11 Ovarian follicular growth and atresia: the relationship between cell proliferation and survival. S. M. Quirk*, R. G. Cowan, R. M. Harman, and C.-L. Hu, *Cornell University, Ithaca, NY*.

Growth factors and steroids play an important role in the process of ovarian follicular development. In cattle, two of the earliest detectable differences between the healthy dominant follicle selected for development to the ovulatory stage versus subordinate follicles destined to undergo atresia are the greater availability of IGF and the greater capacity to secrete estradiol. IGF-1 and estradiol stimulate proliferation of bovine granulosa cells (GC) in vitro and also promote GC survival by increasing resistance to apoptotic stimuli. Our studies show that the ability of IGF-1 and estradiol to increase resistance to apoptosis is intimately tied to their ability to promote progression through the cell cycle. Cell cycle blockade at the G1/S transition by specific inhibitors prevented the protective effects of IGF-1 and estradiol against apoptosis. The protective effect of IGF-1 against apoptosis is mediated by phosphatidylinositol 3-kinase and its downstream target, protein kinase B/Akt. Constitutively active Akt, expressed by a recombinant adenovirus, protected against apoptosis and this effect was dependent upon cell cycle progression. Therefore, the protective effect of estradiol and IGF-1 against apoptosis is dependent upon their ability to promote progression through the cell cycle. The LH surge induces terminal differentiation of GC and their withdrawal from the cell cycle. By 12 h after the LH surge, bovine GC have withdrawn from the cell cycle and become resistant to apoptosis, even in the absence of growth factors. Treatment with a progesterone receptor (PR) antagonist in vitro caused cells to reenter the cell cycle and reversed the resistance to apoptosis, suggesting that PR is required for these effects. Our studies suggest that the susceptibility of GC to apoptosis is dependent upon the cell proliferation cycle. GC from growing follicles are dependent on growth factors for survival, whereas cells that have terminally differentiated are resistant to apoptosis and relatively independent of growth factors for survival.

Key Words: Ovary, Granulosa cells, Cell cycle

12 Control of follicular growth: local interactions and nutritional influences. R. Webb*[1], P. C. Garnsworthy[1], J. G. Gong[2], and D. G. Armstrong[2], [1] *University of Nottingham, Loughborough, UK*, [2] *Roslin Institute, UK*.

Reproductive function is an integrated process encompassing both extraovarian signals and intrafollicular factors. Initiation of primordial follicle growth and the early stages of folliculogenesis can occur without gonadotropins, but FSH may affect the rate of preantral follicle growth. Antral follicle development from 2-4 mm in sheep and cattle is completely gonadotropin dependent. These recruited follicles express a range of mRNAs encoding steroidogenic enzymes, gonadotropin receptors and local regulatory factors and their receptors. As follicles continue to mature, there is a transfer of dependency from FSH to LH, which may be part of the mechanism involved in selection of follicles for continued growth. Locally produced growth factors, such as the IGFs and members of the TGFβ super-family, work in concert with gonadotropins throughout the follicular growth continuum and can have significant effects on follicle selection. Environmental influences such as acute changes in dietary intake also have an impact on ovarian activity. These changes can occur without significant variation in circulating gonadotropin concentrations and can be correlated with changes in circulating concentrations of metabolic hormones including insulin, IGF-I, GH and leptin. For example dietary energy and protein affect the expression of mRNA encoding components of the ovarian IGF system and these changes can regulate the sensitivity/response of follicles towards FSH and contribute to the observed changes in follicular dynamics. The roles of growth factors in follicular development and survival are dependent on gonadotropin status and differentiation state of the follicle, including the extracellular matrix. In conclusion, it is the integration of these extraovarian signals and intrafollicular factors that determine whether a follicle will continue to develop or be diverted into atretic pathways. Funded by DEFRA, SEERAD and BBSRC.

13 Uterine and placental factors regulating conceptus growth in domestic animals. T. E. Spencer* and F. W. Bazer, [1] *Texas A&M University*.

All mammalian uteri contain endometrial glands that synthesize or transport and secrete substances essential for survival and development of the conceptus (embryo/fetus and associated extraembryonic membranes). Analyses of the ovine uterine gland knockout (UGKO) model support a primary role for endometrial glands and, by default, their secretions in conceptus survival and development during the periimplantation and pregnancy recognition period. Endometrial adenogenesis, the process whereby glands develop in the uterus, is primarily a postnatal event in domestic and laboratory animals as well as humans. Endometrial adenogenesis involves differentiation and budding of glandular epithelium (GE) from lumenal epithelium (LE) followed by invagination and extensive tubular coiling and branching morphogenesis throughout uterine stroma to the myometrium. In sheep, endometrial adenogenesis is regulated by pituitary prolactin acting on prolactin receptors that are expressed by the GE. In contrast, expression and functional activation of estrogen receptor alpha in the uterus is a primary regulator of endometrial adenogenesis in the pig. In sheep and pigs, extensive endometrial gland hyperplasia and hypertrophy occur during gestation, presumably to provide increasing histotrophic support for conceptus growth and development. A servomechanism has been proposed in sheep and pigs to regulate endometrial gland development and differentiated function during pregnancy that involves sequential actions of ovarian steroid hormones, pregnancy recognition signals, and lactogenic hormones from the pituitary or placenta. The fact that disruption of uterine development during critical organizational periods can alter the functional capacity and embryotrophic potential of the adult uterus reinforces the importance of understanding uterine developmental biology. Unexplained, high rates of peri-implantation embryonic loss in domestic animals and humans may reflect defects in endometrial gland morphogenesis due to unrecognized defects of uterine growth and development. Knowledge of the basic mechanisms regulating uterine development is expected to develop tools to increase uterine capacity, litter size and neonatal survivability as well as ameliorate certain types of infertility.

Key Words: Conceptus, Growth, Uterus

14 Regulation of the development of fetuses from in vitro produced and cloned embryos. C. E. Farin* and P. W. Farin, *North Carolina State University, Raleigh*.

The establishment of in vitro fertilization and culture systems for mammalian embryos has facilitated the application of embryo technologies in research, industry and clinical settings. Furthermore, the advent of cloning by nuclear transfer has significantly enhanced the potential for genetic modification of livestock. Based on studies in cattle, sheep and mice, it has become apparent that embryos produced using these systems can differ in morphology and developmental potential compared to embryos produced in vivo. Referred to as 'large offspring syndrome', these abnormalities in the development of fetuses, placentas and offspring are particularly evident following transfer of cloned embryos but also occur in pregnancies from embryos produced using in vitro culture alone. The objective of this presentation will be to examine the effects of in vitro production and cloning on embryo and fetal development. Particular emphasis will be placed on exploring physiological and genetic mechanisms that likely contribute to large offspring syndrome.

15 The impact of oocyte quality on development. R. L. Krisher*, *Purdue University, West Lafayette, IN USA*.

Oocyte quality affects early embryonic survival, the establishment and maintenance of pregnancy, fetal development, and even adult disease. Quality, or developmental competence, is acquired during folliculogenesis as the oocyte grows, and during the period of oocyte maturation. Assisted reproductive technologies involving ovarian superstimulation, or collection of immature oocytes followed by maturation in vitro, perturb this process and result in oocytes with reduced quality. In domestic livestock species, offspring have been produced using in vitro oocyte maturation, although only a small percentage of the original pool of immature oocytes is capable of developing to the blastocyst stage and subsequently resulting in pregnancy. In vitro maturation, as it is currently

undertaken, does not support the correct development of oocyte competence. Follicle size affects oocyte quality, potentially implicating mRNA or protein stores as factors involved in oocyte competence. Oocytes from preantral follicles grown in vitro are competent to resume meiosis, although development to the blastocyst stage is reduced. An offspring from oocytes produced using this technique was normal at birth but experienced delayed onset health issues, highlighting the importance of oocyte quality long after embryogenesis. Metabolism may play a critical role in oocyte quality, as glycolytic activity in mature oocytes is correlated with increased embryonic development. Communication between the oocyte and its surrounding cumulus cells is also important for the development of a competent oocyte. Ovarian stimulation causes delayed embryonic development, increased abnormal blastocyst formation, fetal growth retardation and increased fetal loss. Thus, although meiosis and even early development may be completed successfully, there are a variety of processes occurring within the cytoplasm of the oocyte that are required for complete developmental competence. However, the cellular mechanisms that impart oocyte quality are unclear. Until the mechanisms involved in oocyte quality are elucidated, any effort to utilize assisted reproductive technologies in animals for production or biomedical purposes will be inefficient at best.

Key Words: Gamete, Embryo, Assisted reproduction

16 Pre-ovulatory, post-ovulatory and post-maternal-recognition factors that affect establishment and retention of pregnancy in cattle. E. K. Inskeep*, *West Virginia University, Morgantown WV/USA*.

Although fertilization rate is very high when male fertility is normal in most situations that have been studied, pregnancy rates are well below expectations when defined by the birth of live offspring in response to first service. Factors that affect establishment and retention of pregnancy include: (1) preovulatory influences on the follicle and oocyte, (2) early postovulatory uterine and luteal function, (3) concentrations of hormones associated with trophoblastic and endometrial function during maternal recognition of pregnancy, and (4) less-well understood factors during the peri-attachment period. For example, decreased concentrations of progesterone during preovulatory follicular development lead to increased frequency of episodic secretion of LH, increased secretion of estrogen by a persistent follicle, premature resumption of meiosis and a high incidence of embryonic death between the 2- and 16-cell stages in the cow. Using the early-weaned postpartum cow as a model, absence of previous exposure to progesterone causes increased secretion of PGF2α during days 4 to 9 of the first estrous cycle. The elevated PGF2α not only causes luteolysis, but also has a direct embryotoxic effect during the morula to blastocyst transition. Ideal conditions during the peri-attachment period are not clearly defined and factors in pregnancy wastage may vary with species. Nominal increases in secretion of PGF2α between days 30 and 35 may be important for completion of attachment and placentation in the cow. Lower survival of embryos from day 30 to days 45 to 60 in the cow is associated with lower circulating concentrations of progesterone, but association with concentrations of estrogen has varied among experiments.

Key Words: Embryonic mortality, Follicular development, Luteal function

Breeding & Genetics Symposium: Molecular genetics: Lessons from past/new directions

17 Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. C. M. Dekkers*[1], [1]*Iowa State University*.

During the past decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single gene-traits and genes or genomic regions that affect quantitative traits (quantitative trait loci or QTL).This has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by conventional selection (low heritability or traits for which measurement of phenotype is difficult, expensive, only possible late in life, or not possible on selection candidates), as has been demonstrated in a number of simulation studies. The objective here is to review strategies for the use of genes or markers in genetic improvement, to assess the extent to which and how marker and gene information has been used in commercial livestock improvement programs, to assess the successes and limitations that have been experienced in such applications, and to discuss strategies to overcome these limitations. Focus will be on the use of QTL information from experimental populations, on detection, verification, and estimation of effects in commercial breeding populations, and on the integration of molecular data in methods for genetic evaluation and in selection strategies. Types of molecular information that will be considered include gene tests for causative mutations and linked markers in population-wide linkage equilibrium or disequilibrium with the QTL.

Key Words: Marker-assisted Selection, Genetics, Selection

18 Lessons from QTL analyses in mice. D Pomp* and E. J. Eisen, *University of Nebraska,* [2]*North Carolina State University*.

Most phenotypes with economic relevance are multifactorial traits controlled by complex contributions of genetics and environment. Genetic predisposition results from combinations of relatively small effects of sequence variation within a large number of polygenes, known as quantitative trait loci (QTL). Nearly 200 QTL have been reported for growth and body composition traits in the mouse, likely representing 50-100 distinct genes. Molecular biology has yielded significant advancements in understanding these traits at the metabolic and physiological levels. However, little has been learned regarding the identity and nature of the underlying polygenes due to the inherent inaccuracy of QTL localization and the inability to differentiate between co-localization and co-incidence when comparing QTL with potential candidates. This wide gap between our knowledge of physiological mechanisms underlying complex traits and the nature of genetic predisposition significantly impairs QTL discovery. Identification and genetic mapping of key transcriptional, proteomic, metabolomic and endocrine events will uncover large lists of significant positional candidates. However, integration of experimental approaches to jointly evaluate predisposition and physiology will increase success of QTL identification by combining the power of recombination with functional analysis. Measuring physiologically relevant sub-phenotypes (e.g. 10,000 expression phenotypes on an array) within a structured gene mapping population will facilitate pathway-specific prioritization among candidate genes. This would advance our understanding of the genetic architecture of complex traits by testing the hypothesis that genes controlling predisposition to a trait are primarily involved in trans-regulation of the physiological pathways that directly regulate the trait. An integrated ?polygene discovery database? will enable QTL identification and characterization. This will be critical for the success of marker assisted selection in livestock, given the inherent advantages of using directly predictive assays relative to within-family, linked-marker tests.

Key Words: Mouse, QTL, Marker assisted selection

19 Potential use of microarrays and related methodologies in animal breeding. B. Walsh*, *University of Arizona*.

The age of genomics offers biologists with powerful tools few could even dream of twenty years ago. Biology is being transformed by such tools, and animal breeding is no exception. Genome-wide studies of levels of mRNA expression in specific tissues and/or over time can be monitored by microarrays. The rigorous statistical analysis of such arrays is still being fine-tuned, and we will explore some of the resolved, and unresolved, issues. While microarrays offer an approach to gene discovery (i.e., candidate genes), they likely face many of the same issues as QTL mapping in moving from a powerful genetic tool to a particular tool for applied breeding. Microarrays are one tool of functional genomics, a discipline seeking to understand gene and metabolic networks. Another tool are two-hybrid screens that look for interactions between proteins