# ABSTRACTS: INVITED TALKS

**K1. STEM CELLS, SMALL RNAS, AND SELF-RENEWAL.** Haifan Lin. Yale University, New Haven, CT

The hallmark of stem cells is their ability to self-renew meanwhile capable of producing numerous differentiated daughter cells. Previously, we identified the ARGONUATE/PIWI protein family to be essential for stem cell self-renewal in diverse organisms. This protein family can be divided into ARGONUATE and PIWI subfamilies. Recently, we and others discovered that the PIWI subfamily proteins interact with a novel class of non-coding small RNAs that we named PIWI-interacting RNAs (piRNAs). piRNAs are 26~30 nucleotides in length, and are abundantly expressed during spermatogenesis. Individual piRNAs frequently correspond to intergenic and repetitive sequences. In addition, a small number of piRNAs correspond to exonic and intronic sequences. This broad genomic distribution of piRNAs implicates their potential involvement in diverse mechanisms of gene regulation during spermatogenesis. I will present our recent progress on how PIWI subfamily proteins and a subset of piRNAs might be involved in epigenetic and translational regulation that defines the self-renewing fate of germline stem cells and controls other spermatogenic events in Drosophila and mammals.

**PS1. MOLECULAR PHENOTYPING OF THE CYCLING HUMAN ENDOMETRIUM AND HORMONALLY REGULATED GENES IMPORTANT FOR IMPLANTATION.** Linda Giudice. Univ of California - San Francisco, San Francisco, CA

Human endometrium is a dynamic tissue that undergoes proliferation in response to estradiol (E2) in the first half of the menstrual cycle and then differentiation of cellular constituents in the secretory phase, in response to progesterone (P) directly or by paracrine interactions among cell types. Histologic evaluation of endometrium has been the main stay of assessing cycle phase, dating for fertility, and evaluating abnormalities, including endometritis, endometrial hyperplasia, and cancer. However, histologically normal endometrium has been demonstrated to have abnormal expression of specific genes and/or proteins in disorders such as endometriosis, hydrosalpinges, and polycystic ovarian syndrome. We conducted a multi-center clinical study aimed to define the endometrial transcriptome across the menstrual cycle in healthy women and women with endometriosis and compared this to histologic evaluation. Endometrial tissue was obtained from 45 normal subjects undergoing an operative procedure for benign gynecologic conditions and also from some normal volunteers and from 16 subjects with severe endometriosis. RNA was isolated and hybridized to Affymetrix whole genome arrays containing 54,600 genes, and data were subjected to bioinformatic analysis using GeneSpring software and Genontology, and KEGG pathway analysis. Principal Component Analysis revealed clustering of samples into cycle phases, independently of how they were obtained (curetting or biopsy) or indication for surgery. Hierarchical clustering revealed 2 major branches that contained (1) proliferative (PE) and early secretory (ESE) endometrium and (2) mid (MSE) and late (LSE) secretory endometrium. MSE, which corresponds to the window of implantation, revealed numerous genes encoding secretory proteins, cytokines and chemokines, including CXCL14, and members of the innate immune system among others. LSE revealed a transition to a pro-inflammatory phenotype of the immune response and up-regulation of matrix degrading enzymes and the members of the prostanoid family. In women with moderate-severe endometriosis, there was an abnormal molecular transition from ESE to MSE and a decreased response to P. Others have evaluated the endometrial transcriptome during gonadotropin stimulation cycles that reveals advanced maturation of the endometrium and thus dyssynchrony of gene expression in the implantation window. The data underscore the utility of assessing the endometrial transcriptome in understanding biological processes in the endometrium in the window of implantation and the potential utility of this approach for diagnosing endometrial abnormalities. Supported by NIH U54 HD31398 and the NIH Office of Women's Health Research.

**PS2. EXPRESSION PROFILING OF THE CONTROL OF GERM CELL MATURATION BY SERTOLI CELLS- FROM DISCOVERY TO HYPOTHESIS.** Michael Griswold, Qing Zhou, Ying Li, Chris Small, Elisabeth Snyder. Washington State Univ, Pullman, WA

A number of expression databases have been generated from developing tissues and isolated cells of the testis, epididymis and efferent ducts of the male reproductive tract. Expression profiling has become a standard tool for reproductive biologists. Often the application of this technology results in long lists of genes whose expression levels are increased or decreased as a result of experimental protocols. As the discovery phase of this approach reaches maturity the use of this information can be used to stimulate and strengthen new hypotheses and new understandings. The useable information that can be obtained from this enormous amount of data is often related to the precision of the question being asked. As an example, the genes important for murine meiosis can be sorted out of these databases by cross-referencing genes expressed in a similar pattern in the embryonic ovary and the postnatal testis. One of the genes identified in this list was the retinoic acid responsive gene known as stra8. At 14.5 dpc, stra 8 is expressed at high levels in the ovary and not at all in the testis while at 10 days postnatal the expression levels in the male are high and absent in the ovary. Expression arrays of testes from vitamin A treated mice showed a 38 fold induction of stra8 within 24 hr. Subsequent experiments showed that stra8 could be induced in isolated gonocytes in the absence of somatic cells and this induction resulted in the differentiation of gonocytes as measured by the induction of gene markers. These results have led to the hypothesis that in vivo the onset of spermatogonial differentiation and possibly meiosis in the testis is regulated by the controlled exposure of A spermatogonia to retinoic acid at stage VII-VIII of the cycle of the seminiferous epithelium. The Sertoli cells are the endogenous source of vitamin A in the testis in the form of large stores of retinyl palmitate. The endogenous stores and the control of the delivery of retinoic acid can be by-passed by exogenous administration of retinoic acid. Exogenous retinoic acid results in premature stimulation of spermatogonia and ultimately alters the germ cell content of specific cycle stages. The components of the enzymatic and receptor mediated pathway that in Sertoli cells leads to the uptake of serum retinol, the storage of retinyl esters and the ultimate stimulation of spermatogonial differentiation at specific stages of the cycle of the seminiferous epithelium can be inferred from levels and timing of expression of the component genes. Supported by 4 R37 HD10808 and 5 U54 HD 42454 from NIH.

**SA1. SIGNAL TRANSDUCTION AND GAMETE FUNCTION: A 2007 PERSPECTIVE.** Marco Conti. Stanford University, Stanford, CA

Both female and male gametes rely on complex networks of signaling circuits to develop in synchrony with surrounding somatic cells and for the final stages of maturation essential for fertilization. In spite of their largely divergent properties, both male and female gametes rely on cyclic nucleotide signaling for the control of many of their functions. Operating at different stages of gametogenesis, cyclic nucleotide signaling modules are assembled from a large array of components, some of which are unique to gametes. These modules are organized in macromolecular complexes, and their spatial organization within the gamete is essential for efficient signaling. In the male, genetic and biochemical studies have demonstrated that components involved in cyclic nucleotide signaling, such as the soluble adenylyl cyclase which also functions as a bicarbonate sensor, are indispensable for spermatozoon motility and male fertility. In the female, cyclic nucleotide signaling is equally indispensable for the development of both the somatic and germ cell components of the ovarian follicle. A large body of work supports the hypothesis that cAMP signaling is involved in maintenance of oocyte meiotic arrest. During the last five years, receptors, G proteins, adenylyl cyclases, and phosphodiesterases expressed in rodent oocytes have been identified and their function in meiotic arrest and meiotic maturation probed using biochemical and genetic strategies. Novel connections between cAMP and the master regulator of the cell cycle, MPF, have also been elucidated, as they likely play an important role in maintenance of meiotic arrest. Yet some of the biochemical steps involved in oocyte maturation remain poorly understood. Ill-defined signals from the somatic cell compartment maintain the meiotic arrest in oocytes competent to reenter the meiotic cell cycle. Progress has been made in understanding how the LH-dependent cAMP signal propagates from the follicular wall to the cumulus oocyte complex during ovulation. Paracrine and autocrine regulations are required to amplify the initial cAMP signal, to propagate through the different cellular components of the follicle, and to integrate it with other signaling pathways. However, the final steps transferring the signal from the somatic compartment to the oocyte remain controversial. A thorough understanding of the cyclic nucleotide signaling modules operating in the male and female gamete has opened new opportunities for pharmacological intervention on fertility.

**SA2. CLONING OF FOOD ANIMALS AND ENDANGERED SPECIES.** Mark Westhusin, Duane Kraemer, Charles Long. Texas A&M University, College Station, TX

Since the initial report by Wilmut et al (1997) describing the successful production of a cloned sheep (Dolly) by somatic cell nuclear transfer (SCNT), cloned animals representing at least 16 different species have

been reported. These include domestic livestock, rodents, wildlife, exotic and endangered species. Given the history of nuclear transfer, and that prior to Dolly, cloning animals using nuclei derived from adults was thought to be biologically impossible; progress towards the development and application of animal cloning has been both rapid and remarkable. The production of cloned animals is inefficient (in cattle for example, only 10–15% of the embryos transferred result in live offspring). However, even with the low efficiency, cloning animals by SCNT is now utilized in numerous laboratories throughout the world for both basic research and commercial applications. Research applications commonly involve mice simply due to the low costs involved when working with this species. Commercial applications involve employing SCNT as part of the process for producing genetically engineered animals and for the replication and/or conservation of valuable genotypes. In these cases, cloned animals most commonly include cattle, goats, horses, and pigs, although other species such cats, dogs and even deer have attracted attention in terms of potential commercial production. The utilization of SCNT for reproducing exotic and endangered species has garnered much attention, but work has been limited due to the logistics of working with these animals in addition to philosophical differences in opinion as to the benefit of using this technology. Given its current status, the commercialization of animal cloning is poised to grow significantly over the next few years. The rate of growth will depend on continued research to improve the efficiency of this technology and perhaps more important, regulatory issues as pertain to the introduction of products (meat, milk, etc) produced by clones into the market place, and public acceptance of these.

**EX1. REGULATION OF SPERMATOGONIAL STEM CELL HOMEOSTASIS AND THE STEM CELL NICHE IN THE TESTIS.** Derek McLean. Washington State University, Pullman, WA

Spermatogonial stem cells (SSCs) are responsible for the continual production of sperm in the adult male. Adult tissue specific stem cells must generate cells that will differentiate into mature, differentiated cells for a particular function while maintaining a population of cells that retain the stem cell role. This process, stem cell homeostasis, is regulated by systemic factors and factors produced by somatic cells closely associated with the stem cells that along with the extracellular matrix form the stem cell niche. The local environment and the factors that contribute to the SSC niche in the mammalian testis are just beginning to be defined. Investigation of systemic factors that affect the biological activity of SSCs has been used to identify and investigate mechanisms that regulate SSC homeostasis. We have demonstrated that suppression of testosterone production results in the inability of SSCs to colonize the testes of recipient busulfan treated mice. We hypothesized that testosterone induces the expression of factors that contribute to the SSC niche. To test this hypothesis, mice that were treated to suppress testosterone production were injected with testosterone propionate. Following treatment, the expression of glial cell line derived neurotrophic factor (GDNF) in the testis increased within 24 hr. In addition, the expression of ret increased 48 hr after treatment. GDNF is expressed by Sertoli cells and binds to the ret/GFRa1 heterodimer on SSCs and has been shown to regulate SSC homeostasis. In rats in which testosterone has been suppressed, treatment with testosterone induces FSH expression and release in the anterior pituitary. Therefore, to understand the systemic and local regulation of GDNF and ret expression in the testis, current experiments are focused on determining if testosterone directly induces GDNF expression in Sertoli or myoid cells or if FSH expression is increased by testosterone treatment in mice where endogenous testosterone production or signaling has been suppressed. Similarly, possible FSH regulation of GDNF expression in Sertoli cells from adult mice is under investigation. Another model being used to study spermatogonial stem cell homeostasis is vitamin A deficiency. Mice that are deficient in vitamin A have decreased numbers of SSCs compared to age-matched controls. Therefore, we are testing the hypothesis that retinoic acid, either through action in Sertoli cells or directly on SSCs, regulates SSC homeostasis in sexually mature mice. By investigating factors that regulate SSC homeostasis, we intend to identify mechanisms that control differentiation and self-renewal of the cells that are essential for continual sperm production in males. Research supported by the NIH.

**EX2. TAKING EVENTS AS THEY COME: META-ANALYSIS IN REPRODUCTIVE MEDICINE.** John Collins. McMaster University, Mahone Bay, NS, Canada

Review and synthesis are central to good scientific and clinical practice. Traditional narrative reviews have the advantage of broad coverage at the cost of undefined methodology. Systematic reviews make use of explicit methods but they are necessarily limited in scope. The steps in systematic review are (1) refine a scientific or clinical question; (2) design a search procedure to find eligible studies; (3) assess the validity of the eligible studies; (4) extract the data; (5) summarize the data from the eligible studies by means of a meta-analysis or tabulation; and (6) interpret the information in the context of what is known about the original question. While meta-analysis procedures can estimate an overall average effect from continuous study effects such as concentrations and measurements, clinical studies usually involve event outcomes and nonparametric analyses. The events range from benefits (live births in randomized controlled trials of in vitro fertilization) to risks (strokes among hormone users). One duty of the analyst is to determine whether the results of the included studies are not heterogeneous, that is, that they measure the same relationship between treatment and outcome. The validity of a given systematic review is determined by the quality of the individual studies, the rigour with which the systematic methods were applied, and the extent of heterogeneity among the study results.

**EX3. ANGIOGENESIS AND ITS PHYSIOLOGICAL CONTROL IN THE OVARY.** Robert Robinson, Amanda Hammond, Leah Nicklin, George Mann, Morag Hunter. University of Nottingham, Loughbrough, LEICS, United Kingdom

Luteal inadequacy, the insufficient secretion of progesterone, is a major cause of poor embryo development and infertility in a number of species. The follicular-luteal transition is a very dynamic phase involving a large number of processes such as cell differentiation, proliferation, biochemical changes and tissue remodelling. These all enable the corpus luteum (CL) to grow and develop at an extremely rapid rate that equals the fastest growing tumours. Underpinning these processes is angiogenesis, the formation of new blood vessels, which is essential for CL development and progesterone production. Thus understanding the factors that regulate angiogenesis at this critical juncture is essential for the development of novel strategies to alleviate luteal inadequacy and infertility. The two principal growth factors that promote angiogenesis in the ovary are fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF), however their precise role remains to be elucidated. Our investigations have clearly demonstrated that FGF2 plays the more dynamic role during the transition periods in the bovine ovary such as the follicular-luteal transition and CL regression. Namely, FGF2 levels dramatically increase in the follicle after the LH surge and are stimulated by LH in dispersed bovine luteal cells. Conversely, VEGF appears to play a more constitutive role in regulating angiogenesis in the pre-ovulatory follicle and CL. Collectively, this indicates that angiogenic process is initiated by a burst of FGF2 activity induced by the LH surge, while the development and maintenance of this vasculature is more controlled by VEGF. Recently, we have developed a physiologically relevant culture system that mimics luteal angiogenesis. This system consists of all luteal cell types (e.g. endothelial, large and small luteal cells, fibroblasts and pericytes). In this system, endothelial cells start to form clusters on day 2, which then proliferate, followed by the formation of thread-like structures. After 9 days in culture, these tubule-like structures lengthen, thicken and form highly organised intricate networks resembling a capillary bed. Development of this endothelial cell network is stimulated by FGF2 and VEGF both independently (3-4 fold; P less than 0.01) and in combination (10 fold; P less than 0.001). In addition, treatment with either the FGF receptor inhibitor (SU5402) or VEGF receptor 2 inhibitor (SU1498) completely suppressed the formation of these endothelial tubules, emphasising the requirement for both factors. Progesterone production increases with time and is stimulated by LH, which re-enforces the physiological relevance of the culture system at mimicking in vivo luteal function. Exciting preliminary observations have shown that pericytes are closely associated with these endothelial networks and this association is influenced by FGF2 and VEGF. These intriguing interactions open up new opportunities to explore the role of the over-looked pericytes in regulating angiogenesis. In conclusion, the successful development of endothelial cell networks in vitro in a physiologically relevant manner heralds a new era in elucidating the physiological control of the angiogenic process in the developing CL, enabling us to solve those questions still unanswered such as how do blood vessels develop? What is the role of the other luteal cell? What stops the angiogenic process? Funded by BBSRC

**HP1. OOCYTE CONTROL OF MOUSE CUMULUS CELL DEVELOPMENT AND FUNCTION.** John Eppig, Koji Sugiura, You-Qiang Su, Francisco Diaz. The Jackson Laboratory, Bar Harbor, ME

Andy Nalbandov, a pioneer in the field of ovarian function, suggested in the 1970s that oocytes might play an important role in preventing premature follicular luteinization. His idea was not well accepted at that time, but now we know that the role of oocytes in follicular development and function extends far beyond control of luteinization. Oocytes play key roles from the time of follicular formation to ovulation, by controlling granulosa cell proliferation, differentiation, metabolism, and cumulus expansion. The development and function of both the oocyte and its companion follicular somatic cells depend upon complex bidirectional interactions between the two cell types. For example, normal oocyte development cannot occur without the complicity of follicular granulosa cells and this can be instructed by the oocyte. It has been known since the classic experiments of Biggers, Brinster, and Whitten that glucose is a

poor energy fuel for mammalian oocytes and embryos, and products of glycolysis, such as pyruvate, are provided to oocytes by their companion cumulus cells. We found that oocytes stimulate elevated levels of mRNAs encoding enzymes essential for glycolysis by cumulus cells. Furthermore, the combination of oocyte-derived BMP15 and FGFs mediate the effects of oocytes on the expression of mRNAs encoding glycolytic enzymes and on glycolysis by the cumulus cells. Similarly, oocytes are deficient in their ability to synthesize cholesterol, but oocyte-derived BMP15 promotes cholesterol biosynthesis by cumulus cells. Thus oocytes outsource metabolic functions to cumulus cells when the oocytes are deficient in the ability to carry out these pathways themselves. Besides controlling metabolism in cumulus cells, oocytes also play key roles in promoting the processes that differentiate cumulus cells from mural granulosa cells that line the follicular wall. The development of cumulus cells requires signals from oocytes. On the other hand, differentiation of mural granulosa cells is dependent upon stimulation with follicle-stimulating hormone (FSH). Paracrine factors produced by the oocyte prevent the expression of transcripts characteristic of the mural phenotype in cumulus cells and promote the expression of transcripts that define the cumulus cell phenotype. Both the suppression of mural transcripts and promotion of the cumulus transcripts require the activation of SMAD2/3 in cumulus cells by oocytes. In contrast, FSH stimulates expression of mural transcripts, but suppresses levels of cumulus transcripts. Thus, oocyte-stimulated SMAD2/3 signaling and FSH establish opposing gradients of influence in the follicle. These factors specify the mural and cumulus granulosa cell phenotypes that are pivotal for appropriate endocrine function and oocyte development, respectively, during the preantral to antral follicle transition. This research was supported by the US NICHD, grants HD23839 and HD44416.

**HP2. JOURNEYS FROM INHERITANCE PATTERNS OF OVULATION RATE IN SHEEP TO FACTORS INFLUENCING OOCYTE-SOMATIC CELL COMMUNICATION IN MAMMALS.** Kenneth McNatty, Susan Galloway, Jennifer Juengel, George Davis. Victoria University of Wellington, Wellington, Wellington, New Zealand; AgResearch Invermay Agricultural Centre, Dunedin, Otago, New Zealand; Agresearch Wallaceville Animal Research Centre, Upper Hutt, Wellington, New Zealand; University of Otago, Dunedin, Otago, New Zealand

From extensive analyses of prolificacy records of sheep and controlled breeding experiments, several flocks have been identified in which inheritance patterns were indicative of major genes affecting ovulation rate. Subsequently, genetic linkage mapping and physiological studies have led, in several cases, to the likely chromosomal location and putative candidate genes responsible for the phenotypes. To date, several different point mutations in the BMP15 gene on the X-chromosome and one each in the GDF9 and ALK6 genes on chromosomes 5 and 6 respectively have been identified. The BMP15 mutations (alleles) were identified in several different lines or breeds of sheep in New Zealand (NZ), (FecXI, FecXH), Ireland (FecXG, FecXB) and France (FecXL) and for the GDF9 mutation (allele) in Irish sheep (FecGH). Animals heterozygous for the BMP15 and GDF9 mutations have higher ovulation rates (i.e. 40-100%) than the wild-types whereas those homozygous for either of these mutations are anovulatory with streak-like ovaries. The mutation in ALK6 (FecBB) has been identified in several different breeds in Australia, NZ, Indonesia, India and China. Animals heterozygous and homozygous for the ALK6 mutation have ovulation rates about 75 and 150% higher respectively, than the wild types. In NZ, a sheep breed has been identified with another putative major gene on the X chromosome (FecX2W) which increases ovulation rate by 20-40%. The inheritance pattern of this mutation is unusual as it is maternally imprinted and silenced in daughters inheriting the putative gene from their dams and in the daughters of sires that inherit it from a dam actively expressing the gene. Thus, the increased ovulation rate only occurs in ewes inheriting the putative gene from a sire that inherited it from a carrier dam that did not have an increased ovulation rate (i.e. a silenced carrier). Within the ovine ovary, BMP15 and GDF9 mRNA and protein are localized to the oocyte and ALK6 to the oocyte, cumulus and granulosa cells. In vitro studies demonstrated that BMP15 and GDF9 may act synergistically on cumulus/granulosa cells to influence proliferation and steroidogenesis and that each factor influences the gonadotropin responsiveness of somatic cells. Whilst the mutation in FecX2W animals is not known, recent results show that follicular development and the expression level of ALK5 in oocytes and ALK6 in both oocytes and granulosa cells differs between carrier and non-carriers suggesting some localised effects for the putative gene. In vivo studies suggest that within the ovary, both BMP15 and GDF9 are likely to have paracrine actions since immunization of sheep or cattle against these proteins or peptides can inhibit or stimulate follicular development in a dose-responsive manner. Oocytes or somatic cells with the ALK6 mutation undergo precocious follicular maturation and ovulate and form corpora lutea at significantly smaller diameters than the wild-type. Precocious maturation seems to occur as a result of the oocyte developing more rapidly during the preantral growth phase and the granulosa cells undergoing differentiation with an earlier responsiveness to pituitary hormones. Collectively, the evidence from studies in sheep is that the level of oocyte-somatic cell communication in mammals has a profound effect on the proliferative and differentiative events during ovarian follicular development and also on the number of follicles that ovulate.

**MA1. NONE BUT OURSELVES CAN FREE OUR MINDS: SCIENCE IN A MAJORITY WORLD.** Trevor Archer. National Institute of Environmental Health Sciences, Research Triangle Park, NC

Abstract not available at time of publication.

**TF1. A LAB OF YOUR OWN.** Teresa Woodruff. Northwestern University, Evanston, IL

The move from a postdoctoral fellow to principal investigator is an exciting, challenging and sometimes daunting transition. What makes a good lab? How do you manage people? How do you organize and operate start up money and grant dollars? How do you communicate your ideas effectively? What are the keys to success? These are questions we all have asked at some time during our career. Unfortunately, there are no definitive answers. But, there are some guidelines that can be used. This talk will provide you with some concepts and approaches to the development of your first laboratory. Bring your questions! We will have a lively dialogue that may ensure that your reproductive science lab is as strong as it can be.

**MS1. SPECIES COMPARISONS IN MOLECULAR AND FUNCTIONAL ATTRIBUTES OF THE ANDROGEN AND ESTROGEN RECEPTOR.** Vickie Wilson. U. S. EPA, Research Triangle Park, NC

While endocrine disrupting compounds (EDCs) have the potential to act via several mechanisms of action, one of the most widely studied is the ability of environmental chemicals to interact directly with either the estrogen (ER) or androgen receptor (AR). In vitro screening assays designed to identify hormone mimics or antagonists typically use mammalian (rat, human) androgen receptors (AR). Although the amino acid sequences of receptors from nonmammalian vertebrates are not identical to the mammalian receptors, it is uncertain whether these differences affect interactions of potential endocrine disrupting chemicals (EDC) with the receptors. It is known that specific mutations in the human AR result in abnormal male sexual phenotypes as the structural changes in AR protein decrease the affinity of the receptor for endogenous androgens. This suggests that individuals or species with minor variations in the AR sequence may be more or less susceptible to some toxicants and that even subtle differences within species might affect the organism's response to EDCs. Some studies indicate that steroid hormone receptors from non-mammalian vertebrates not only differ from mammalian receptors structurally but also in their binding affinities for some steroids and environmental chemicals. With these issues in mind, and to test the hypothesis that EDC's bind steroid receptors from mammalian and non-mammalian vertebrates and invertebrates with similar affinity, we are developing AR and ER binding assays from representative species from several classes of vertebrates and invertebrates. Recombinant receptors from multiple species are being examined in order to be able to draw significant conclusions as to the ability to extrapolate data across species. Current studies compare the affinity of both endogenous steroids and EDCs to recombinant fish (fathead minnow and rainbow trout) and human AR and ER. These types of studies are critical for determining the necessity of including receptors from other species in in vitro assessments and will impact the number and scope of assays needed to perform a thorough assessment. Ultimately, they will increase confidence in risk assessment decisions which involve extrapolation across species. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

**MS2. EPIGENETIC TRANSGENERATIONAL ACTIONS OF ENDOCRINE DISRUPTORS ON REPRODUCTION AND DISEASE: THE GHOSTS IN YOUR GENES.** Michael Skinner. Washington State University, Pullman, WA

Transgenerational effects of environmental toxicants, such as endocrine disruptors, significantly amplify the impact and health hazards of these compounds. One of the most sensitive periods to endocrine disruptor exposure is during embryonic gonadal sex determination when the germ line is undergoing a re-methylation process. The objective of the research was to investigate endocrine disruptor actions on testis development and male reproduction. The model endocrine disruptors tested were vinclozolin, which acts as an anti-androgenic compound, and methoxychlor, that has metabolites that are both weak estrogenic and anti-androgenic compounds. Previous studies have shown that methoxychlor and vinclozolin can effect embryonic testis development at the time of testis morphogenesis and that this causes an increase in germ cell apoptosis in

the adult. Interestingly, observations demonstrate that this spermatogenic defect is transgenerational (F1, F2, F3 and F4 generations) and are hypothesized to be due to a permanent altered DNA methylation of the germ-line through an epigenetic action of the endocrine disruptor. Abnormal testis development and germ cell differentiation caused by endocrine disruptors was found to be in part due to inappropriate control of the testis transcriptome. The expression of over 200 genes were found to be altered in the embryonic testis and surprisingly this altered transcriptome was similar for all vinclozolin generation (F1-F3) males. In addition to detection of the male testis disorder, as the animals age transgenerational effects on numerous other disease states were observed including tumor development, prostate disease and kidney disease. Recent observations also suggest transgenerational effects on behaviors such as sexual selection are associated with evolutionary biology. Therefore, the transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to alter the epigenetic (i.e DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease development transgenerationally in a heritable manner. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational disease states is a new paradigm in disease etiology and evolution not previously considered.

**MS3. HOW DOES EARLY LIFE SOCIAL ENVIRONMENT SCULPT OUR GENES?** Moshe Szyf, Ian C.G. Weaver, Nadine Provencal, Patrick McGowan, Gustavo Turecki, Richard Tremblay, Michael Meaney. McGill University, Montreal, QC, Canada; GRIP University of Montreal, Montreal, QC, Canada

Maternal care has long term effects on health outcomes and behavior, which last through life. How is maternal behavior, which is restricted to early childhood, memorized through life long after maternal behavior is gone? We will present a hypothesis, which is based in our work on maternal care in rats that epigenetic processes program critical genes in response to maternal care. This stable epigenetic programming is nevertheless reversible in adult brain by treatment with either the HDAC inhibitor TSA or central infusion of the essential amino acid methionine a precursor to S-adenosyl-methionine (AdoMet) the donor of methyl-groups for DNA methylation. A molecular mechanism responsible for this dynamic equilibrium of the DNA methylation pattern is proposed here. High levels of maternal licking/grooming (LG) behavior over the first week of postnatal life results in induction of the transcription factor NGFI-A. Binding of NGFI-A to the exon $1_7$ GR promoter within the 6-day-old offspring permanently marks the promoter sequence for histone acetylation and facilitates DNA demethylation which is triggered by MBD2. We will also present preliminary data from our human studies suggesting that epigenetic differences correlate with behavioral differences in humans as well. Our data illustrates a possible mechanism for how behavioral cues mediate epigenetic programming of genes within the brain. We propose that the epigenome mediates between the dynamic environment and our static genomes and provides a molecular link between nurture and nature.

**MS4. DEVELOPMENT OF THE FEMALE REPRODUCTIVE TRACT.** Richard Behringer, Grant Orvis. University of Texas M.D. Anderson Cancer Center, Houston, TX

The reproductive tract of female mammals derives from the Müllerian duct that forms during embryogenesis. The Müllerian duct gives rise to the oviducts, uterus, cervix, and upper portion of the vagina. We will present studies on the mechanisms of Müllerian duct formation, using molecular, fate-mapping, and experimental embryological studies in transgenic mice. Our studies show that the Müllerian duct forms predominantly by proliferation of cells located at its caudal tip. We suggest that these cells represent a stem cell pool that resides in a highly specialized niche between the Wolffian duct and the coelomic epithelium. Fate-mapping studies of the Müllerian duct epithelium using a Wnt7a-Cre transgenic mouse line are underway to determine which cell types this tissue gives rise to in the adult female reproductive tract.

**MS5. INTERACTION BETWEEN ANDROGENS AND LOCAL SIGNALING MOLECULES IN WOLFFIAN DUCT MORPHOGENESIS.** Humphrey Yao, Jessica Tomaszewski, Avenel Joseph, Denise Archambeault. University of Illinois, Urbana-Champaign, IL

One of the most fascinating and yet mysterious events in male sex organ development is how a straight Wolffian duct is transformed into various parts of the male reproductive tract such as epididymis, vas deferens, and seminal vesicles. Initial formation of the Wolffian duct, also known as the mesonephric duct, is not a sexually dimorphic event as this duct is found in embryos of both sexes. It has become clear that transcription factors and signaling molecules including Emx2, Foxc1 Gata3, Pax2, Wt1, and Wnt9b control the establishment of the Wolffian duct in a non sex-specific manner. However, the maintenance and further regional specification of the Wolffian duct becomes male-specific and requires androgens produced by the developing testis. In the female embryo, lack of testosterone production results in degeneration of the Wolffian duct. During embryogenesis, the Wolffian duct differentiates from one straight duct into a complex segmented tract with unique characteristics within individual segments. How androgen alone facilitates this regional differentiation of the Wolffian duct remains unknown. We hypothesize that a segment-specific signaling field is established along the Wolffian duct and is responsible for formation of individual components of the male reproductive tract. We have identified inhibin beta A (Inhba) as a signaling molecule specific to the anterior Wolffian duct mesenchyme and required for proper morphogenesis of the epididymis. The mesenchyme-derived Inhba is essential for proliferation and differentiation of the Wolffian duct epithelium. Loss of Inhba resulted in a significant decrease in epithelial proliferation and, eventually, absence of the epithelial coiling in the epididymis. Most interestingly, androgen production was normal in the absence of Inhba indicating the epididymal phenotypes were not caused by androgen deficiency. We further demonstrated that the maintenance of Inhba expression required testosterone, consistent with the findings that testosterone is the upstream regulator. Taken together, we have discovered Inhba as the first mesenchyme-derived factor to regulate transformation of the anterior Wolffian duct into epididymis in response to testosterone. It remains to be determined how Inhba becomes expressed exclusively in the anterior Wolffian duct (Supported by NIH-HD46861 and the March of Dimes Birth Defects Foundation).

**MS6. COMPARATIVE EPIDIDYMAL PROTEOME OF MONOTREMES AND OTHER MAMMALS INCLUDING HUMANS.** Jean-Louis Dacheux, Russell Jones, Clémence Belleannée, Valérie Labas, Maya Belghazi, Françoise Dacheux. INRA, Nouzilly, 37380, France; INRA-CNRS, Nouzilly, France; The University of Newcastle, Newcastle, NSW, Australia

The two epididymal functions preserved across mammalian species are the maturation of the testicular sperm in the proximal epididymis and the maintenance of their viability in the distal part of this organ. There is a division of labour along the epididymis to carry out these functions involving sequential modification of the composition of the luminal milieu in numerous regions by the secretion and absorption of specific proteins. We are examining the protein composition of the epididymal fluids in order to identify which proteins have been conserved during mammalian evolution. This report presents an analysis of the epididymal proteome and secretome on several domestic mammals, monotreme and human. Using a microcannulation technique, the proteins present and secreted in the lumen fluid by the epithelium from various epididymal regions were analysed and identified by 2D gel electrophoresis and LC MS/MS from all these different species. Several hundred epididymal proteins were identified and for several of them, their luminal concentrations along the epididymal tube were quantified. Among these most distant species in mammalian evolution, several proteins or protein functionalities have been conserved, although their abundance in the epididymal luminal fluid varies considerably between species. Further, the variation among species in the secretory activity and luminal protein composition along the epididymis indicates that the regionalisation of the epididymides is specific to species, although it is small among the monotremes. The monotreme and human epididymides show the least regionalization in the luminal composition and the secretion of protein. This comparative approach illustrates that evolution has produced different strategies of providing a protein environment for sperm maturation and storage in the epididymis.

**MS7. NOVEL GLYCOLYTIC ENZYMES ARE ESSENTIAL FOR SPERM FUNCTION AND MALE FERTILITY.** Deborah O'Brien. University of North Carolina School of Medicine, Chapel Hill, NC

There are a surprising number of glycolytic enzyme variants in mammalian sperm, including several with restricted expression in the male germline. New features of this central metabolic pathway continue to be uncovered. We recently identified two novel aldolase A variants in mouse sperm that are encoded by intronless retrogenes and a third splice variant with a distinctive N-terminus. Multiple glycolytic enzymes are localized in the principal piece, which is the longest segment of the sperm flagellum. At least four of these enzymes are anchored to the fibrous sheath, along with a number of key components of signal transduction pathways. This compartmentalization and enzyme diversity suggests that energy production in mammalian sperm may be regulated by novel mechanisms. Our gene targeting studies of two isozymes expressed only during spermatogenesis indicate that glycolysis is essential for maintaining sperm motility and male fertility in the mouse. Males lacking glyceraldehyde 3-phosphate dehydrogenase-S (GAPDHS) are infertile and produce sperm that do not exhibit progressive motility. Although indistinguishable from wild-type sperm at the light microscopic level, sperm lacking GAPDHS have subtle ultrastructural defects in the fibrous sheath, with wider spacing between some of the circumferential ribs. ATP

levels of GAPDHS-null sperm are only 10% of wild-type levels immediately after isolation and decline further within 30 min. These low ATP levels cause deficits in protein phosphorylation during epididymal maturation and capacitation, which may contribute to the observed defects in motility, zona binding and in vitro fertilization. We also produced mice lacking phosphoglycerate kinase 2 (PGK2). This isozyme catalyzes the reaction immediately after GAPDHS in the glycolytic pathway in sperm. We expected the reproductive phenotype of PGK2-null mice to be identical to GAPDHS-null males, without defects in the fibrous sheath since PGK2 is not tightly bound to this cytoskeletal structure. As expected, sperm motility and male fertility are severely impaired in mice lacking PGK2 and fibrous sheath ultrastructure is indistinguishable from wild-type sperm. However, these males sire occasional small litters. In further comparisons, we found that motility and ATP levels are consistently higher for PGK2-null sperm during the first 30 min after isolation from the cauda epididymis. These studies suggest that glycolysis may be regulated by distinct mechanisms in male gametes and confirm the importance of this metabolic pathway for sperm energy production and function. Supported by NICHD/NIH through U01 HD45982 and cooperative agreement U54 HD35041 as part of the Specialized Cooperative Centers Progam in Reproduction and Infertility Research.

**MS8. GENETICS OF SPERMATOGENESIS AND FERTILIZATION IN THE NEMATODE *C. Elegans*.** Steven L'Hernault, Elizabeth Gleason, Katherine Hill, Paul Hartley, Tim Kroft, Miriam Ratliff, Guang-dan Zhu. Emory University, Atlanta, GA

The ultimate function of a spermatozoon is to successfully fertilize an egg and this requires a unique cell surface. We take a genetic approach to analyzing how the spermatozoon acquires its unique cell surface. Our studies are performed in the nematode *C. elegans* because its unusual reproductive biology facilitates recovery of suitable mutants. Like the acrosome found in flagellated spermatozoa, amoeboid *C. elegans* spermatozoa contain a specialized secretory vesicle (MO) that fuses with the cell surface and makes it competent for fertilization. Many of the mutants in our collection affect biogenesis and function of the MO and their study is giving us insight into the origin and function of this organelle. The MO, like the acrosome, is assembled by the coalescence of a large number of vesicles. We discovered that the SPE-39 protein, which has an ortholog in humans and other animals, mediates this process. *spe-39* mutants are sterile because sperm cannot function if they are unable to form MOs. Once formed, MOs become acidified and this occurs at the spermatid stage. MO, like lysosome and acrosome, acidification is blocked by bafilomycin, which is a specific inhibitor of the vacuolar (V) ATPase. Since the V-ATPase is essential, it is a challenge to study its role during spermatogenesis in animals. We discovered that the severe MO defects observed in *spe-5* mutants are caused by mutation of a V-ATPase B subunit that is required only during spermatogenesis. Nine mutants make normal-appearing spermatozoa that are fertilization-defective and two produce a MO resident protein that is defective (Xu and Sternberg 2003; Cell 114: 285; Chatterjee et al. 2005 Development 132: 2795). We are currently studying three other fertilization-defective mutants, *fer-14*, *spe-16* and *spe-42*. *fer-14* and *spe-42* both encode transmembrane proteins while *spe-16* encodes an ubiquitin E3 ligase. We are currently analyzing the spermatozoon cell surfaces of these three mutants and will determine whether their defects are associated with the MO.

**MS9. MUTAGENESIS AND PHENOTYPE-DRIVEN APPROACHES FOR IDENTIFICATION OF MALE FERTILITY GENES.** Mary Ann Handel, Laura Reinholdt, Fengyun Sun, Carl Lessard, Sheila Bornstein, Heather Lothrop, Suzanne Hartford, John Schimenti, John Eppig. The Jackson Laboratory, Bar Harbor, ME; Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada; Cornell University, Ithaca, NY

Male infertility contributes to roughly half of human couple infertility, yet causes, which can be genetic and/or environmental, are poorly understood. One obstacle is that we have not yet identified the full repertoire of genes whose function is required for male fertility. Targeted mutagenesis strategies, such as gene knock-outs, are a useful approach to defining genetic requirements for male fertility, but some prior knowledge of the targeted gene is necessary. However, in cases when infertility was an unexpected outcome of gene disruption, such strategies have led to the identification of genes previously unsuspected to play a role in fertility. In contrast to gene-directed approaches such as knock-outs and gene traps, phenotype-driven approaches do not rely on previous knowledge of gene identity and are almost wholly unbiased. The ReproGenomics Program at The Jackson Laboratory (http://reprogenomics.jax.org/) identifies novel genes required for fertility. Phenotype analyses for male fertility are based on breeding performance and/or gonad size; practical considerations dictate a strategy of screening for infertility rather than reduced fecundity. This program has identified phenotypes affecting male germ cell differentiation and function from spermatogonia to ejaculated sperm, providing valuable resources for dissection of developmental pathways at all stages. One recovered mutation affects survival of germ cells at the onset of adult Sertoli cell function. Another affects progress of spermatocytes out of meiotic prophase I; the affected germ cells do not exhibit competence to undergo the meiotic divisions. A large cluster of recovered mutations affect the post-meiotic spermiogenic differentiation of germ cells. These models for human syndromes of oligoasthenoteratozoospermia provide interesting insight into the etiology and clinical management of these syndromes. Many of these mutations have been distributed to the scientific community. The mutated gene has been identified for about 20% of the mutations produced. The staff of ReproGenomics is actively pursuing the positional cloning of some and also collaborates with outside end users to facilitate fine mapping and positional cloning of several mutations. Mice carrying any of the mutations are available to any interested scientists, and the ReproGenomics Program maintains a cyropreserved sperm bank for all mutations recovered and regionally mapped. To date, considering mutations that affect males and/or females, the ReproGenomics Program has produced roughly 10% of all mutations known to affect fertility in mice, providing novel experimental approaches for resolving problems of infertility in the clinic. (Supported by the NIH, HD42137)

**MS10. CLONING AND EXPRESSION PROFILING OF TESTIS-EXPRESSED SMALL RNAS.** Wei Yan. University of Nevada School of Medicine, Reno, NV

Recently identified non-coding small RNAs have been suggested to regulate gene expression through mRNA degradation, translational suppression, and chromatin modification. A striking feature of spermatogenesis is that meiotic and haploid male germ cells exhibit high transcriptional activity, strictly regulated spatiotemporal expression and profoundly repressed translation. If small RNAs are indeed important regulators of gene expression, one would expect that the testis expresses numerous small RNAs and some of them should be testis-specific. Furthermore, if these small RNAs play essential roles in spermatogenesis, aberrant small RNA expression may be involved in male infertility and small RNAs may also serve as good drug targets for future male contraceptives. Cloning and preliminary characterization of testis-expressed small RNAs represent the first step toward these ultimate goals. We attempted to clone small RNAs with sizes ranging from 20-30 nt from the mouse testis using a modified cloning method that we established. We identified 141 miRNAs and 585 piRNAs from the mouse testis. Among them, 29 miRNAs and 496 piRNAs are novel. Genes encoding these miRNAs and piRNAs are distributed across the entire genome and most of them exist as clusters. Expression profiling for 122 miRNAs and 64 piRNAs revealed that the 5% of miRNAs and 30% of the piRNAs were exclusively expressed in meiotic and haploid male germ cells in the testis. In addition to the size difference, precursors for the majority of miRNAs (94%) and only a small portion of piRNAs (17%) can form stem-loop structures. Paired expression of sister miRNAs derived from both the 5' and 3' strands of the precursor miRNAs appears to be a unique feature of testis-expressed miRNAs, whereas only piRNAs display expression of multiple overlapping homologs from a single locus. Our preliminary data indicate that the testis is the organ with the highest expression of miRNAs and piRNAs both in number and in abundance. Differences in biogenesis and expression profiles between miRNAs and piRNAs strongly suggest that the regulation of their expression and their physiological roles may be substantially distinct.

**MS11. AVIAN EMBRYONIC STEM CELLS, PRIMORDIAL GERM CELLS AND TRANSGENIC CHICKENS.** James Petitte. NC State University, Raleigh, NC

Transgenic animals have become important tools for biological research. In mammals, several methods are available to introduce foreign DNA into the mammalian genome. Gene transfer in birds is a relatively complicated process because of the unique aspects of avian reproduction, and avian transgenics has largely been limited to the use of retroviral/lentiviral vectors. However, the temporal and spatial aspects of the development of avian primordial cells (PGCs) provides easy access to the germline and several methods can be used to produce germline chimeras. Initially, the ability to make germline chimeras has given rise to efforts to establish avian embryonic stem cells (ESCs)lines. In the chick, ESCs can be cultured from the area pellucida of the unincubated embryo. These cells show several features of stem cells including the capacity to give rise to all somatic tissues. However, competency to give rise to the germline has been difficult to achieve, most likely due to the mechanisms of avian germline development. As an alternative to embryonic stem cells, the long term culture of avian PGCs hold significant promise for non-viral methods of manipulating the avian genome. Using a combination of STO feeder layers, conditioned media, fibroblast growth factor-2 and stem cell factor, stable lines of chick PGCs have been established from single embryos. These lines express several markers of germ cells and have been in continuous culture for over a year. Many of the lines retain their ability to

populate the germinal ridge when injected into early embryos and can give rise to functional gametes at sexual maturity. Like that observed in mammals, under specific culture conditions, PGCs can give rise to embryonic germ (EG) cells. However, as observed with ESCs, avian EG cells only give rise to somatic cells. Hence, the long-term culture of primordial germ cells opens new applications for avian germ cell biology, avian transgenics, germplasm preservation and stem cell biology.

**MS12. MICRORNAS EXHIBIT HIGH FREQUENCY GENOMIC ALTERATIONS IN HUMAN OVARIAN CANCER.** Lin Zhang, Jia Huang, Nuo Yang, Joel Greshock, M Megraw, Antonis Giannakakis, S Liang, T Naylor, A Barchetti, M Ward, G Yao, A Medina, A O'Brien-Jenkins, D Katsaros, Artemis Hatzigeorgiou, P Gimotty, Barbara Weber, George Coukos. University of Pennsylvania, Philadelphia, PA

MicroRNAs (miRNAs) are endogenous noncoding RNAs, which negatively regulate gene expression. To determine genomewide miRNA DNA copy number abnormalities in cancer, 283 known human miRNA genes were analyzed by high-resolution array-based comparative genomic hybridization in 227 human ovarian cancer, breast cancer, and melanoma specimens. A high proportion of genomic loci containing miRNA genes exhibited DNA copy number alterations in ovarian cancer (37.1%), breast cancer (72.8%), and melanoma (85.9%), where copy number alterations observed in >15% tumors were considered significant for each miRNA gene. We identified 41 miRNA genes with gene copy number changes that were shared among the three cancer types (26 with gains and 15 with losses) as well as miRNA genes with copy number changes that were unique to each tumor type. Importantly, we show that miRNA copy changes correlate with miRNA expression. Finally, we identified high frequency copy number abnormalities of Dicer1, Argonaute2, and other miRNA-associated genes in breast and ovarian cancer as well as melanoma. These findings support the notion that copy number alterations of miRNAs and their regulatory genes are highly prevalent in cancer and may account partly for the frequent miRNA gene deregulation reported in several tumor types.

**MS13. WHY SIZE MATTERS: NOVEL MECHANISMS PROTECT EARLY EMBRYOS FROM THE STRESS OF CELL VOLUME PERTURBATIONS.** Jay Baltz. Ottawa Health Research Inst. and Univ. of Ottawa, Ottawa, ON, Canada

Control of size is a fundamental property of cells. Animal cells control their volumes osmotically, by regulating intracellular osmolyte concentrations. A cell's immediate response to deviations from its preferred volume is to adjust intracellular ion concentrations, but this is not a good long-term solution for many cells. Instead, they use "organic osmolytes"—small, uncharged organic compounds—to provide intracellular osmotic support and regulate cell volume. There is good evidence that preimplantation embryos require such organic osmolytes for cell volume regulation and viability, and that this is a key requirement for normal embryo development. Raising osmolarity to the same level as in the oviduct blocks mouse embryo development in vitro. However, this developmental arrest could be eliminated when any of several potential organic osmolytes were present, among the most effective of which was glycine. However, none of the organic osmolyte transporters found in other mammalian cells was present in early embryos. Instead, early mammalian embryos control their cell size by mechanisms that are fundamentally different from those in somatic cells. One is a glycine transporter called GLYT1 that mediates the accumulation of glycine as an organic osmolyte, and is required for embryo viability and cell volume regulation. It is restricted to a short period of oocyte and cleavage-stage development. Independent cell volume regulation first appears in the maturing oocyte just after ovulation is triggered, and includes a re-setting of oocyte volume and activation of GLYT1. Thus, the emerging model is that oocytes become capable of independent volume regulation just before they are ovulated and fertilized, and that the early cleavage-stage embryo then uses cell volume regulatory mechanisms that are unique to embryos until around the time of compaction.

**MS14. IMPACT OF STRESS AND STRESS ENZYMES ON EARLY MAMMALIAN DEVELOPMENT.** Daniel Rappolee. Wayne State Univ, Detroit, MI

Homeostatic responses, but also differentiation, are induced during transient stress in preimplantation embryos and trophoblast stem (TS) cells. In embryos and TS cells, the homeostatic responses include slower cell accumulation via decreased cell cycle entrance and increased apoptosis. These responses are largely mediated by the stress-signaling enzymes, stress-activated protein kinase (SAPK) and AMP-activated protein kinase AMPK. Both of these kinases are induced rapidly by stress and enter the nucleus. During stress in TS cells, SAPK is necessary to induce and maintain nuclear HAND1 and AMPK is necessary to cause loss of nuclear ID2. Together these stress enzymes are necessary for the induction of placental lactogen (PL)1 through regulation of HAND1 and ID2. The loss of cell growth rate and ID2 protein are reversible, if stress is removed. Therefore, stress, acting through stress enzymes, can cause differentiation to mediate the next essential developmental event. But, is this just a quirk of a single ensemble of proteins? A global analysis of the kinetics of the stress response of TS cells using microarrays suggests that the induction of PL1 is a small part of a larger differentiation program activated by stress. The transcription factors mediating differentiation of early primary and secondary trophoblast giant cells are induced by 24hr of stress. These include hairy enhancer of split (HES)1, retinoic activated (STRA)13, and GATA2. But, transcription factors marking later-arising placental lineages of spongiotrophoblasts (TPBPa, MASH2) and syncytiotrophoblasts (GCM1, TEF5) are not induced. Besides PL1, other early endocrine factors are also induced. These include proliferin, PLP-M, and PLP-E, but not later endocrine factors such as PLII and PLP-A. Also, stress induced, AMPK-dependent loss of the transcription factors ID2 and CDX2 in blastocysts and 2-cell embryos as well as TS cells, suggests that mechanisms for mediating stress responses may be shared, although biological outcomes are likely to be different.

**MS15. SUSCEPTIBILITY OF GENOMIC IMPRINTING TO EMBRYO CULTURE.** Mellissa Mann. CHRI/University of Western Ontario, London, ON, Canada

The efficacy and safety of human assisted reproductive technologies (ARTs) have come under intense questioning. Accumulating evidence suggests that children conceived by ARTs may be at increased risk for developing genomic imprinting disorders. This may be related to the fact that the timing of many ART procedures coincides with crucial events that regulate genomic imprinting during oocyte growth and early embryo development. Our research investigates specific ART procedures, including in vitro embryo culture, in a mouse model system to determine their effects on genomic imprinting, a mechanism that directs parental-specific expression of a group of developmentally important genes. To determine of the effects of ART-induced stress, imprinted gene expression and DNA methylation were examined after in vitro preimplantation culture. Following culture in Whitten's medium, the normally silent paternal H19 allele was aberrantly expressed and undermethylated in cultured blastocysts. Loss of H19 imprinting persisted in midgestation conceptuses, with placental tissues displaying a greater loss of imprinting than the embryo proper where imprinted expression and DNA methylation for the most part were preserved. These data indicate that mechanisms operating to maintain imprinting in the mammalian preimplantation embryo are labile and subject to environmental stress. Furthermore, tissues of trophectoderm origin are unable to restore genomic imprints and suggest that mechanisms that safeguard imprinting may be more robust in the embryo than placenta. These results provide a cautionary note for the culture of human embryos in ART. Further investigations are required to determine the scope of ART effects on genomic imprinting and development. (Lalor Foundation)

**MS16. BIOLOGY OF *GnRH* NEURONAL MIGRATION IN THE LIVING ZEBRAFISH EMBRYO.** Nancy Wayne. UCLA School of Medicine, Los Angeles, CA

Gonadotropin releasing hormone (GnRH) neurons are the command cells in the central nervous system (CNS) that control reproductive physiology and sexual behavior in all vertebrates studied to date. These neurons are born outside of the CNS and migrate into the forebrain during embryogenesis. Inappropriate migration of these neurons during development results in infertility in adulthood. Mechanisms that regulate GnRH neuronal migration and cell physiology are poorly understood because these neurons are relatively few in number and scattered in the hypothalamus and adjacent brain areas, making it unfeasible to study their biology in living tissue of wildtype animals. To overcome this obstacle, we generated a stable line of transgenic zebrafish in which the GnRH promoter drives expression of green fluorescent protein (GFP). This new animal model provides us with the unique opportunity to identify GnRH neurons for analyses of neuronal migration and electrical activity in the intact, living embryo. The aim of this work is to reveal the functional relationship between neuronal migration and spontaneous action potential firing in vivo. My collaborators and I are using a combination of experimental tools to understand the biology of GnRH neuronal migration: molecular biology to manipulate gene expression; optical imaging to monitor neuronal migration and axonal pathfinding; and whole-cell patch clamp electrophysiology to analyze electrical activity.

**MS17. MINIMAL REQUIREMENTS FOR FERTILITY; HOW MANY GnRH NEURONS DO YOU REALLY NEED?** Allan Herbison. University of Otago, Duendin, Otago, New Zealand

The key importance of reproduction to the survival of any species suggests that the biological processes underlying fertility should be robust and exhibit a degree of functional redundancy. In mammals, fertility is governed by the activity of a small population of approximately 1000 GnRH neurons that must, improbably, migrate from the nose into the brain during embryogenesis to become functionally active. Using a novel

transgenic mouse line, we investigated the minimal GnRH neuron requirements for puberty, ovulation and fertility.

The GnRH neurons fail to migrate into the brain of the GNR23 mutant mice in an allele-dependent manner; adult hemizygous GNR23 mice have only approximately 300 GnRH neurons in their forebrain whereas homozygous GNR23 mice have around 80 GnRH neurons in total. The GnRH neurons that do migrate, arrange themselves in the normal scattered distribution of the GnRH neuron population and extend axons to all of their normal targets. The GnRH neurons trapped within the nose die after birth. The reproductive phenotype of GNR23 mice is provided below.

(1) Male hemizygous and homozygous GNR23 mice exhibit normal levels of fertility despite the reduced size of their otherwise normal testicles and reduced plasma FSH concentrations.

(2) Female hemizygous GNR23 mice (200 GnRH neurons) exhibit normal levels of fertility despite generating an LH surge that is reduced in magnitude by approximately 80%. This indicates that substantial redundancy is likely to exist at the level of the ovary with respect to ovulation.

(3) Female homozygous GNR23 mice (80 GnRH neurons) are either infertile or severely sub-fertile. These mice typically fail to get pregnant and remain in persistent estrous. They also fail to ovulate in response to exogenous steroids and have ovaries that show no evidence of ovulation. Using the immediate-early gene c-Fos as an indicator, it appears that <10 GnRH neurons are activated at the time of the surge in homozygous GNR23 mice. Presumably this results in insufficient levels of GnRH within the portal vessels to create an LH surge.

(4) Despite the severe reproductive deficits found in adult females, puberty (as assessed by vaginal opening and first estrus) was observed to occur at the correct time in female GNR23 mice.

Together, these observations indicate that control of fertility by the GnRH neuronal system is extremely robust with 80% of the population being redundant. Indeed, less than <10% of the GnRH neuron population is compatible with normal fertility in males. Females, that must exhibit more complex cyclical behavior, have a greater requirement but still manage to drive normal fertility with as few as 200 GnRH neurons (~20% of population). Intriguingly, puberty, including first ovulation, is normal with only 80 GnRH neurons suggesting an even more robust mechanism for GnRH neuron activation of puberty in female mice. The inference from these studies is that individuals presenting at the clinic with hypogonadotropic hypogonadism must have pathologies impacting upon the great majority of their GnRH neurons.

**MS18. NEUROBIOLOGICAL CONTROL OF PULSATILE *GnRH* RELEASE IN MAN.** Stephanie Seminara. Massachusetts General Hospital, Boston, MA

The hypothalamic hormone GnRH is episodically released into the hypophysial portal circulation and stimulates the release of the gonadotropins LH and FSH. These gonadotropins in turn stimulate the processes of gametogenesis and sex steroid production at the level of the gonads. The GnRH neuron has stood as the central information integrator for pubertal onset. Yet exactly how that integration occurs and what positively and negatively modulates the function of the GnRH pulse generator have been challenging to understand. Gene networks, environmental cues, and endogenous signals all play a role. Today we will examine some of these key elements that play a role in the neuroendocrine control of reproduction, with particular emphasis on the role of human genetics in elucidating the modulators of GnRH secretion.

**MS19. OOCYTE TRANSCRIPTIONAL REGULATORS IN OVARIAN HEALTH AND DISEASE.** Aleksandar Rajkovic. Baylor College of Medicine, Houston, TX

Oogenesis is a specialized and regulated process essential for ovarian development, embryogenesis and homeostasis. Pathologic changes in both regulatory and structural components of this pathway affect ovarian differentiation, maintenance, and early embryogenesis leading to premature ovarian failure and early embryo losses. A basic understanding of the biologic modifiers important in oogenesis, especially those, which act on the transcriptional level, would further our understanding of oocyte biology as well as provide insight into premature ovarian failure, reproductive life span, menopause, ovarian tumors and early embryonic losses. Identification and characterization of genes preferentially expressed in oocytes is useful in unraveling oocyte-specific pathways and their contribution to ovarian pathology. Using variety of approaches, we and others, have identified several germ cell specific transcriptional regulators, such as Figla, Sohlh1, Lhx8, and Nobox. Germ cell transcriptional regulators act as pro-survival factors and regulate genes essential for folliculogenesis and early post-fertilization embryo development. Transcriptional regulators upstream of Sohlh1, Lhx8 and Nobox, will give us more insight into the elusive molecular mechanisms that guide female gonadal differentiation. In mice, deletion of Figla, Sohlh1, Lhx8 and Nobox leads to ovarian failure. Rapid loss of oocytes observed in mouse model systems parallels some cases of non-syndromic ovarian failure in humans, and we show that transcriptional pathways in mice are mutated in some cases of human ovarian failure.

**MS20. SYSTEMATIC ANALYSIS OF GENES INVOLVED IN THE OOCYTE-TO-EMBRYO TRANSITION.** Minoru Ko. NIH/National Institute on Aging, Baltimore, MD

The transition from oocyte to embryo, marked by the first wave of transcription called zygotic genome activation (ZGA), is one of the most important events in animal development. Recent large-scale EST projects and DNA microarray studies in mouse have identified many novel genes that are expressed during the ZGA. Among these genes, we have been studying Zscan4 that encodes a SCAN domain and four zinc finger domains. Zscan4 is expressed exclusively in late 2-cell embryos and embryonic stem (ES) cells. The loss-of-function study by the siRNA technology indicates an important function of Zscan4 for progression from 2- to 4-cell stages and subsequent embryo development. Interestingly, Zscan4 is not expressed in the inner cell mass (ICM) of blastocysts — putative in vivo counter part of ES cells. We found that the Zscan4 is reactivated during the blastocyst outgrowth and subsequently expressed in only a few percent of undifferentiated ES cells. We will discuss the details of Zscan4 and other genes that show similar expression patterns. This research was supported by the Intramural Research Program of the NIH, National Institute on Aging.

**MS21. THE EMBRYO GLASS CEILING: ASSESSMENT OF NEW TECHNOLOGIES FOR EMBRYO EVALUATION IN IVF LABORATORIES.** Richard Scott. Reproductive Medicine Associates of New Jersey, Morristown, NJ

Abstract not available at time of publication.

**MS22. REGULATION OF SPERM-EGG INTERACTIONS: NEW INSIGHTS INTO HOW MAMMALIAN EGGS PREVENT POLYSPERMY.** Janice Evans. Johns Hopkins Univ., School of Public Health, Baltimore, MD

Fertilization triggers the initiation of development and establishment of blocks to prevent fertilization by multiple sperm (polyspermy). Polyspermic fertilization will result in a triploid embryo, which will typically die during early development. The incidence of polyspermy is 1-2% based on studies of a variety of mammalian species, and in humans, triploidy is detected in ~10% of spontaneously aborted human conceptuses, and the majority of triploid human embryos do indeed appear to be the result of two sperm fertilizing an egg. To prevent fertilization by more than one sperm, mammalian eggs utilize blocks to polyspermy, established on the zona pellucida and the egg plasma membrane. Thus, after fertilization, these structures undergo a dramatic change in function — from being receptive to sperm to being unreceptive to sperm. Our work has been examining this post-fertilization change in egg membrane function and what factors regulate the establishment of the membrane block to polyspermy. Part of our work has focused on Ca2+ and Ca2+-dependent effector molecules. We have found that membrane block establishment, like other changes occurring upon fertilization and egg activation, is affected by the increase in cytosolic Ca2+ concentrations. Mouse eggs that experience only one sperm-induced Ca2+ transient establish a membrane block that is less effective than do eggs that experience normal sperm-induced Ca2+ transients, but that is more effective than do eggs with completely suppressed Ca2+ increases. The sperm-induced increases in cytosolic Ca2+ in the egg appear to regulate the timing of membrane block establishment, as this block is established more slowly in eggs that experience one or no sperm-induced Ca2+ transients. We are also interested in what Ca2+-dependent effectors work downstream from Ca2+ to lead to membrane block establishment. Because Ca2+/calmodulin-dependent protein kinase II (CaMKII) appears to be the key regulator of Ca2+-dependent egg activation events, particularly cell cycle resumption, we examined whether that CaMKII was a Ca2+-dependent effector leading to membrane block establishment. We have found that that eggs treated with a CaMKII inhibitor establish a less effective membrane block to polyspermy than do control eggs. But, on the other hand, expression of a constitutively active CaMKII in eggs is not sufficient for membrane block establishment, despite the fact that these CaMKII-activated eggs undergo other egg activation events; this provides evidence that different pathways downstream from Ca2+ lead to the different egg activation responses. Adding to this are other aspects of our studies that suggest that membrane block establishment may be regulated by other factors working in conjunction with Ca2+. A Ca2+ independent pathway that is linked somehow with sperm-egg interaction also seems to contribute to membrane block establishment, based on studies of ICSI-fertilized eggs, eggs activated by injection of soluble sperm components, and of fertilized eggs with suppressed sperm-induced Ca2+ transients. We also have observed a correlation between abnormalities in sperm-associated remodeling of the egg cortical actin cytoskeleton and abnormalities in the membrane block. Ongoing studies are examining Ca2+-dependent and Ca2+-independent signaling pathways as well as

sperm-associated remodeling of the egg cortex and membrane, as these three cellular phenomena are part of our model for how mammalian eggs prevent polyspermic fertilization.

**MS23. DISSECTING THE ROLE OF ODORANT RECEPTORS IN NAVIGATING HUMAN SPERM.** Marc Spehr. Ruhr-University Bochum, Bochum, NRW, Germany

In addition to odorant receptor (OR) expression in sensory neurons of the olfactory epithelium, some OR proteins are also found in ectopic tissues, e.g. in testis. In mammals, such testicular ORs have been attributed a potential function as molecular switches of unique sperm swimming behaviors. Using a combination of recombinant receptor expression in HEK293 cells, single cell calcium imaging, sperm accumulation assays, flagellar beating and video motion analysis, we recently identified and functionally characterized a human testicular OR, OR1D2 (a.k.a. hOR17-4), that specifically responds to a subset of structurally similar floral odors (e.g. bourgeonal) and triggers chemotactic and chemokinetic sperm responses *in vitro*. In addition, we here report functional description of two novel putative sperm ORs. With respect to their individual physiological roles *in vivo*, however, several crucial questions remain. First, are these ORs actually functional in human sperm? Second, do they trigger common or distinct signaling pathways, evoking similar or OR-specific behavioral responses? Third, is the popular "one cell - one receptor" rule that applies to olfactory neurons also applicable to sperm? Comparative analysis of receptor-specific activation profiles reveals non-overlapping molecular receptive fields as well as distinct susceptibility to antagonistic odors. Activation of individual ORs in human sperm mediates distinct behavioral response patterns. Our findings thus support the notion that OR-dependent signaling pathways could play a substantial role in various pre-fusion aspects of sperm physiology.

**MS24. THE SPERM ANNULUS IS A SEPTIN-RINGED ORGANELLE CRITICAL IN PRODUCING FERTILIZATION-COMPETENT SPERM.** Gary Hunnicutt. Population Council, NY, NY

The sperm annulus has long been known simply as an electron-dense ringed structure of the sperm that separates the principal piece (PP) from the midpiece (MP) of the tail. Studying septin 4 knockout mice, we discovered that the sperm from these mice lacked the annulus, could not swim, and could not undergo protein tyrosine phosphorylation. We showed that septin 4 was indeed a component of the annulus and Ihara, et al. further demonstrated that septins 1, 6, and 7 also were a part of the annulus. It has been hypothesized that the annulus acts as a membrane domain barrier that prevents mixing of proteins and lipids in the MP with proteins and lipids in the PP. Using our sept4-/- mice, we tested this hypothesis by determining the localization patterns of the freely diffusing transmembrane protein, EMMPRIN (CE9, basagin, CD147) on sperm before and after epididymal passage. EMMPRIN moves from the PP on testicular/caput sperm into the MP on corpus/cauda sperm. Although EMMPRIN localized exclusively within the PP membrane of wt testicular sperm, EMMPRIN showed multiple patterns on testicular sperm from the sept4-/-, often being detected throughout the entire length of the tail, just the MP, or sometimes within the head as well. WT sperm removed from either the corpus or cauda showed EMMPRIN localized exclusively within the MP. Concomitantly with epididymal transit, EMMPRIN was proteolytically clipped from 40 kDa to 26 kDa. In sept4-/- corpus/cauda sperm, EMMPRIN again showed multiple localization patterns, but the most striking observation was that the EMMPRIN's fluorescence intensity was markedly reduced. Immunoblots of wt and sept4-/- sperm for EMMPRIN confirmed that EMMPRIN detection was dramatically lost in the sept4-/- sperm and its proteolytic processing did not mimic that seen in wt sperm. We hypothesized that the loss of EMMPRIN in the sept4-/- sperm might result because EMMPRIN can diffuse into the membrane of the residual body (RB) and then is lost when the RB is shed. We examined cytoplasmic droplets (CD) – RB remnants – on testicular and caput sperm, and found EMMPRIN associated with the CD membranes. Taken together, our results suggest that: 1) the annulus is required for membrane domain maintenance; 2) domain segregation to the PP of the tail prevents the loss of membranes proteins that otherwise might diffuse onto and be lost with the shedding of the RB; and 3) keeping proteins within specific domains may ensure their proper posttranslational modifications by keeping them in close apposition to their modifying effectors.

**MS25. PROGESTERONE REGULATION OF UTERINE EPITHELIAL PROLIFERATION AND DIFFERENTIATION.** Jeffrey Pollard. Albert Einstein College of Med, Bronx, NY

Estrogen synthesized during pro-estrus in mice or in the proliferative phase in human's causes the uterine epithelium to undergo cell proliferation. Progesterone, synthesized either upon copulation in mice or during the secretory phase in humans blocks estrogen-induced proliferation and causes differentiation such that the epithelium will accept an implanting blastocyst 1. We have studied the mechanism of action of the sex steroid hormones in regulating these processes in uterine epithelial cells. We have demonstrated that E2 regulates the cell cycle via two parallel processes. The first activates the canonical cell cycle pathway through causing localization of cyclin D1 to the nucleus where it phosphorylates pRb and cause cell cycle progression. This in turn is regulated via an IGF-IR mediated activation of PI3-kinase that results in an inhibitory phosphorylation of GSK-3β, the kinase responsible for phosphorylating cyclin D1 and causing its nuclear egress 2. The second pathway involves the regulation of the binding of mini-chromosome maintenance (MCM) proteins to the origin of DNA replication 3. Both these pathways are blocked by P4, thereby inhibiting DNA replication 2, 3. In turn, P4 induces the expression of over 200 genes in the uterine epithelium 4. Many of these gene products fall into defined pathways suggesting that these may be important for implantation. This talk will discuss these mechanisms of P4 action and extend the observations to humans.

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**MS26. NON-CLASSICAL ESTROGEN SIGNALING IN THE MOUSE UTERUS.** Sanjoy Das. Vanderbilt University, Nashville, TN

Early (phase-I) and late (phase-II) estrogenic responses in the uterus have been recognized for more than 60 years, yet mechanisms involved in their regulation remain controversial. One concept is that an early events(s), occurring within the first 6 h, prepares the uterus for later (18-30 h) increase in DNA and protein synthesis and cell proliferation. An alternate view is that the late growth phase is a result of the continuous presence of the stimulus. Discussion of either concept usually makes an assumption that all of the responses are dependent upon ligand interaction with two estrogen receptor isoforms (ERalpha and ERbeta). However, increased gene expression following injection of estrogens in mice lacking ERalpha or in which ligand-dependent ER-activity has been silenced by an ER-antagonist, ICI 182,780 (ICI), has shown this to be an oversimplification. Accumulating evidence now suggests that estrogen regulates diverse but interdependent signaling pathways in uterine biology via ER-dependent and ER-independent manners. While our long standing hypothesis is that estrogenic certain early responses are ER-independent, late responses are ERalpha-dependent, and a cross-talk between the two phases is necessary for a full complement of estrogenic responses in the uterus. In this regard, we observed that early regulatory genes [Wnt4/Wnt5a/Ctnnb1, Hspa5 (old name grp78/bip) and Nol5 (old name Sik similar protein)] that are known to be elicited by estradiol-17beta (E2) in the mouse uterus without involving ERs as a phase-I response, are intimately associated with the control of ERalpha-mediated mechanisms in uterine biology. In this regard, Wnt/Ctnnb1 induced canonical Wnt-signaling appears to be essential for the onset of estrogen-regulated phase-II response in the mouse uterus, and studies further show that there is a cross-talk between the Wnt-signaling downstream effectors (TCF/LEF) and ERalpha at the level of chromatin for gene regulation in the uterus. Additionally, Hspa5 undergoes molecular interaction with ERalpha and appears to be crucial for ERalpha-dependent gene transcription and phase-II responses by estrogen. With respect to Nol5, a nucleolar protein, also complexes and recruits together with ERalpha for gene transcription via ERE/AP-1 sites on the promoters of estrogen-responsive uterine genes. Overall, these results establish a molecular relationship between the two-phase estrogenic responses in the uterus via non-classical target proteins.

**MS27. DECIDUALIZATION: AN EMERGING ROAD MAP.** Milan Bagchi, Mary Laws, Quanxi Li, Athi Kannan, Cyril Ramathal, Wei Wang, Indrani Bagchi. University of Illinois at Urbana-Champaign, Urbana, IL

The steroid hormones, progesterone (P) and estrogen (E) are critical regulators of embryo implantation. In the mouse, implantation is initiated upon the attachment of the embryo to the uterine luminal epithelium on day 4 of pregnancy. Embryo attachment is followed by extensive proliferation of uterine stromal cells and their differentiation into morphologically and functionally unique decidual cells. P and E play pivotal roles in preparing the uterus for embryo attachment and subsequent decidualization. The cellular actions of these hormones are

mediated through their cognate nuclear receptors, which are ligand-inducible transcription factors. These receptors regulate the expression of specific gene networks that in turn control the stromal proliferation, differentiation, and angiogenesis that occur in pregnant uterus during decidualization. Generation of a comprehensive map of the pathways underlying implantation would require identification and functional characterization of the steroid-regulated molecules that control these events. To achieve this goal, we performed gene expression profiling using DNA microarray technology to identify genes whose expression in the uterus is regulated by P and E. We have identified several genes that are markedly induced in response to these hormones in uterine stromal cells during decidualization. To understand the role of the microarray-derived steroid-regulated genes in pregnant uterus, we are analyzing the functional contribution of selected pathways using knockout mouse models and in vitro primary culture systems. Our recent results on these studies will be presented.

**MS28. QUESTIONS RAISED BY PLACENTAS OF LASER-INTERRUPTED TTTS.** Kurt Benirschke. UCSD Medical Center, San Diego, CA

The mechanism by which the 'twin-to-twin transfusion syndrome' (TTTS) arises was first clearly delineated when Friedrich Schatz observed it in 1886 and illustrated its placental causes. The syndrome occurs only in monochorionic (MZ) twin and is the result of one or more arterio-venous, transvillous anastomoses. DeLia et al. showed in 1989 that fetoscopic laser interruption of these one-way anastomoses can alleviate this maladaptation of the twins. The ablation has since been practiced in numerous medical centers, with variable but usually beneficial results. The actual mechanism why such anastomoses should develop is unclear, although the frequent marginal or velamentous insertion of the umbilical cord has been implicated by some investigators. An alternative view is that perhaps 'unequal' splitting of the blastocysts/disk takes place from the beginning. While vascular connections occur often in DZ cattle (with occasional freemartinism) and regularly in callithricid gestations, they differ in size and character and they merely induce blood chimerism. When successful laser ablation has occurred, the two fetuses continue to grow, hydramnios disappears, the anhydramnios of the ('stuck') donor twin is cured (presumed amnion nodosum disappears) and the outcome is mostly beneficial for the twins. The placentas of such twins after delivery weeks or months later have been of interest. It is generally then possible to identify the laser coagulation sites and to observe some areas of degeneration and villous infarction, but a variety of unusual changes are also encountered. Importantly, in many cases they indicate the persistence of intact placental villous tissue below the site of vascular ablation, despite the fact that the surface vessels are completely occluded. This is contrary to our understanding of the nature of villous perfusion by fetal vessels, and while it has been described in some detail before, these connections appear to be of no further detriment to fetal development. One important aspect of TTTS is the discrepancy of fetal cardiac size (Naeye has drawn attention to this). When twins are aborted and permission for autopsy is not granted it is then possible to sonographically assess the cardiac discrepancies. Finally, I will present some date to indicate that not only is there a possible increase in TTTS consequent to ART, but the reason for the frequent 'splitting' of one implanted blastocysts is currently unknown.

**MS29. EMERGING PARADIGMS IN THE RELATION BETWEEN THE FETUS AND ITS PLACENTA.** Karen Downs. University of Wisconsin, Madison, WI

Brachyury (T) protein identifies a new architectural and functional component in the murine allantois, the future umbilical component of the placenta. In the absence of Brachyury, this feature, called the Allantoic Core Domain (ACD), is defective. Consequently, the allantois does not grow far enough to form the placenta, and the umbilical vasculature does not form. I will present data which highlight the characteristics of the ACD, and its role in unifying the embryonic and extraembryonic components of the murine conceptus through a common axis.

**MS30. PLACENTAL VASCULAR DISEASE IN MOUSE MODELS OF INTRAUTERINE GROWTH RESTRICTION.** S. Lee Adamson. Mount Sinai Hospital, Toronto, ON, Canada; Samuel Lunenfeld Research Institute, Toronto, ON, Canada

Placental vascular abnormalities causing deficient placental perfusion contribute to common complications of human pregnancy including preeclampsia and intrauterine growth restriction (IUGR). Blood flow pulsatility, quantified using the Doppler Resistance index, is often elevated in the uterine and/or umbilical circulations in preeclamptic or growth restricted pregnancies. This change in hemodynamics is associated with placental vascular abnormalities which presumably elevate vascular resistance and thereby impair perfusion. However, the causes of placental vascular abnormalities in human pregnancy as well as the relationships between structural and hemodynamic changes are poorly understood. In our lab, we are using genetically-altered mice as new models to explore the etiology of abnormalities in placental vascularization and hemodynamics. We are using ultrasound biomicroscopy (30-40 MHz; Vevo770, VisualSonics, Canada) to non-invasively image and record Doppler blood velocity waveforms of isoflurane-anesthetized pregnant mice. We have monitored blood velocity in the uterine artery and intraplacental arterial canals of the mother, and in the yolk sac and umbilical circulations of the embryo both in normal pregnancy and in mouse models of intrauterine growth restriction (IUGR). Dr. Junwu Mu has shown in the uterine artery that peak (PSV) and end-diastolic velocities (EDV) increase and the Doppler Resistance Index (RI= (PSV-EDV)/PSV) decreases during gestation in mouse as in human pregnancy. In the umbilical artery, PSV increases progressively from the day the heart starts to beat (E8.5) until term (E18.5) whereas PSV in the vitelline artery to the yolk sac increases until E13.5, and then remains stable. In the umbilical artery, EDV increases from zero to become detectable as early as E15.5 and is detectable in nearly all embryos at term. Umbilical waveforms are similar to those observed in first trimester human pregnancy. Similar to findings in human IUGR, Drs. Junwu Mu and Carole Watson, and Ms. Shathiyah Kulandavelu observed elevated resistance indices in the umbilical and uterine arteries of two mouse models of IUGR; eNOS knockouts from Jackson Laboratories, and transgenics with perinatal hepatic overexpression of IGFBP-1 created by Drs. Victor Han and Carole Watson. In collaboration with Dr. John Sled (Mouse Imaging Centre, Toronto), Ms. Monique Rennie and Ms. Kathie Whiteley are using X-ray micro-computed tomography (μCT) to image and quantify vascular branching patterns in the utero-placental and umbilico-placental circulations following the installation of an X-ray opaque contrast agent. Preliminary results suggest this method can be used to detect normal developmental changes in placental vascularity as well as vascular defects in IUGR models. We conclude that ultrasound biomicroscopy and μCT provide viable methods for quantifying placental hemodynamics and vascularity throughout pregnancy in mice. Our early results show that there are strong parallels in placental structure and hemodynamics between mice and humans in both normal and IUGR pregnancies. Funding support from the Canadian Institutes of Health Research is gratefully acknowledged.

**MS31. GENETIC ANALYSIS OF GONADOTROPIN ACTIONS IN THE MOUSE.** T. Rajendra Kumar. Univesity of Kansas Medical Center, Kansas City, KS

Pituitary-derived gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH) are heterodimeric glycoprotein hormones. They regulate gonadal growth, differentiation and steroidogenesis. Men with mutations in genes encoding the beta subunits, or gonadotropin receptors present various degrees of infertility. To model loss of function mutations involving gonadotropin signaling, we made null mutations in the hormone-specific beta subunits and created mice lacking either FSH or LH. We have used these genetic models to study how FSH and LH regulate somatic- and germ cell interactions in the testis. FSH beta null male mice are hypogonadal but fertile. The Sertoli cell proliferation and number are reduced; consequently, the null mice have reduced sperm number. The Leydig cell number and serum testosterone are unaffected. We have identified FSH-responsive genes using suppressive subtractive genetic screens, pathway-specific and global gene expression profiling, candidate gene approach and the FSH beta null mice as a unique genetic resource. Together, these approaches are aiding us to place different signaling components downstream of FSH receptor in Sertoli- and indirectly in germ cells. We have also investigated the consequences of loss-of-LH function in the testis. LH beta null mice are hypogonadal, demonstrate severely suppressed testosterone levels and are infertile. The Leydig cell number is reduced in these null mice. Whereas the fetal Leydig cell lineage is unaffected, postnatal Leydig cell development is blocked in mutants. Immunophenotyping followed by confocal microscopy analysis identified that the interstitium predominantly consists of immature Leydig cells. Spermatogenesis is blocked at the round spermatid stage and the mutant tubules show enhanced apoptosis in multiple germ cell types. Pharmacological rescue of LH beta null mice provided a convenient platform to identify LH- and testosterone-responsive genes. Thus, these gonadotropin ligand null mice are novel genetic tools to investigate signaling pathways in somatic and germ cells in the gonads.

**MS32. DOES VARIATION IN SIZE OF THE OVARIAN RESERVE AND FSH SECRETION IMPACT FERTILITY?** James Ireland, George Smith, Alex Evans, Pat Lonergan, Fermin Jimenez-Krassel, Janet Ireland, Danielle Scheetz, Joe Folger. Michigan State Univ, East Lansing, MI; University College Dublin, Dublin, Ireland

Development of methods to monitor size of the ovarian reserve and determination of the role of the ovarian reserve in fertility would greatly benefit family planning and human clinical reproductive medicine. The bovine dominant follicle model was chosen to address these problems not only because cattle like women have multiple FSH-induced follicular

waves leading to dominant follicle development during relatively long reproductive cycles, but also because they have a highly variable number of healthy oocytes in the ovarian reserve throughout their reproductive life. Moreover, while humans require decades to deplete their ovarian reserves, the number of healthy oocytes in the ovarian reserves of cattle is reduced nearly 90% by the time they are 5 years old. We recently demonstrated that cattle can be reliably phenotyped through counting (via ovarian ultrasonography) peak number of antral follicles (>3 mm in diameter) growing during the 2 or 3 follicular waves occurring during 21-day estrous cycles. Our recent studies show that 11-12 mo old heifers with consistently (repeatability = 0.95) low (<15) versus high (>25) follicle numbers during follicular waves have many characteristics previously associated with aging and infertility, including not only a greatly diminished number of healthy oocytes and follicles in the ovarian reserve, but also higher circulating FSH concentrations, lower serum concentrations of inhibin-A, anti-mullerian hormone (AMH) and progesterone during estrous cycles, and reduced number of high quality oocytes and embryos in response to superovulation. Based on these data, coupled with the well established important role for FSH in regulation of LH receptors in granulosal cells of dominant follicles, we hypothesized that chronically high circulating FSH concentrations in animals with relatively low antral follicle counts during follicular waves (diminished ovarian reserve) negatively impacts the LH response system (receptor plus signaling cascade), and in turn, growth, differentiation, and (or) function of dominant ovulatory follicles. New preliminary data indirectly supports this hypothesis and demonstrates that capacity of granulosal cells isolated from dominant follicles to luteinize, and responsiveness of such cells to LH, are both markedly reduced in animals with low vs high antral follicle counts during follicular waves. Consequently, these preliminary findings in the bovine model support the possibility that chronically high circulating FSH concentrations during follicular waves may diminish responsiveness of dominant follicles to LH, which in turn may reduce dominant follicle function in animals with low antral follicle counts and diminished ovarian reserves. Taken together, our observations are important because they show for the first time that ultrasonography can be used reliably to identify (phenotype) cattle of similar ages that have many phenotypic traits usually associated with aging and infertility. Moreover, these results establish the bovine as an appropriate model to determine not only the mechanisms whereby the variation in the ovarian reserve and FSH secretion may impact oocyte quality and embryo development, but also whether the variation in the ovarian reserve and circulating FSH concentrations is associated with infertility.

**MS33. DESIGN OF A LONG-ACTING RECOMBINANT FSH.** Irving Boime. Washington Univ School of Medicine, St. Louis, MO

The glycoprotein hormone family consists of the pituitary proteins lutropin (LH), follitropin (FSH), thyrotropin (TSH), and the human placental hormone chorionic gonadotropin (CG). They are heterodimers, which share a common α subunit but differ in their hormone-specific β subunits. All of the subunits are glycosylated, containing asparagine-linked oligosaccharides and, in the case of the CGβ subunit, O-linked carbohydrates are also present in a discrete region at the carboxyl end (termed carboxyl terminal peptide or CTP). The complexity of the gonadotropin structure has been a reservoir of numerous fascinating structure-function investigations. The use of recombinant DNA mutagenesis and transfection protocols not only has elucidated new biological findings regarding the glycoprotein hormones, but has led to the design of therapeutic agents. For example, the CTP of the CGβ subunit is essential for the relatively long half-life of hCG in the circulation. Currently, a major issue regarding the clinical use of FSH to induce follicle development in Assisted Reproduction Protocols is its short half-life in vivo, which necessitates multiple injections. Using recombinant DNA techniques, the CTP of the hCGβ was fused to the human FSHβ subunit coding sequence, thereby generating an FSH agonist that exhibited a prolonged circulating half-life and enhanced bioactivity in vivo. Treatment with such long-acting gonadotropins would be less stressful for the patient, requiring fewer injections. Recent clinical trials have demonstrated the expected clinical efficacy of this novel analog. Such a therapeutic agent represents a powerful example of how examining the biology of these hormones can lead to a generation of agonists to aid the clinician and patient in the more comfortable and efficient treatment of reproductive disorders.

**MS34. GENOMIC IMPRINTING, NUTRIENT SUPPLY AND FETAL GROWTH.** Miguel Constancia. The Babraham Institute, Cambridge, Cambridgeshire, United Kingdom

Genomic imprinting is an epigenetic form of gene regulation that results in only one parental allele being expressed. Several imprinted genes play important functions in control of fetal size, with paternally expressed genes promoting growth and maternally expressed genes inhibiting it. These opposing effects are best explained by the kinship theory which proposes that imprinting evolved as a result of a genetic conflict between parental genomes over allocation of maternal resources. We recently described novel mechanisms by which imprinted genes control resource allocation to the fetus, with dual effects on fetal demand and placental supply systems (1-5). Imprinted gene knock-outs are excellent models to study functional adaptations of mismatched growth between placenta and fetus. We found that small placentas can respond to fetal demand signals by increasing transport efficiency. This is achieved through up-regulation of key nutrient transporters (e.g. System A amino-acids; glucose) or selective overgrowth of cell types involved in nutrient exchange. Conversely, we found that big (overgrown) placentas comply with fetal growth demand or maternal constraint by reducing transport efficiency through down-regulation of key nutrient transporters. The placenta appears thus to be able to alter its size, structure and transporter abundance in response to the fetal nutrient demands for growth. We identified the glucose transporter Slc2a3 and the imprinted system A amino-acid Slc38a4 transporter as central players in this dynamic system that is able to respond to changes in the fetal environment. Preliminary analyses of knock-outs of these transporters support the hypothesis of their involvement in fetal growth and placenta function. Our results suggest that the placenta acts a sensor of fetal demand. We suggest that fetal demand is the main determinant of nutrient supply through the placenta, with nutrient supply being adjusted to match the fetal demand irrespective of the size of the placenta. Imprinted genes are important components of nutrient supply and demand during mammalian development. References: 1. Constancia et al. 2002 Nature 417:945-948; 2. Sibley et al. 2004 Proc. Natl. Acad. Sci. 101:8204-8208; 3. Constancia et al. 2004 Nature 432: 53-57; 4. Constancia et al. 2005 Proc. Natl. Acad. Sci. 102:19219-19224; 5. Angiolini et al. 2006 Placenta 27: S98-102

**MS35. PROFILING DNA METHYLATION IN HUMAN PLACENTA BY MSNP.** Benjamin Tycko, Le Jiang, Alexandra Spadola. Columbia University, New York, NY

As the primary site of maternal-fetal exchange the placenta is a major factor in fetal growth and pregnancy outcome. Convergent lines of evidence from mouse models and human syndromes have implicated epigenetics, particularly DNA methylation, as an important determinant of placental function. For example, several genes that show allele-specific DNA methylation (ASM) due to parental imprinting have well established roles as growth rheostats for the placenta. To gather more complete information on DNA methylation in human placentas, we have now applied a novel genome scanning method, called MSNP. This method (Yuan et al., Cancer Res 2006, 66: 3443-3451) adapts Affymetrix SNP Arrays for methylation profiling, producing a "bar-code" readout of the methylation status at thousands of SNP-tagged loci. Our data reveal specific patterns of DNA methylation that distinguish human placenta from other human organs, and uncover other differences that distinguish normal placental tissue from androgenetic hydatidiform moles. A unique advantage of the MSNP method is that it reveals not only net gains and losses in DNA methylation in comparing two biological samples, but also differences in ASM. From the MSNP data we are compiling lists of SNP-tagged loci that show ASM in human placentas, and sorting through these loci to ask which are regulated by parental imprinting and which have ASM independent of imprinting.

**MS36. CONSERVATION OF IMPRINTING IN SWINE AND COMPARATIVE ASPECTS OF IMPRINTING.** Jorge Piedrahita, Steve Bischoff, Shengdar Tsai. North Carolina State University, Raleigh, NC

Imprinted genes have been identified in placental mammals, and marsupials but not in egg-laying mammals (monotremes), birds, fishes, reptiles, or amphibians, suggesting that the phenomena of genomic imprinting and placentation have co-evolved. While marsupials are not placental mammals per se, they develop a non-invasive yolk-type placenta that, in the case of the opossum, is functional for a period of 3 days. The parental-conflict hypothesis proposes that paternal imprinting evolved as a way to enhance fetal growth, and maternal imprinting evolved as a way to control fetal growth. The hypothesis is supported by uniparental pregnancies, direct experimental evidence from null knockout mice, as well as human conditions associated with aberrant imprinting, all of which indicate that dysregulation of imprinted genes have a drastic effect on placental morphology and function. Thus, the evolutionary and experimental evidence supporting the co-evolution of placental function and imprinting reinforces the importance of concentrating on this family of genes when searching for candidate genes capable of affecting placental efficiency and fetal growth. This is important not only for human disorders such as intrauterine growth restriction and pre-eclampsia but also to elucidate the placental abnormalities commonly seen in somatic cell nuclear transfer clones of certain species, and to understand their impact upon litter size in species such as swine. As the placenta is one of the most divergent organs among placental mammals (ranging from highly invasive haemochorial to non-invasive epitheliochorial), we have utilized comparative genomics to better understand the role of imprinted

genes in placental function. We have elected to focus our efforts on the non-invasive porcine placenta and have undertaken a systematic approach to identify and compare the family of imprinted genes in this species. Pregnancies generated from embryos derived from only maternal DNA (gynogenotes or parthenotes) produce small fetuses and placentas; in contrast, pregnancies generated with only paternal DNA (androgenotes) have large, overgrown placentas but only vestigial fetuses. As such, we conducted transcriptional profiling experiments of day 30 control and parthenogenetic porcine embryos using Affymetrix Porcine GeneChip microarrays. Four tissues were profiled: brain, fibroblast, liver, and placenta. At $q<0.05$, we identified 13 known imprinted genes differentially expressed in at least one of the four tissue types: DCN, DIRAS3, IGF2, MEST, NDN, NNAT, PEG3, PEG10, PHLDA2, PLAGL1, SGCE, SLC38A4, and SNRPN. DIRAS3 was detected as differentially expressed in all tissues except placenta, MEST and SGCE in all tissues except liver, and IGF2 (known to be imprinted in swine) in all tissues except brain. NNAT was detected as differentially expressed only in brain and fibroblast. NDN, PEG10, PLAGL1, and SNRPN were detected as differentially expressed in all four tissues. To confirm the expression analysis results we followed up our screen for epigenetic asymmetry by interrogating the imprinting status of a subset of differentially expressed genes by pyrosequencing using an interbreed model. Our data also supports the lack of imprinting of GABRA5 and GNAS. These findings point to the usefulness of comparative models to clarify the tissue-specific epigenetic asymmetry of mammalian imprinted loci. This research was supported by USDA-CSREES grant 524383, and NIH grant HD048510 to JP and by a NSF Graduate Research Fellowship to ST. This work was performed as part of an initiative from the Center for Comparative Medicine and Translational Research (CCMTR) at the North Carolina State University College of Veterinary Medicine.

**MS37. TRANSCRIPTIONAL REGULATION OF INFLAMMATORY MEDIATORS IN ENDOMETRIOSIS.** Robert Taylor, Fritz Wieser, Jean-Louis Vigne. Emory School of Medicine, Atlanta, GA; University of California, San Francisco, San Francisco, CA

Endometriosis affects more than 7 million women in North America and accounts for ~$1 billion in medical and surgical health expenses annually. A variety of theories has been promulgated to explain the derivation of extrauterine implants of endometrial tissue that define this syndrome, but the precise pathogenic mechanisms responsible for pelvic pain and infertility associated with endometriosis remain unknown. Investigations from our laboratory have supported the hypothesis that recruitment of immune cells is a critical etiological step in disease establishment. The implantation of endometriosis lesions on ectopic peritoneal surfaces requires attachment and invasion of stromal and epithelial cells into the extracellular matrix of the host tissue. These proliferating cells attract immune cell infiltrates, particularly macrophages, via the selective production of chemokines. Recruitment of macrophages exacerbates implant growth and fibrosis via paracrine secretion of mitogens, cytokines, oxygen free radicals and prostaglandins. In addition, environmental endocrine disruptors have been invoked as putative etiologic agents. We observed that peritoneal concentrations of RANTES (Regulated on Activation Normal T cell Expressed and Secreted) was highly correlated with the presence of endometriosis in women and this protein accounts for ~70% of monocyte chemotaxis in Boyden chamber assays containing pelvic fluid from endometriosis patients. Endometriosis stromal cells are a potent source of RANTES secretion and RANTES mRNA expression. Proinflammatory cytokines, particularly IL-1beta and TNF-alpha, activate RANTES gene transcription in these cells through an NF-kappaB-dependent mechanism. Estradiol enhances this effect at transcriptional and post-transcriptional levels. An environmental toxicant, 2,3,7,8-TCDD (dioxin), also appears to activate RANTES gene expression directly in endometriosis stromal cells. RANTES mRNA production also can be negatively regulated in these cells. PPAR-gamma ligands inhibit RANTES expression. Prolonged exposure to medroxyprogesterone acetate or treatment with the non-steroidal anti-inflammatory drug, sulindac, represses RANTES transcription via interference with NF-kappaB. It is hoped that identification and characterization of the latter pathways will provide opportunities for new drug discovery and refinement of the treatment of endometriosis. Supported by NIH grant HD37321.

**MS38. IN VITRO MODELS OF ENDOMETRIOSIS.** Craig Witz. Univ of Texas Health Sci Ctr, San Antonio, TX

There is mounting evidence that retrograde menstruation and implantation of endometrial fragments are the primary factors responsible for development of the majority of endometriotic lesions. Many crucial questions concerning the initial interaction of endometrial cells with the peritoneum remain unsettled. Until recently, the manner of endometrial cell attachment to the peritoneum was controversial. Specifically, it was debated whether endometrial cells attach to intact, viable mesothelium (i.e. the epithelium lining of the peritoneum). Tissues serving as a surrogate for the peritoneum, such as the amnion and the chicken chorioallantoic membrane, have been used to evaluate the genesis of the early endometriotic lesion. These studies have led some investigators to postulate that PMCs are a barrier to attachment of ectopic cells to the peritoneum. These investigators hypothesize that trauma to the mesothelial lining is a prerequisite for endometrial adhesion. Studies using whole explants of human peritoneum, as well as peritoneal mesothelial cell (PMC) monolayer cultures, demonstrate that whole fragments of proliferative, secretory, and menstrual phase endometrium, as well as cultured endometrial stromal and epithelial cells (ESCs and EECs, respectively) adhere to intact PMCs within one hour. There is a significant variability in the rate of endometrial binding to PMCs. This variability is mostly dependent on the source of endometrium rather than the source of PMCs. Following attachment to the peritoneum, the early endometriotic lesion is rapidly invasive. Endometrial invasion through PMCs begins within 6 hours. By 24 hours, the growth of PMCs over the invaded endometrium is well established. There is evidence that attachment of endometrial cells to PMCs grown on basement membrane (BM) preparations leads to an increased rate of invasion through the BM compared to binding to the BM alone. Following binding of ESCs and EECs to PMCs, there are alterations in gene transcription by both endometrial cells and PMCs that may contribute to endometrial invasion. This suggests that PMCs play an integral role in endometrial invasion into the peritoneum. We have developed a model that allows for quantification of endometrial invasion into the peritoneum. This model has been used to evaluate growth factors and cytokines that may increase or decrease endometrial invasiveness into the peritoneum. Elucidation of factors involved in endometrial cell-PMC attachment, and characterization of changes in both PMCs and endometrial cells following binding, will further our understanding of the genesis of the early endometriotic lesion.

**MS39. ALTERED ENDOMETRIAL GENE EXPRESSION IN A BABOON MODEL OF ENDOMETRIOSIS.** Asgerally (Asgi) Fazleabas, Julie Hastings, Kevin Jackson, Linda Giudice, Julie Kim, Hugh Taylor, Xiang Zhang, Shuk-Mei Ho. University of Illinois, Chicago, IL; Univ of Cincinnati Med Ctr, Cincinnati, OH; University of California, San Francisco, CA; Yale University, New Haven, CT; Northwestern Univ, Chicago, IL

Endometriosis is one of the most common causes of chronic pelvic pain and infertility. However, the causative factors associated with reduced fecundity have not been clearly elucidated. Due to the limitations associated with controlled studies in women with endometriosis, we have developed an induced endometriosis model in the baboon by inoculating the peritoneal cavity with menstrual endometrium on two consecutive menstrual cycles. To determine if the presence of endometriotic lesions influenced the gene expression in the eutopic endometrium, endometrial tissues were harvested at 1,3,6,9,12 and 15 months (m) from the same cohort of animals during the window of uterine receptivity. Microarray expression profiling for elucidation of molecular players participating in the maturation of the endometrium during the window of implantation was done on an Affymetrix platform. Independent hierarchical clustering analysis with all samples revealed two major dendogram branches comprised of a) 1 and 3m and b) 6m, 15m, spontaneous endometriotic samples, together with controls. A Heatmap of gene expression revealed dysregulation of many genes 1 and 3m after induction of disease, with lower levels of differential gene expression in 6m, 15m and spontaneous endometriotic samples. These data further suggest that the eutopic endometrium undergoes sequential changes that are initially characterized by a dominance of estrogen (E2) regulated genes, such as FOS, CYR61 and EMMPRIN followed by the development of progesterone (P) resistance within the endometrium and a decrease in P regulated genes such as HOXA10 and Calcitonin. Overall, our studies imply that the genes showing transient dysregulation during the initial phases of the disease are E2 regulated genes, while P regulated genes are permanently down regulated, as the disease progresses. These changes in gene expression were correlated with aberrant endometrial morphology and epigenetic changes. Increased levels of *DNMT1* mRNA and protein were observed in endometria from baboons with disease compared to controls. Bisulfite sequencing revealed significantly increased levels of methylation in the F1 region within the promoter of the *HOXA10* gene. MSRF identified 14 hypo- and two hyper-methylated DNA fragments in baboons with endometriosis. Hypomethylation and subsequent increased levels of genes involved in cell growth and differentiation (i.e.,*WNT2B*) may dysregulate differentiation of the secretory endometrium of baboons with disease Furthermore, these changes in gene expression and morphology were associated with an altered steroid receptor distribution. Both estrogen receptors (alpha and beta) were significantly decreased in the stromal cells while PR levels were reduced in the glandular epithelial cells during the transition phase. Also, although PR was present in the stromal cells, this receptor was less responsive to ligand stimulation. The chaperone immunophilin FKBP52 is critical for P action and the effects of FKBP52

are primarily controlled by PRA. The absence of FKBP52 also skews the uterus towards an exaggerated E2 response during the window of receptivity. In our endometriosis model the decrease in both PR and FKBP52 is associated with the development of P resistance further validating our hypothesis that endometriosis associated infertility is a result of diminished P action in the eutopic endometrium. Using a well established non-human primate model, we have demonstrated that the presence of endometriotic lesions results in an altered eutopic endometrial environment. In many ways these changes replicate those reported for women with endometriosis. By sequential analysis of the eutopic endometrium in the same animals during the progression of the disease, we have also shown that early in the disease process there is a transitory dominance of an estrogenic phenotype, but as the disease progresses a more permanent P4 resistant phenotype results. [NIH HD 40093]

**MS40. DISRUPTION OF THE SPERM-SPECIFIC *LDHC* GENE IS DETRIMENTAL TO SPERM FUNCTION AND MALE FERTILITY.** Erwin Goldberg, Fanny Odet, Chongwen Duan, William Willis, Eugenia Goulding, Aisha Kung, Mitch Eddy. Northwestern University, Evanston, IL; National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC

The lactate dehydrogenase isozymes are key catalysts in the glycolytic pathway of energy metabolism and it is well known that the distribution of the LDH isozymes vary in accordance with the metabolic requirements of different tissues. There are three subunit types (A, B and C) and exquisite tissue specificity is exemplified by the *Ldhc* gene. It is abundantly expressed in male germ cells during spermatogenesis and encodes the only LDH isozyme for which activity can be detected in spermatozoa. Spermatozoa rely almost exclusively on aerobic glycolysis to produce the ATP necessary for capacitation, motility and fertilization. Why *Ldhc* gene expression has been conserved in mammalian testes where *Ldha* and *Ldhb* genes are also transcribed remains an enigma. We have chosen targeted disruption of the *Ldhc* gene as the method to address the question of why testes and sperm need this unique form of LDH. Preparation of a targeting construct proved challenging since the intronic sequences are composed of 43% repetitive elements. The final construct contained Neo sequences bounded by loxP elements for conditional expression with TK for selection. Redundancy in this metabolic pathway would predict a wild-type phenotype in the mutant animal. Reliance on LDHC for glycolysis in germinal epithelial cells would predict impaired spermatogenesis most likely during pachytene of the first meiotic division or in early spermatids which prefer lactate over glucose as energy substrate. We hypothesized that disruption of ATP production via this metabolic pathway would impair motility and fertilizing capacity of spermatozoa similar to that observed with GAPDHS null mice. However, here we demonstrate that targeted disruption of *Ldhc* affects only male fertility. LDHC has been studied as an immunocontraceptive in females. The present results not only satisfy proof of principle but also suggest that this protein may be a useful target in developing a male contraceptive. This research was supported by NIH HD05863 (EG) and in part by the Intramural Research Program of the NIH, National Institutes of Environmental Health Sciences (EME).

**MS41. DYNAMIC REGULATION OF CELL CYCLE AND PLURIPOTENCY IN THE FETAL MALE GERM LINE.** Patrick Western, Denise Miles, Jocelyn van den Bergen, Ruili Li, Matt Burton, Andrew Sinclair. Murdoch Childrens Research Institute, Parkville, Melbourne, Victoria, Australia

There is a close biological relationship between germ cells and pluripotent cell types including embryonic stem cells, embryonic germ cells and embryonal carcinoma cells, which underlie testicular cancer. A small group of key developmental genes, which control differentiation potential, are restricted to pluripotent cells and the developing germ line. These genes are also typically reactivated in germ cell derived embryonal carcinoma. Our data has shown that several pluripotent genes are down regulated in fetal male germ cells in a pattern that may be coincident with mitotic arrest. However, the timing and molecular basis of mitotic arrest in fetal male germ cells remained poorly defined. We have now accurately defined the time of male germ cell arrest in inbred and out bred mouse strains using flow cytometry. To determine how mitotic arrest of fetal germ cells proceeds, we have analyzed the regulation of the key genes and proteins controlling G1-S phase progression. Finally we have examined the expression of the pluripotent genes at the transcriptional and post-transcriptional levels in germ cells progressing through mitotic arrest. Our data shows that mitotic arrest of male germ cells occurs between E12.5 and E14.5, that this arrest involves several key G1-S phase regulators and that the pluripotent genes are suppressed at the transcriptional and post-transcriptional levels during this developmental phase. Based on this data and the reactivation of pluripotent genes in germ cell cancers, we hypothesize that down regulation of pluripotent genes is required for normal male germ cell differentiation and effective exit from the cell cycle. To test this hypothesis we are currently examining the function of pluripotent genes in fetal germ cells. Using a candidate gene approach we are also exploring the mechanisms involved in initiating mitotic arrest and pluripotent gene regulation in fetal germ cells.

**MS42. GERMLINE DEVELOPMENT AND GERM CELL TUMORS IN THE ZEBRAFISH SYSTEM.** James Amatruda, Joanie Neumann, Katherine Lillard, Liliana Carbajal. UT Southwestern Medical Center, Dallas, TX

The development of the germline requires precise control of the specification and migration of primordial germ cells, and of gonadal stem cell self-renewal and differentiation. Defects in these processes are linked to infertility and to germ cell cancers. Despite intensive investigation, the genes controlling normal and abnormal germline development are incompletely understood. To address these problems, we are using zebrafish as a genetically tractable, relevant vertebrate model of germline development. Zebrafish germ cells are well characterized, and the key pathways governing the development of primordial germ cells are highly conserved from fish to human. To discover the genes underlying the development of germ cell tumors, we introduced random mutations into the zebrafish genome and recovered a line of fish that reproducibly develops testicular germ cell tumors at high penetrance. The tumors resemble human seminomas and recapitulate many features of human TGCTs, including post-pubertal onset, and an apparent progression from carcinoma in situ to invasive disease. The mutation is dominantly inherited, and homozygotes are affected more severely than heterozygotes. We have localized the mutation to a chromosome via interval haplotype mapping, and we are attempting to clone the mutant gene through analysis of linked markers in affected kindreds. In parallel work, we are investigating the role of specific molecular pathways in normal and abnormal germ cell development. We are also using in vitro culture of zebrafish germ cells as a tool to understand the regulation of gametogenesis, and as a platform for facile manipulation of the zebrafish genome.

**MS43. TRANSCRIPTION-INDEPENDENT STEROID SIGNALING IN THE OVARY.** Stephen Hammes. University of Texas Southwestern Medical Center, Dallas, TX

Classical steroid receptors mediate many transcription-independent (nongenomic) steroid responses in vitro. A major problem in this field has been to justify the biological significance of these nongenomic processes, since dissecting the relative importance of genomic versus nongenomic steroid receptor-mediated signaling is difficult. To circumvent these concerns, our laboratory studies steroid-induced maturation, or meiotic resumption, of oocytes. Oocyte maturation is an important, biologically relevant, steroid-mediated phenomenon that is well-accepted to occur completely independent of transcription. We have used steroid-triggered Xenopus laevis oocyte maturation as a model to study nongenomic steroid signaling, and have shown that: 1) contrary to dogma, androgens, rather than progestins, are the physiologic regulators of Xenopus oocyte maturation; 2) androgen-triggered maturation is mediated by classical androgen receptors; 3) steroids rapidly alter constitutive G protein signaling in a "Release of Inhibition" fashion to promote meiotic progression; 4) steroids trigger activation of the cytoplasmic protein Paxillin, which then regulates maturation by enhancing MAPK signaling. Finally, we have demonstrated that, under some conditions, steroids can promote mouse oocyte maturation independent of transcription, suggesting that the process of nongenomic steroid-triggered maturation may be conserved from lower to higher vertebrates.

**MS44. G PROTEIN SIGNALING AND FUNCTIONS OF THE NOVEL PROGESTERONE MEMBRANE RECEPTORS IN REPRODUCTIVE TISSUES.** Peter Thomas, Emmanouil Karteris, Jon Levine, Yefei Pang. Univ of Texas at Austin, Port Aransas, TX; Brunel University, Uxbridge, United Kingdom; Northwestern University, Evanston, IL

Although there is extensive evidence that steroids can exert rapid, nongenomic (i.e. nonclassical) actions initiated at the cell surface by binding to specific membrane receptors, the identities of most steroid membrane receptors remain unclear. A novel membrane progestin receptor (mPR) family with seven-transmembrane domains, unrelated to nuclear steroid receptors and comprising three subtypes, alpha, beta and gamma, was recently discovered in fish and other vertebrates. The mPRs are expressed in a wide variety of reproductive tissues. Both recombinant and wild type mPRs display high affinity, saturable, displaceable and specific progestin binding, typical of steroid membrane receptors. Binding and immunoprecipitation studies with [35 S]GTPgammaS and G-protein alpha subunit antibodies, respectively, show that mPRs are directly coupled to G-proteins and activate them upon progestin treatment. The mPRs typically activate pertussis-sensitive inhibitory G proteins (Gi), to down regulate adenylyl cyclase activity. Recent studies

suggest mPRs have important roles in a variety of reproductive functions. Clear evidence has been obtained that mPRs are intermediaries in progestin induction of oocyte maturation in fish and amphibians. The receptor is also involved in progestin stimulation of fish sperm motility. In mammals, the mPRs have been implicated in progesterone regulation of uterine function in humans and GnRH secretion in rodents. The alpha and beta mPR subtypes and their intracellular signaling pathways through Gi have been identified in human myometrial cells. Activation of mPRs in the myometrial cells leads to transactivation of PR-B, the first evidence for cross-talk between membrane and nuclear PRs. Progesterone activation of the mPRs can also lead to a decrease in the expression of the nuclear steroid receptor coactivator SRC2. The data indicate the presence of an unusual signaling pathway mediated by mPRs in human myometria that may result in a functional progestin withdrawal, shifting the balance from a quiescent state to one of contraction at term. Both mPR subtypes are also present in the preoptic anterior hypothalamic region of the rodent brain and in immortalized GnRH-secreting neurons, GT1-7 cells. The finding that rapid down regulation of luteinizing hormone secretion by progesterone also occurs in PRKO mice indicates it is mediated by a novel progesterone receptor. Activation of G proteins and down regulation of adenylyl cyclase activity and GnRH release by GT1-7 cells was observed after progesterone treatment, which was abrogated after transient transfection with siRNA for mPR alpha. The results suggest progesterone decreases GnRH secretion through a mPR/Gi/cAMP pathway.

**MS45. EXTRA-NUCLEAR ESTROGEN RECEPTOR SIGNALING.** Ellis Levin. UC-Irvine and the Long Beach VAMC, Long Beach, CA

Steroid hormones produce rapid effects that result from engaging specific receptors localized most often to the plasma membrane. Membrane-initiated steroid signaling (MISS)1 includes rapid signal transduction that leads to the modification of existing proteins and cell behaviors. Rapid signaling through calcium, amine release, and kinase activation also impacts the regulation of gene expression by steroids, sometimes requiring integration with nuclear steroid receptor function. In this and other ways way, the integration of all steroid actions in the cell co-ordinates outcomes such as cell fate, proliferation, differentiation, and migration. The nature of the receptors is of intense interest and recent data suggests that extra-nuclear and nuclear steroid receptor pools are the same proteins. Recent insights as to the structural determinants for membrane localization and function, and interactions with G proteins and other signal molecules in confined areas of the membrane lead to a fuller understanding of how steroid receptors such as the estrogen receptor (ER) effect rapid actions. Increasingly, the relevance of rapid signaling for the in-vivo functions of ER has been established. This impacts reproductive organ development and function, cardiovascular and bone biology, and steroid-responsive cancer biology. Receptors in other cell sites including mitochondria support important effects of estrogen that are relevant to breast cancer. Overall, there must be integration of the signals generated at each localized ER pool to produce a net steroid action.