin α gene family and demonstrated its potential role in transporting oocyte-specific nuclear proteins and its requirement during early embryogenesis (WV)

**Impact:** Identification of the novel importin α and understanding its functions in controlling early events of embryonic development may ultimately lead to the development of new strategies to improve the efficiency of nuclear transfer and reproduction in cattle (WV).

***Objective 2. Refine methods for production of genetically enhanced animals to improve livestock production efficiency.***

**Accomplishment:** Studied genetic imprinting in naturally reproducing pigs and compared gene expression in DNA methylation in cloned and control pigs.

**Impact:** The understanding of the mechanism of gene expression differences in cloned vs control animals has the potential to improve the survival of cloned animals and therefore increase cloning efficiency (CT).

**Accomplishment:** We improved the efficiency of stem cell generation (CT).

**Impact:** Improving the efficiency of embryonic stem cell derivation has major implication in the generation of bovine embryonic stem cells which are still not available due to culture condition inadequacy (CT).

**Accomplishment:** The accomplishments of these studies are the development of a novel differentiation culture system that is capable of producing large numbers of germ-like cells that undergo advanced stages of development from embryonic stem cells. These cells represent an important germ cell source for the production of transgenic animals, leading to the improvement of livestock production (GA).

**Impact:** Genetically engineered animals serve as animal models for numerous diseases and have helped in their treatments (GA).

**Accomplishment:** Preliminary results indicate absence of the transgene in control animals after co-habitation and post-mating with transgenic animals. This work provides a critical first step toward providing rigorous scientific data for risk assessment of transgenic livestock (IL).

**Impact:** The production of α-lactalbumin and IGF transgenic swine allow for improvement of lactation in swine production systems. These observations have profound effects on increasing the efficiency of milk and meat production. Further, the risk assessment on the safety of these animals will provide needed information to enable their entrance into the food supply (IL).

**Accomplishment:** Adipose-derived stem cells demonstrated a clear osteogenic differentiation and similar expression and pattern of the two osteogenic genes most abundant in MSCs (*COL1A1* and *SPARC*). However, the higher abundance of *SPP1* and *BGLAP* and the different behavior of *SPP1* in MSCs suggest a different transcription profile between the two cell types (IL).

**Impact:** The ability to isolate and differentiate mesenchymal lineage stem cells *in vitro* and then transplant them back into live animals with corresponding proper differentiation will allow stem cell therapy for production parameters such as lactation and muscle growth in livestock (IL).

**Accomplishment:** The cytokine Noggin can upregulate Nanog expression sufficiently to permit further studies that will help identify more optimal conditions (MD).

**Impact:** The identification of cytokines which extend expression of pluripotency-related transcription factor NANOG in bovine embryonic cells will aid greatly in the derivation of embryonic stem cells in cattle, goats and other livestock. ESC in domestic species will provide an invaluable tool in genetic engineering of transgenic animals for improved production traits, disease resistance and production of biopharmaceuticals (MD).

**Accomplishment:** Identified genes whose products might be playing a role in epigenetic control of molecular reprogramming of gene expression in cloned bovine embryos (MS).

**Impact:** Identified epigenetic errors in cloned bovine embryos could be used to improve the cloning efficiency (MS).

**Accomplishment:** Developed a successful procedure for cryopreservation of porcine embryos using a microdroplet procedure. In addition, we have established divergent survival rates for cryopreserved embryos from Chinese Meishan and white crossbred lines of swine. This difference in survival rate occurs within the first 24 hours following thawing (NE).

**Impact:** Results from freezing studies with embryos from different lines of swine could provide improved methods for cryopreservation of swine embryos so that valuable germplasm, including genetically altered animals, can be maintained for future use (NE).

**Accomplishment:** Generated Yucatan SCNT clones (NC).

**Impact:** Cloning of the Yucatan pigs increases the usefulness of SCNT (NC).

**Accomplishment:** Isolated neural stem cells, amniotic fluid stem cells, bone marrow mesenchymal stem cells and have preliminary evidence of isolation of porcine induced pluripotent cell (iPS) (NC).

**Impact:** Availability of various porcine stem cells facilitates generation of transgenic swine. Also, the ability to isolate and differentiate mesenchymal lineage stem cells *in vitro* and the transplant them back into live animals with corresponding proper differentiation will allow stem cell therapy for production parameters such as lactation and muscle growth in livestock (NC).

**Accomplishment:** Methylation patterns of the scNT blastocysts show more similarity to the fibroblast donor cell line used for scNT as compared to the IVF blastocysts (UT).

**Impact:** Failed demethylation of key genes is a likely cause of failed embryo/fetal development following somatic cell nuclear transfer (UT).