nursing after 48 h from birth did not restore uterine MMP9 or ESR1 signals at PND 14 to levels observed for colostrum-fed gilts. Zymographic analyses indicated an increase ($P<0.05$) in uterine MMP9 gelatinolytic activity in animals that nursed from birth to PND 14 in comparison to gilts that were fed replacer over the same period. Overall, results indicate that normal induction of porcine uterine MMP9 and ESR1 requires ingestion of colostrum during the first 48 h of life. Data support the idea that maternally-driven lactocrine signaling for two days from birth may be essential to establish an optimal developmental program in neonatal uterine tissues. Depriving neonates of such lactocrine-acting factors could alter the developmental trajectory of uterine tissues with negative reproductive consequences in adulthood. (Support USDA-NRI 2003-35203-1357 and 2007-35203-18098; NSF-EPS 0814103)

170. Transforming Growth Factor β Receptor Type 1 Is Essential for Female Reproductive Tract Development and Function. Qinglei Li, Luz M. Martinez, Julio E. Agno, and Martin M. Matzuk. Baylor College of Medicine, Houston, TX, USA

The transforming growth factor β (TGFβ) superfamily, the largest family of growth factors in mammals, plays key roles in numerous biological processes. TGFβ ligands bind to their type 1 and type 2 receptors and activate intracellular SMAD transducers to initiate signal transduction. Although recent studies have achieved tremendous insights into this growth factor family in female reproduction, the functions of the receptors in vivo remain poorly defined, partially due to receptor redundancy or lethal phenotypes of genetically engineered ubiquitous null mouse models. TGFβ type 1 receptor (TGFBR1), also known as activin receptor-like kinase 5 (ALK5), is the type 1 receptor for TGFβ ligands. *Tgfbr1* null mice die embryonically, precluding functional characterization of TGFBR1 postnatally. To study TGFBR1-mediated signaling in female reproduction, we generated a mouse model with conditional knockout (cKO) of the TGFBR1 in the female reproductive tract using anti-Mullerian hormone receptor type 2 (*Amhr2*) promoter-driven Cre-recombinase. We found that *Tgfbr1* cKO females are sterile during a 6-month fertility test. To uncover the causes of the sterility, we performed histological, functional, and molecular analyses of the female reproductive tract. Strikingly, we discovered that the *Tgfbr1* cKO females developed oviductal diverticuli which phenocopy the cKO of DICER1, the RNase III that processes microRNAs in the cytoplasm. These findings suggest that the microRNA pathway is derailed in the oviduct in the absence of TGFBR1. Further studies demonstrated that embryo development and transit to the uterus are severely compromised in the *Tgfbr1* cKO mice due to the formation of the oviductal diverticuli. Thus, we identified essential roles of TGFβ family signaling through TGFBR1 in female reproductive tract development and function. Further understanding of the regulatory significance of TGFBR1-mediated signaling in the female reproductive tract may help to discover novel therapeutic approaches for infertility treatment. Supported National Institutes of Health Grant HD33438.

171. Estrogen Stimulates De Novo Synthesis of Cholesterol in Mouse Uterus During Implantation. Yuechao Zhao, Quanxi Li, Indrani C. Bagchi, and Milan K. Bagchi. University of Illinois at Champaign-Urbana, Urbana, IL, USA

In mice, the coordinated actions of estrogen (E) and progesterone (P) via their respective nuclear receptors lead to successful establishment of embryo implantation. It is known that ovarian E stimulates the P-primed uterus to undergo receptive transformation to initiate the attachment of blastocyst to luminal epithelium. Subsequently, uterine stromal cells undergo a unique differentiation process, known as decidualization. However, the molecular mechanisms underlying the actions of E during implantation remain unclear. In this study, we sought to identify the E-induced gene networks that operate in uterine stromal cells during implantation. Ovariectomized mice were treated with a well-established regimen of E and P that mimics the hormonal sequence prior to implantation and creates a receptive uterus. The global gene expression profiling was performed using stromal cells isolated from P-primed uterus treated with or without E. We identified several hundred genes whose expression was significantly altered in response to E. We grouped differentially expressed genes into biological pathways using Ingenuity Pathway Analysis. Strikingly, we found that the expression of 18 genes associated with cholesterol biosynthesis was significantly up regulated in stromal cells upon administration of E to P-primed uterus. These genes included several key enzymes that participate in the cholesterol biosynthesis as well as the sterol regulatory element-binding protein 2 (Srebp2), which is the master regulator that controls the expression of these enzymes. To explore the potential role of Srebp2 in implantation, we monitored its uterine expression during early pregnancy using immunohistochemistry. The Srebp2 protein was induced in uterine stromal cells on day 4 of pregnancy, and its expression was further enhanced in differentiating stromal cells at days 5-7 of gestation, suggesting that regulation of cholesterol biosynthesis is potentially critical for the decidualization process. To investigate this possibility, we utilized an in vitro decidulization system in which primary undifferentiated stromal cells, isolated from mouse uterus prior to implantation, were allowed to undergo differentiation in the presence of E and P. We observed that the expression of mRNAs encoding Srebp2 and several cholesterol biosynthesis-related enzymes was markedly elevated during the in vitro differentiation. Importantly, the level of endogenous cholesterol was markedly increased in stromal cells undergoing decidualization. Furthermore, the treatment of these cells with inhibitors of cholesterol biosynthesis resulted in the down-regulation of decidual biomarkers, such as decidual prolactin-related protein and connexin 43, indicating impairment in uterine stromal cell differentiation. Collectively, these studies uncover a unique pathway in which E stimulates the de novo biosynthesis of cholesterol by promoting Srebp2 expression. It is conceivable that the cholesterol synthesized within the pregnant uterus acts as the precursor of local production of hormonal signals critical for stromal decidualization during early pregnancy.

172. Protein Phosphatase-Type 2A (PP2A) Is Involved in the Initial Events of Human Sperm Capacitation. Patricio Morales, Janetti R. Signorelli, and Emilce S. Diaz. University of Antofagasta, Antofagasta, Chile

During mammalian sperm capacitation, an increase in protein phosphorylation in tyrosine and serine/threonine residues has been described. The role of protein kinases, including tyrosine kinases and PKA, in this process is well documented. However, little is known about the role of protein phosphatases in this event. PP2A is serine threonine phosphatase whose activity has been detected in human and primate sperm extracts. Its role during sperm capacitation is not known. The aim of this work was to study the involvement of PP2A in the regulation of human sperm capacitation. To accomplish this, human sperm samples, obtained from normal donors according to the WHO guidelines, were selected by a Percoll gradient and then resuspended in a non-capacitating medium (modified Tyrode medium without BSA and bicarbonate). The pH was 7.4 and the osmolarity was adjusted between 280 and 300 mmol/kg. Immediately thereafter, some sperm aliquots were incubated at 37°C and 5% CO2 as follow: a) in non-capacitating medium plus 90 nM endothal; b) in capacitating medium (2.6% BSA and 25 mM bicarbonate); c) in capacitating medium plus 90 nM endothal; d) capacitating medium plus inhibitor solvent. At different periods (0, 15, 30, 60 and 300 min), sperm aliquots were withdrawn to evaluate the percent of capacitated sperm using the chlortetracycline fluorescence assay. The results indicate that incubation with endothal very rapidly increased the percent of capacitated sperm. Similar results were observed using 1 nM okadaic acid. The effect of the inhibitors took place only when the sperm were incubated in capacitating medium. The increase in sperm capacitation was especially impressive during the first half hour of incubation; thereafter, there was no difference between sperm incubated in capacitating medium plus the inhibitors versus sperm incubated in capacitating medium without the inhibitors. In addition, the effect of endothal was evidenced by an increase in protein phosphorylation in threonine residues by mean of western blot. Using a polyclonal rabbit antibody the presence of PP2A was confirmed in human sperm. These results suggest that PP2A may have an important role in regulating the initial events of the human sperm capacitation process. This research was supported by Fondecyt Project 1080028.

173. Glycodelin-A Primes Zona Pellucida-Induced Acrosome Reaction of Human Spermatozoa via Downregulation of Extracellular Signal Regulated Kinases and Enhancement of Zona Pellucida-Induced Calcium Influx. William S.B. Yeung, Philip C.N. Chiu, Kai-Fai Lee, and Ben S.T. Wong. The University of Hong Kong, Hong Kong, China

Spermatozoa interact with different isoforms of glycodelin during their passage towards the oocytes in the female reproductive tract. The only known action of glycodelin-A, one of the glycodelin isoforms, is inhibition of spermatozoa-zona pellucida binding, an action paradoxical to general belief that the maternal body should promote fertilization. Therefore, we studied other actions of glycodelin-A on spermatozoa. The objective of this report was to investigate the mechanism of action of glycodelin-A on zona pellucida-induced acrosome reaction, a crucial step in the fertilization process. Glycodelin-A did not affect spontaneous acrosome reaction. Pre-treatment of human spermatozoa with glycodelin-A enhanced zona pellucida-induced acrosome reaction, i.e. the increase in the percentage of acrosome-reacted spermatozoa after sequential glycodelin-A and zona pellucida treatment was significantly higher than the sum of treatments with glycodelin-A and zona pellucida alone. Native human zona pellucida protein-3 (ZP3) and ZP4 were purified. Glycodelin-A primed ZP3-, but not ZP4-induced acrosome reaction. Other glycodelin isoforms and deglycosylated glycodelin-A did not have such priming activity. Glycodelin-A treatment increased cAMP level and protein kinase-A (PKA) activity, but decreased that of extracellular signal-regulated kinase (ERK). Treatment with inhibitors of ERK simulated the priming activity of glycodelin-A. Calcium influx is an important event in zona pellucida-induced acrosome reaction. Therefore, the action of glycodelin-A on calcium influx was studied by fluorescence imaging of individual spermatozoon using Fluo-4AM dye. Compared with the untreated control, glycodelin-A induced a stronger and more rapid calcium influx in spermatozoa upon exposure to solubilized zona pellucida. In conclusion, glycodelin-A sensitizes human spermatozoa for zona pellucida-induced acrosome reaction through activation of the cAMP/PKA-ERK signaling leading to enhanced zona pellucida-induced calcium influx. (This work is supported in part by a research grant from the University of Hong Kong.)

174. STIM1 Is Required for Normal Fertilization in the Pig. Kiho Lee, Chunmin Wang, John M. Chaille, and Zoltan Machaty. Purdue University, West Lafayette, IN, USA

The oscillation in the intracellular free Ca2+ and the depletion of the intracellular stores stimulates an influx of extracellular Ca2+. This Ca2+ influx is responsible for sustaining the long-lasting Ca2+ oscillation. A previous report from our lab indicates that STIM1, a known Ca2+ sensor in somatic cells is expressed in porcine oocytes and is essential for store-operated Ca2+ entry. Because a Ca2+ entry through the plasma membrane is critical for the maintenance of Ca2+ oscillation during fertilization, we hypothesized that STIM1 function has implications for subsequent embryo development. In this study, the inactivation of STIM1 in oocytes and its effect on early embryo development after fertilization was investigated using the pig as a model. Gilt ovaries were obtained at a local abattoir and immature oocytes were collected by aspirating mid-size follicles. The oocytes were matured in vitro in a TCM199-based medium. First, matured oocytes (34 hours after the beginning of