**6 National Research Initiative (NRI) in reproduction: Challenges for success.** W. W. Thatcher*, *University of Florida*, *Gainesville*.

Program managers developed a dynamic system for the NRI to receive scientific inputs on priorities for research to reduce infertility and improve reproductive management (e.g., http://www.biolreprod.org/cgi/rapidpdf/biolreprod.105.048686v1) including strengthening programs and training. The reduction in funded grants at an increased rate per grant reflects the need for congressional infusion of funds to the NRI. Presently, panels are still able to identify excellent and very good proposals. Should this NRI funding strategy continue, or should award level be fixed with the RFA, precluding panel mandated budget cuts? Should there be bi–annual submission deadlines and possible two tier funding levels? The increased percent of applied research grants reflects the importance of transition research to shareholders. Does the single panel review process accommodate both basic and applied proposals? Partnerships between NRI and commercial enterprises should be developed for developmental application of research results. Does the EPSCoR strengthening program really meet the USDA mandate to implement a competitive grants program of priority mission areas? An alternative would be joint regional grants of excellence that have both synergistic and strengthening effects. Should panel service be limited so re–submitted proposals are considered by an independent set of agricultural oriented reviewers or should panelist turnover be reduced to sustain evaluation criteria? Innovative joint funding with the NIH/NRI, to incorporate large animal models for human biomedical relevance, should be applied to a multiplicity of areas (i.e., Table 1 in http://www.adsbm.msu.edu/whitepaper.html) without compromising support to the NRI agricultural enterprise. Basic research needs a visionary focus on areas of need. Investigator teams should strive towards shortening the interval from a ″biological observation″ to implementation in ″food production systems″. Such successes become the focus for an aggressive joint education effort of Congress by universities, commercial entities, scientific societies, stakeholders, and the public for congressional support of the NRI.

**Key Words:** NRI, Grants, Congress

## Triennial Reproduction Symposium: Concurrent Techniques Sections — Molecular Techniques and Statistics

**7 RNA interference: a new approach to *in vivo* study of gene function.** R. V. Anthony* and J. D. Cantlon, *Colorado State University*, *Fort Collins*.

Definition of hormone function was classically accomplished by ablation-replacement studies. However, as our knowledge of the complexity of hormones and growth factors has grown, it has become increasingly difficult to clearly define the necessity and function of many of the hormones, growth factors and regulatory proteins under investigation. The use of homologous recombination within mouse embryonic stem cell lines allows functional gene ablation, and has been used extensively during the past 15 years to define specific gene function. The use of similar methodologies in livestock species has yet to yield an efficient approach. In contrast, the parallel development of our understanding of naturally occurring RNA interference with the development of efficient virus-based vectors for gene transfer holds great potential for effectively ″knocking down″ specific gene function. Short-hairpin (sh) RNA-encoding cassettes, typically consisting of inverted repeats separated by a loop sequence, followed by a short poly(T) string to signal transcription termination, are inserted downstream of a RNA polymerase III promoter within the viral-vector of choice. Several virus vectors are useful for delivery of shRNA expression cassettes, each with particular attributes. Both adenovirus and lentivirus-derived vectors provide a high rate of infectivity in most mammalian cell types, with lentiviral vectors allowing stable integration into the host genome if the study of long-term effects is needed. Upon transcription a shRNA is generated and the loop is recognized by the processing enzyme Dicer, generating ″guide″ sequences. Guide sequences are incorporated into the RNA-induced silencing complex (RISC), which targets mRNA for degradation if recognized by the guide sequence. For each mRNA of interest, design and testing of a number of shRNA, along with adequate controls, are required to identify the most efficient construct before proceeding to *in vivo* use. This technology, which has been used effectively in rodents, may become the method of choice for defining gene function in livestock.

**Key Words:** RNA interference, shRNA, Viral-mediated infection

**8 Interpretation of microarray data: Trudging out of the abyss towards elucidation of biological significance.** G. W. Smith*[1], G. J. M. Rosa[1], P. M. Coussens[1], R. Halgren[1], A. C. O. Evans[2], M. Mihm[3], P. Lonergan[2], and J. J. Ireland[1], [1]*Michigan State University*, *East Lansing*, [2]*University College Dublin*, *Dublin, Ireland*, [3]*University of Glasgow*, *Glasgow, UK*.

The recent development of tools for expression profiling in livestock has availed reproductive biologists new opportunities to examine global changes in gene expression during key developmental timepoints, in response to hormonal treatments, and as a tool for phenotyping or predicting developmental potential. Such experiments often yield lists of tens to hundreds to thousands of regulated genes/transcripts of interest. Some argue such technological advances signal a move from hypothesis driven research to descriptive discovery research and information overload at the expense of biological significance. One can easily spend hours and hours staring into the abyss, wondering if results are real and what they mean. Microarrays can be more than a high throughput and expensive screening tool. Many factors contribute to success of expression profiling experiments and yield of interpretable data including nature of the hypothesis/objective of study, platform, complexity of tissue of interest, experimental design and incorporation of best available strategies for data processing, analysis, and interpretation. Beyond mere assessment of significant differences in transcript abundance between tissue A and B, current experimental and statistical approaches for microarray data provide opportunities for studying variation in transcriptional activity across multiple experimental groups and time points, for building classification models for use in diagnosis and outcome prediction, and for clustering genes and subjects to study gene pathways and networks and to unravel/search for hidden patterns, respectively. Although challenging due to limited annotation/ontology classification for a large proportion of genes in livestock species, functional categories of co-regulated genes and gene pathways can be mined, and hypotheses about common regulatory elements/functional significance formulated. We have applied cDNA microarray technology to studies of follicular growth, oocyte quality and the periovulatory period in cattle. Strategies to facilitate analysis and interpretation of microarray data will be discussed, using select examples from our data sets and other sources.

**9 Statistical power calculations.** R. Lenth*, *University of Iowa*, *Iowa City*.

The talk will focus on how to do meaningful power calculations and sample-size determination for common study designs. There are important guiding principles: (1) Certain types of retrospective power calculations should be avoided, as they add no new information to an analysis. (2) Effect size should be specified on the actual scale of measurement, not on a standardized scale. (3)

Rarely can a definitive study be done without first doing a pilot study. Sample-size guidelines for pilot studies will also be briefly discussed. Finally, I will present some examples, using Java applets that I have developed and that are available on the web at http://www.stat.uiowa.edu/~rlenth/Power/.

**Key Words:** Statistical power, Sample size

**10 Procedures for statistical treatment of binomial and categorical data.** R. Quaas*, *Cornell University*, *Ithaca, NY*.

The analysis of discrete observations has always presented problems with a variety of methods of varying complexity but typically none simple. Unless, that is, one ignores the categorical nature of the data and does a usual analysis of variance. Surprisingly this often does quite well yielding ″p values″ quite similar to more appropriate methods, e.g. logistic regression for binomial data. If one is interested in estimates, however, these may be more interpretable using an appropriate non-linear method. Currently there is readily available software for the analyses. A series of case studies will be presented.

**Key Words:** Categorical data, Binomial data

## Triennial Reproduction Symposium: Symposium II — Reproductive Immunology

**11 Regulation of immune cells in the uterus during pregnancy in ruminants.** P. J. Hansen*, *University of Florida*, *Gainesville*.

Pregnancy results in a change in number and function of immune cells in utero that potentially affect fetal survival and uterine defense mechanisms postpartum. These changes are driven by local signals from the conceptus as well as from hormonal changes mediated by the placenta or maternal system. In sheep, for example, macrophages accumulate in the uterine endometrium during pregnancy. Use of a unilaterally-pregnant model, in which pregnancy is surgically confined to one uterine horn, has revealed that accumulation is due to both systemic signals (numbers of cells in the non-pregnant uterine horn of the unilaterally-pregnant ewe higher than amounts in uteri of non-pregnant ewes) and locally-produced signals (number of cells in the uterus of unilaterally-ligated ewes higher in the pregnant horn than in the non-pregnant horn). Gamma-delta T cells also accumulate in uterine epithelium during pregnancy as a result of unidentified systemic signals. These cells may participate in growth of the conceptus, immunosuppression, or placental detachment at parturition. One of the key regulators of uterine immune function is progesterone. In sheep, progesterone can block tissue graft rejection in utero when injected to achieve concentrations too low to directly inhibit lymphocyte proliferation. Progesterone probably inhibits uterine immune responses in sheep indirectly by inducing secretion of a member of the serine proteinase inhibitor family called uterine serpin from the endometrial epithelium. Uterine serpin can block lymphocyte proliferation in vitro in sheep and natural killer cell mediated abortion in vivo in mice. Uterine serpin is also present in cattle, goats and pigs but its role in immune function in these species has not been documented. The relevance of changes in uterine immune function to the reproductive and immune status of ruminants has not been established. There is little evidence for immunological causes of pregnancy loss (except for cloned fetuses) but the downregulation of uterine immune function during pregnancy is likely to lead to a postpartum uterus with compromised capacity for preventing establishment of infectious disease.

**Key Words:** Pregnancy, Ruminants, Immunology

**12 Why is the fetal allograft not rejected?** C. J. Davies*, *Washington State University*, *Pullman*.

In viviparous species the fetus must be protected from a potentially hostile maternal immune system. The major histocompatibility complex (MHC) is a genetic region that encodes MHC class I (MHC-I) and class II (MHC-II) proteins, which present peptide antigens to T lymphocytes and induce graft rejection. MHC-II proteins are only expressed on professional antigen presenting cells. However, classical MHC-I proteins are expressed on all nucleated somatic cells. Protection of the fetus from immune-mediated rejection involves down-regulation of classical MHC-I antigen expression on trophoblast cells, which form the external epithelial layer of the placenta, and maintenance of an immunologically favorable, immunosuppressive, environment in the uterus. Normally, bovine trophoblast cells do not express MHC-I antigens prior to day 120 of pregnancy. However, third trimester trophoblast cells in the interplacentomal and arcade regions of the placenta express both classical MHC-I proteins, that could potentially induce fetal rejection, and non-classical MHC-I proteins. A human non-classical MHC-I antigen, HLA-G, is an important immunoregulatory factor required for maintenance of pregnancy. In cattle, third trimester MHC-I expression has no adverse effects and probably contributes to placental separation at parturition. However, somatic cell nuclear transfer (SCNT) fetuses, the majority of which are aborted between days 30 and 90 of pregnancy, had trophoblast cell expression of MHC-I antigens prior to day 34 of pregnancy. In conjunction with increased trophoblast MHC-I expression, SCNT pregnancies exhibited a marked increase in the number of stromal lymphocytes in the uteri of surrogate dams. A retrospective study found that SCNT pregnancies established using MHC-I homozygous cell lines, where the immunological barrier is greatly reduced, had significantly improved fetal survival from day 28 to term (51% survival for MHC-I homozygous and 5% for MHC-I heterozygous SCNT fetuses). Consequently, it appears that the high rate of fetal mortality in SCNT pregnancies is due, at least in part, to inappropriate expression of trophoblast MHC-I antigens and immune-mediated placental rejection.

**Key Words:** Abortion, Immunology, Bovine