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 and 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 the 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 neonatal uterine tissues with negative reproductive consequences in adulthood. (Support USA-NU-2003-35203-1337 and 2007-35203-18098; NSF-EPS 0814103)

170. Transforming Growth Factor β Receptor Type 1 Is Essential for Female Reproductive Tract Development and Function. Qingle Li, Liz 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 respective type 1 and type 2 receptors, and the receptor complex activates intracellular kinase cascades 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. The TGFβ type 1 receptor (TβR-I) also known as activin receptor-like kinase 5 (ALK5), is the type 1 receptor for TGFβ ligands. TβR-I null mice die embryonically, precluding functional characterization of TGFβR1 postnatally. To study TGFβR1-mediated signaling in female reproduction, we generated a mouse model with a conditional deletion of the TβR-I allele in the oviductal tract using anti-Mullerian hormone receptor type 2 (Amhr2) promoter-driven Cre recombinase. We found that TβR-I 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 TβR-I cKO mice showed a dramatic decrease in oocytes and decidualization, which phenocopies the phenotype of DICER1, the RNAIII that processes microRNAs in the cytoplasm. These findings suggest that the microRNA pathway is derailed in the oviduct in the absence of TGFβR1. Further studies demonstrated that embryo development and transit to the uterus are severely compromised in the TβR-I cKO mice due to the formation of the oviductal diverticuli. Thus, we identified essential roles of TGFβ family signaling through TβR-I in female reproductive tract development and function. Further understanding of the regulatory significance of TGFβR1-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 embryonic development. 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 decidualization remain unclear. In this study, we sought to identify the E-induced gene networks that operate in uterine stromal cells during decidualization. Overexpressed stromal cells were treated with a well-established regimen of E and P that mimics the hormone milieu in which primary undifferentiated stromal cells, isolated from mouse uterus prior to implantation, were incubated. These studies demonstrated that E stimulated the expression of 18 genes associated with cholesterol biosynthesis and signaling leading to enhanced zona pellucida-induced calcium influx. (This work is supported by NIDDK grant R01DK080080).

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

During mammalian sperm capacitation, an increase in protein phosphatase activity in tyrosine and serine/threonine residues has been described. The role of protein kinases, including tyrosine kinases in the capacitation 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 osmolality was adjusted between 280 and 300 mOsm/g. Immediately thereafter, some sperm aliquots were incubated at 37°C and 5% CO2 as follow: a) In capacitating medium; b) In capacitating medium plus 90 nM endothal; c) In capacitating medium plus 90 nM endothal; d) In capacitating medium plus inhibitor solvent. At different periods (0, 15, 30, 60 and 90 minutes), sperm samples were withdrawn to evaluate the percent of capacitated sperm using the chlorotetracycline fluorescence assay. The results indicated that incubation with endothal, which is very recently increased activity of protein phosphatase, did not affect capacitation. On the other hand, endothal was 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 a stabilization of capacitation. These results suggest that PP2A may have an important role in regulating the initial events of human sperm capacitation process. This research was supported by Fondecyt Project 1080042.


In mammalian fertilization, the zona pellucida plays a key role in sperm-oocyte fusion by engaging the calcium influx pathways. Glycodelin-A, a protein secreted by the growing oocyte, plays a crucial role in triggering the acrosome reaction of spermatozoa. This review explores the mechanisms underlying the role of glycodelin-A in sperm capacitation, acrosome reaction, and zona pellucida binding, acting on paracrine action to control the 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 serum protein isolated from normal donors blocked glycodelin-A primed ZP3, but not ZP4-induced acrosome reaction. Other glycodelin isomers and deglycosylated glycodelin-A did not have such priming activity. Glycodelin-A treatment increased both ZP3 and 5% CO2 as

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 successive acrosome reaction and sperm-oocyte fusion. A previous report 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 the pregnancy) were activated with parthenogenetic stimulation. After 24 hours of culture, embryo development was assessed. The results showed that STIM1-mediated Ca2+ oscillation is critical for embryo development after fertilization.