3 Regulation of oocyte meiotic maturation. F. J. Richard*, *Université Laval*, *Québec, QC, Canada*.

Mammalian oocytes are arrested at prophase of the first meiotic division before induction of maturation by the preovulatory luteinizing hormone surge. In vitro, oocyte maturation occurs spontaneously. The first meiotic arrest is characterized by a large nucleus called the germinal vesicle. One important signalling molecule for resumption of meiosis is cAMP. High levels of cAMP block spontaneous meiotic resumption. Research investigating the regulation of oocyte cAMP has led to the discovery of new receptors, G proteins, cyclases and phosphodiesterases. Leydig insulin-like 3 (INSL3), a polypeptide growth factor of the insulin family, is expressed in theca cells. INSL3 activates LGR-8 (leucine-rich repeat-containing G protein-coupled receptor 8) which is expressed in the oocyte. LGR-8 is coupled to the inhibitory G protein, thus leading to a decrease in cAMP production. Treatment with INSL3 initiates meiotic progression of oocytes in preovulatory follicles, demonstrating the importance of cAMP management for meiotic resumption. Furthermore, microinjection of an anti-Gs protein into mouse oocytes resulted in meiotic resumption, suggesting that meiotic arrest of the oocyte was dependent on Gs activity. The orphan Gs-linked receptor GPR3 is expressed in the oocyte. The oocytes of null-GPR3 mice resume meiosis when still in their follicles, suggesting that GPR3 is involved in the control of cAMP production, and thus meiotic maturation. Cyclic nucleotides are synthesized by cyclases and degraded by phosphodiesterases. Mouse and rat oocytes express isoform 3 of adenylyl cyclase. In the mouse, the null mutation results in approximately 50% of the oocytes resuming meiosis, demonstrating the importance of the synthesis of cAMP in controlling nuclear maturation. The null mutation of the major PDE expressed in mouse oocytes (PDE3A) results in female sterility due to ovulation of GV-arrested oocytes that cannot be fertilized. Maintenance of meiotic arrest is explained by constitutive cAMP signalling associated with undetectable cAMP-PDE activity. Collectively, these results are starting to illuminate the key players involved in the control of oocyte cAMP and thus, nuclear maturation.

Key Words: Oocyte, Maturation, cAMP

Triennial Reproduction Symposium: The USDA-NRI in Reproduction — Relevance to Production Agriculture

4 The National Research Initiative (NRI) competitive grants program in animal reproduction: Changes in priorities and scope relevant to U.S. animal agriculture. M. A. Mirando*, *Cooperative State Research, Education, and Extension Service, United States Department of Agriculture*, *Washington, DC*.

The NRI is the USDA's major competitive grants program and is administered by the Cooperative State Research, Education, and Extension Service (CSREES). The NRI was authorized by the U.S. Congress in the 1990 Farm Bill at a funding level of $500 million; however, the maximal NRI appropriation was $181.17 million in fiscal year (FY) 2006. Across all programs, the NRI is mandated to use 30% of its funding to support mission-linked research. Since its inception in 1991, the NRI has funded competitive grants in the discipline of animal reproduction. Before 2004, the Animal Reproduction Program funded a broad range of projects encompassing almost every sub-discipline in reproductive biology of farm animals, including aquatic species important to the aquaculture industry and laboratory animals. During FY 2004, the NRI Animal Reproduction Program narrowed the focus of its funding priorities to five issue-based topics in an effort to make greater measurable improvements in a few high impact areas over the next 10 years. Funding priorities were narrowed further in FY 2006 to three sub-disciplines based, in part, on recommendations that emerged from a stakeholder workshop conducted by CSREES in August, 2004. In FY 2003, Congress authorized expenditure of up to 20% of funds appropriated to the NRI to support projects that integrate at least two of the three functions of research, education, and extension-outreach. In FY 2004, the Animal Reproduction Program included a funding priority for integrated projects focused primarily on infertility in dairy cattle. The program funded its first integrated project in FY 2005. During FY 2002, increased emphasis on justification for use of model systems (e.g., laboratory animals and in vitro systems) was included in the NRI Request for Applications (RFA). In FY 2006, applications proposing to primarily utilize nonagricultural animal models were excluded from the program. Currently, all proposed studies must be thoroughly justified in terms of relevance to U.S. animal agriculture and relevance to program priorities identified within the RFA.

Key Words: Reproduction, Grants, Funding

5 A researcher's perceptions of USDA funding in reproduction. J. J. Reeves*, *Washington State University*, *Pullman*.

Through the 1970's, NIH was the only source of federal competitive research funding for Animal Scientists in reproduction. This required couching domestic animals as models for basic research on human reproduction. The first USDA Competitive Research Grants Program was initiated in 1978 under the auspices of the Competitive Research Grants Office. Again, Animal Scientists could only get funds for research in reproduction through the Animal Health Special Grants Program, which began in 1980. Dedicated funding for animal reproduction did not start until 1985 and was available primarily in the reproductive efficiency and physiology areas of the Animal Science Program. Funding for individual grants and duration of funding were similar between NIH and USDA, typically in the range of 3 years with total direct costs of $150,000. USDA funding in reproduction permitted directing research more toward the animal industry and less toward human reproductive problems or animal health problems. The names of these programs have changed over time, the National Research Initiative (NRI) Competitive Grants Program started in 1991 with a program in Animal Reproduction. Successful funding of individual grants has been based on an industry problem with a sound hypothesis and basic technology. The USDA review system has been based on external (ad hoc) reviewers as well as a primary and a secondary panelist reviewer. This review system may drop the external reviewers. USDA did not change the award size for individual grants until 2001 when it gradually increased through 2003. It then markedly increased individual grants in 2004 to a funding level of $300,000-$500,000 over 3 to 4 years. This is good in some respects but results in funding many fewer grants. Policies based on funding the best designed and presented proposals in priority areas should continue. The number of grants funded per year is approaching a low critical number, with an average of only 10 new grants funded per year. At the present funding level it will be difficult for even the best scientist to sustain a research career based only on USDA funding.

Key Words: USDA, Grants, Reproduction

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.