

revealed that the majority of changes occurred between Days 10 and 12 of SEP. To compare transcript levels between CG-rescued and regressing CL, previously banked rhesus GeneChip array data (NCBI GEO Series GSE10367) from CL collected during the mid- to late and very late luteal phase were analyzed with time-matched intervals in SEP. Comparing RMA-normalized transcripts from the natural cycle (Group 1, CL days 10, 12, 14-16, and 18-19) with those from luteal rescue (Group 2, hCG-treated samples) revealed 7677 transcripts changing in expression pattern >2-fold (one-way ANOVA, FDR correction; $P < 0.05$) between the two groups. Clustering of samples revealed that the SEP samples possessed the most related transcript expression profiles. Regressed CL (days 18-19, around menses) were the most unlike all other CL. KEGG analysis indicated the most affected pathway was Steroid Biosynthesis; an acute stimulatory response to hCG during SEP was observed for HSD3beta2 and CYP19 mRNA, whereas a chronic stimulatory response occurred for StAR, CYP11A1, CYP17A1, and LHCGR mRNA as measured by real-time PCR. Acute stimulation by CG was noted for PGR, and GCR α mRNA, while acute inhibition by CG was measured for VEGF, CRHBP and 11 β HSD2 mRNA. Chronic stimulation was detected for RLN1, CAT, and PGRMC1 mRNA; while message for 11 β HSD1 was chronically inhibited during SEP. Transcript levels for the prostaglandin F2 α receptor PTGFR, which are up-regulated during luteolysis, were mostly down-regulated by SEP treatment. The most significantly absent pathway (negative z-score) following SEP treatment includes a group of genes whose products promote cell-death. By further comparing the genome-wide changes in luteal gene expression during rescue in SEP, with those in CL during luteolysis in the natural menstrual cycle, it is possible to identify key regulatory pathways promoting fertility. R01 HD20869, RR00163, T32-HD07133 (CVB), China Scholarship Council (CSC; LX).

128. Liver X Receptor Modulation of Gene Expression Leading to Pro-Luteal Effects in Primate Luteal Cells. Randy L. Bogan and Jon D. Hennebold. Oregon Health & Science University, Oregon National Primate Research Center, Beaverton, OR, USA

The expression of genes necessary for reverse cholesterol transport (RCT) and cholesterol metabolism is controlled by the liver x receptors (LXR) alpha and beta, members of the nuclear hormone receptor superfamily. Previously, we reported an acute induction of RCT component expression occurs (mRNA and protein) coinciding with the fall in progesterone (P4) synthesis at the end of the luteal phase (i.e., functional regression) in the rhesus macaque corpus luteum (CL). Additionally, we identified three endogenous LXR ligands (22R-hydroxycholesterol and 27-hydroxycholesterol, or 22ROH and 27OH, respectively; and desmosterol) that are present in the CL during the luteal phase. Therefore, LXR regulation of gene expression may be a key determinant of functional regression in the primate CL. To test this hypothesis, we used dispersed primate luteal cell cultures obtained from mid-luteal stage CL (days 7-8 post LH-surge, peak P4 production) to determine whether synthetic and endogenous LXR ligands: 1) increase expression of known LXR target genes, 2) inhibit expression of cholesterol uptake and steroidogenic genes, and 3) increase cholesterol efflux. The well characterized synthetic LXR agonist T0901317 (T09, 1 μ M) as well as the endogenous ligands 22ROH, 27OH, and desmosterol (1 or 5 μ M, physiologic range) were incubated with luteal cells for 24 hr. To facilitate uptake of sterols, 0.5% (w:v) beta-cyclodextrin (beta-CDX) was added to the culture media. T09 caused significant ($p < 0.05$) increases compared to vehicle in mRNA levels of known LXR target genes including the cholesterol efflux proteins ATP binding cassette subfamily A1 (*ABCA1*), and G1 (*ABCG1*), as well as *LXRalpha* itself ($n = 8$). T09 also caused a significant decrease in 3beta-hydroxysteroid dehydrogenase (*HSD3B2*) mRNA compared to vehicle ($p < 0.05$), but had no effect on low and high density lipoprotein receptors, or steroidogenic acute regulatory protein expression. Surprisingly, beta-CDX itself caused a significant ($p < 0.05$) decrease in *ABCA1*, *ABCG1*, and *LXRalpha* expression in vehicle-treated cells. As beta-CDX is known to deplete cells of sterols, this indicates that there is a high degree of basal LXR gene expression controlled by endogenous sterols. LXR target gene responsiveness to the endogenous ligands varied between luteal preparations. When replicates were divided based on initial *LXRalpha* mRNA levels (as an indicator of endogenous LXR activity), the 4 cultures with the lowest basal *LXRalpha* expression had significant ($p < 0.05$) increases in *ABCA1* and/or *ABCG1* mRNA levels in response to endogenous ligand (5 μ M) treatment. The cultures with the highest *LXRalpha* levels did not respond to endogenous ligands with statistically significant increases in LXR target gene expression, indicating that luteal cells with higher LXR levels may be refractory to further stimulation. Cholesterol efflux assays were used as a functional endpoint of LXR activation. T09 and the 5 μ M doses of 22ROH, 27OH and desmosterol significantly ($p < 0.05$, $n = 5$) increased cholesterol efflux compared to controls. Collectively, these data indicate that: 1) activation of LXR-mediated RCT may inhibit luteal steroidogenesis by increasing cholesterol efflux and decreasing *HSD3B2* expression ultimately causing functional regression, and 2) there is a high degree of basal LXR activity in the primate CL that may need to be repressed during the luteal phase to maintain P4 synthesis. This research was supported by NICHD R01-HD42000 and NCRR RR00163.

129. Factor(s) Secreted from Bovine Luteal Cells Alter Function of Bovine T Lymphocytes. Koji Toyokawa and J.L. Pate. Pennsylvania State University, State College, PA, USA

The overall goal of this research is to understand the function of the bovine corpus luteum (CL), ultimately leading to improved reproductive efficiency in the dairy industry. The specific research focus is to understand how T lymphocytes (T

cells) in the bovine CL are regulated during the estrous cycle. We hypothesized that T cell function could be modified not only by cell-cell contact but also via secreted factor(s) from the CL. There are two major protein secretory pathways, termed conventional and unconventional secretory pathways. Most secreted proteins transit through the ER-Golgi system (conventional secretion), whereas some secreted proteins bypass the traditional secretory pathway (unconventional), and the latter secretory pathways are not inhibited by brefeldin A (BFA). In order to test our hypothesis, we utilized BFA to block secretion of conventionally secreted proteins, which also results in upregulation of proteins secreted by unconventional pathways. In this study, conditioned media from luteal cells alone (LC) and from luteal cells treated with BFA (LC+BFA) were utilized to understand autocrine and/or paracrine interactions between T cells and luteal cells. The primary objectives of this study were to determine T cell responses and cytokine production stimulated by factor(s) secreted from luteal cells. First, the efficacy of BFA in altering protein secretion by luteal cells was examined. Secretion of IL-4, one of the conventionally secreted cytokines, was significantly inhibited in LC+BFA compared to conditioned medium from LC (50% reduction, $P < 0.01$). Secondly, we determined if conditioned medium from luteal cells would mediate T cell responses, and further examined if BFA-restricted secretion would alter function of T cells. Conditioned media from LC and LC+BFA significantly stimulated T cell proliferation compared to media alone with or without BFA ($P < 0.01$). In addition, LC+BFA-conditioned medium stimulated even higher T cell proliferation than LC-conditioned medium alone (30% and 20%, respectively, $P < 0.01$). However, addition of PGF2 α to the culture medium had no additive or synergistic effect on T cell responses. Finally, we investigated if factor(s) secreted from LC- and LC+BFA-conditioned media altered cytokine expression in T cells. Gene expression of IL2, IL2R, IFN γ and TGF β was examined in T cells cultured in LC- or LC+BFA-conditioned medium. Unexpectedly, expression of IL2 was significantly downregulated in T cells cultured in LC- and LC+BFA-conditioned media compared to medium alone ($P < 0.01$), whereas expression of IL2R and IFN γ in T cells was not changed. However, expression of TGF β (anti-inflammatory cytokine) in T cells was also downregulated in LC- and LC+BFA-conditioned media ($P = 0.05$ and 0.08, respectively). In conclusion, this study provides evidence that autocrine and/or paracrine factor(s) are involved in activation of T cells by luteal cells. Furthermore, it is speculated that different factor(s) may be secreted from luteal cells via unconventional secretions, and factor(s) secreted from luteal cells stimulated by BFA may have more potent stimulatory effects on T cell proliferation. However, the exact molecular mechanisms and identification of luteal-derived factors to modulate T cell functions remain unknown. This project was supported by National Research Initiative Competitive Grant no. 2004-35203-14789 from the USDA Cooperative State Research, Education, and Extension Service to JLP.

130. Olfactomedin 1 (Olfm 1) in Fallopian Tube May Modulate Tubal Ectopic Pregnancy in Humans: Evidence from Immunohistochemistry and an In Vitro Coculture Model. Suranga P. Kodithuwakku, William S.B. Yueng, Pak Chung Ho, and Kai Fai Lee. The University of Hong Kong, Hong Kong, Hong Kong SAR

Olfactomedins are secretory glycoprotein constituted in the extracellular matrix (ECM) of various cell types. Recent studies suggested that Olfm-1 is down-regulated during the window of implantation (WOI) in the human endometrium and up-regulated in pathological condition like endometriosis and recurrent spontaneous abortions. Ectopic pregnancy is a gynaecological emergency and fertility threatening phenomenon which occurs in 1-2% of normal pregnancies and shows an increasing trend. Yet, tubal ectopic pregnancy accounts for more than 98% of the cases and the distal part of the fallopian tube accounts for 85-95% of all tubal pregnancies. Our unpublished data demonstrated that the expressions of Olfms are steroid hormone-dependent and Olfm-1 affects spheroid attachment onto endometrial epithelial cells in vitro. In the present study, we investigated the spatiotemporal expression of Olfm-1 protein and the transcript of different isoforms (Olfm-1, -2, -3, and -4) in normal Fallopian tubes and tubes with ectopic pregnancies from human samples. Furthermore, an in-vitro-trophoblastic-spheroids and Fallopian-tube-epithelial-cells co-culture model was developed to investigate the possible role of Olfm-1 in tubal ectopic pregnancies using a human Fallopian tube epithelium cell line (OE-E6/E7) and recombinant Olfm-1 protein. Olfm-1 mRNA in the ampullary region showed a significantly lower level ($p < 0.05$) at luteal phase ($n = 12$) than the follicular phase ($n = 15$); whereas there was no significant difference in the infundibullary or isthmic regions between the two phases. The Olfm-1 protein was strongly expressed in the epithelium of the three regions in the human Fallopian tube. The expression of Olfm-1 protein in the infundibullary region is significantly higher ($p < 0.05$) than in the isthmic and ampullary regions in both the follicular (H-SCORE = 3.6 ± 0.3 vs 2.5 ± 0.3 vs 2.3 ± 0.6 , respectively, $n = 10$) phases of the cycle. Interestingly, the ampullary tubal ectopic sections ($n = 10$) showed a significantly lower level of Olfm-1 expression in the epithelium (H-SCORE = 1.3 ± 0.2 when compared to normal ampullae ($p < 0.05$)). A trophoblastic-spheroids (JAR) and human-Fallopian-tube-epithelial-cell (OE-E6/E7) co-culture system was established. Treatment of OE-E6/E7 with recombinant human Olfm-1 for 24 hrs dose-dependently (0.01-1 μ g/ml) reduced JAR spheroids attachment to the OE-E6/E7 monolayers when compared to the untreated controls. Furthermore, activation of the Wnt-signalling pathway using Wnt3a or LiCl was associated with reduced Olfm-1 expression in OE-E6/E7 and increased spheroids attachment to OE-E6/E7 cells, suggesting a possible interaction of Olfm-1 with the Wnt-signalling pathway in the tubal ectopic pregnancy. In sum, Olfm-1 in the human Fallopian tube may function to reduce tubal ectopic embryo attachment. Activation of the Wnt-

signaling pathway and down-regulation of Olfm-1 expression favour the spheroid/embryo attachment onto human Fallopian tube epithelium. [This project is supported in part by an RCG grant HKU7514/05M to PCH]

131. Potential Role of *Wnt* in Neonatal Uterine Development. Kanako Hayashi, Shin Yoshioka, Edmund B. Rucker, Thomas E. Spencer, Paul S. Cooke, Francesco J. DeMayo, John P. Lydon, and James A. MacLean. Southern Illinois University School of Medicine, Carbondale, IL, USA; University of Kentucky, Lexington, KY, USA; Texas A&M University, College Station, TX, USA; University of Illinois at Urbana-Champaign, IL, USA; Baylor College of Medicine, Houston, TX, USA

WNT genes encode secreted glycoproteins that control cell fate, mortality, proliferation, differentiation, and tissue growth, and subsets (*Wnt4*, *Wnt5a*, and *Wnt7a*) are involved in Mullerian duct patterning and differentiation during embryonic development of the female reproductive tract. The present study investigated potential role of *Wnts* during postnatal mouse uterine development, because disruption of endometrial adenogenesis and mesenchymal specification and differentiation can cause permanent fertility problems in the adult. The expression of 19 *Wnt* genes and 10 *Fzd* receptors was determined by RT-PCR. The spatial expression patterns of the most highly expressed genes: *Wnt4*, *Wnt5a*, *Wnt7a*, *Wnt7b*, *Wnt11*, *Wnt16*, *Fzd6* and *Fzd10* were further examined by *in situ* hybridization in the neonatal mouse uterus. *Wnt4*, *Wnt5a* and *Wnt16* were localized in the endometrial stroma, whereas *Wnt7a*, *Wnt7b* and *Wnt11* were in the uterine epithelia of neonatal mice. Exposure of mice to estrogen or progesterone during critical development periods inhibits endometrial adenogenesis. Diethylstilbestrol (DES) or progesterone-induced disruption of endometrial gland development was associated with reduction or ablation of *Wnt4*, *Wnt5a*, *Wnt7a*, *Wnt11*, *Wnt16* and *Fzd10* mRNA during endometrial morphogenesis. Next, we have characterized postnatal uterine morphogenesis by conditionally ablating the expression of *Wnt11* in the uterine epithelium of mouse after birth using progesterone receptor (PR) Cre knockin mouse. To increase the efficiency in the production of homozygous null alleles and to decrease the incidence of mosaic deletion, *PRcre/+ Wnt11^f-* mice were crossed with *PRcre/+Wnt11^f-* mice to *PR/+Wnt11^ff* mice. *Wnt11^fd* mice did not show disrupted endometrial gland development, and adult *Wnt11^fd* mice had normal estrus cycles and were fertile. However, we observed disorganized luminal epithelium on postnatal (P) day 7 in *Wnt11^fd* mice, and levels of *Wnt4*, *Wnt5a*, *Wnt7a*, *Wnt7b* and *Wnt16* in neonatal uteri were decreased in *Wnt11^fd* mice compared to those in *Wnt11^ff* mice. Interestingly, ablation of *Wnt11* appears to increase the numbers of endometrial glands on P21 and P28. *Wnt7a* was stimulated on P14 and P21 in uteri of *Wnt11^ff* mice, whereas *Wnt5a* and *Wnt16* were suppressed by loss of *Wnt11*. These results implicate that temporal and spatial expression of *Wnts* are critical factors for endometrial adenogenesis via autocrine and paracrine effects on neonatal uterine development. Further, *Wnt11* actions may involve changes in the expression of other members of the *Wnt* system to regulate uterine morphogenesis. Supported by NIH HD058222.

132. Steroidal Regulation of Uterine Macrophage Migration Inhibitory Factor Expression Is Mediated via miRNA-451. Warren B. Nothnack and Caitlin Healy. University of Kansas Medical Center, Kansas City, KS, USA

Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine which regulates cell proliferation, angiogenesis and immune cell trafficking. Within the uterus, MIF is expressed primarily by uterine epithelial cells in a cycle stage-dependent fashion. Despite characterization of the pattern of uterine MIF expression in a variety of species, virtually no information exists on MIF regulation. As such, the objective of this study was to examine steroidal regulation of Mif in the mouse uterus. Two-month-old, ovariectomized female mice (N=6/treatment group/time point) were treated with estrogen (E2; 1 µg/kg BW) or E2 + progesterone (P4; 2 mg/kg BW) then sacrificed at 0, 4, 8, and 24h post steroid treatment. Uterine Mif was examined by qRT-PCR, Western blot analysis, and immunohistochemical localization. E2 alone, or plus P4, significantly (P<0.05) increased Mif mRNA expression at all time points. Assessment of Mif protein revealed that in contrast to the effect on transcript expression, E2 significantly decreased Mif protein expression by approximately 35% and 65% at 4h and 8h post steroid treatment, respectively, while at 24h, Mif protein levels were approximately 20% below 0h values. Mif localized primarily to luminal and glandular epithelial cells with low stromal expression. The discordant pattern of expression between transcript and protein suggested to us that uterine Mif expression may be regulated by microRNAs (miRs). miR451 is a putative regulator of MIF expression which we have shown to be regulated by E2 within the uterus in a pattern that is inversely correlated to the pattern of Mif uterine protein expression. To test the hypothesis that miR451 regulates Mif protein expression, we transfected human endometrial epithelial cells (HES), with pre-miR-451 precursor, non-targeting precursor (pre-miR-NT; negative control) or transfection buffer alone and assessed miR451 transcript expression by qPCR while MIF protein expression was assessed by Western analysis. Transfection with pre-miR-451 precursor resulted in a significant increase in HES cell expression of mature miR451 which was associated with a significant reduction in MIF protein expression. To further verify that miR451 targets the MIF 3'UTR, HES cells were co-transfected with a Renilla-MIF 3'UTR vector containing the wild-type miR451 seed sequence or a Renilla-MIF 3'UTR mutant vector (mutant miR451 seed sequence), a control vector containing firefly luciferase, and either pre-miR-451 or pre-miR-NT precursors. HES cells co-transfected with constructs which contained the wild-type 3'UTR and pre-miR-451 exhibited significantly less luciferase activity (Renilla normalized to firefly)

compared to cells co-transfected with the mutant 3'UTR and pre-miR-451. Cells co-transfected with the mutant 3'UTR and either the pre-miR-451 or pre-miR-NT precursors did not exhibit a significant change in luciferase activity compared to controls. Collectively, these data are interpreted to suggest that miR451 is capable of binding to the 3'UTR of MIF *in vitro* and suppressing luciferase reporter activity. These observations, coupled with the MIF Western blot data strongly suggest that MIF expression is regulated by miR451. In summary, we demonstrate for the first time that E2 regulates uterine Mif expression and that this regulation appears to be mediated in part by miR451.

133. Estrogen Action Is Required for Expression of MMP-26 in the Secretory Phase of the Menstrual Cycle in Rhesus Macaques. Camila Contin Diniz de Almeida Francia, Christopher S. Keator, Kunie Mah, Lindsay Ohm, and Ov D. Slayden. Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA

Matrix metalloproteinase 26 (MMP-26) is a novel matrilysin-like endopeptidase that may play a role during embryo implantation, but our knowledge on MMP-26 regulation remains inadequate. In women, endometrial MMP-26 is up-regulated by progesterone (P) during the early and mid secretory phase. However, MMP-26 expression is refractory to P stimulation in the late secretory phase and the mechanism underlying MMP-26 down regulation is not known. In this study we examined hormone regulation of MMP-26 in ovariectomized artificially cycled rhesus macaques. The samples were obtained from animals assigned to other studies at the Oregon National Primate Research Center. The animals were treated with a controlled estradiol (E2) and P regimen to mimic the menstrual cycle. To stimulate the artificial cycles an E2-releasing capsule was placed subcutaneously (SC) to induce the proliferative phase and after 14 days of E2 priming, a P-releasing capsule was placed SC to induce the secretory phase of the cycle. Removal of the P implant on day 28 completed the cycles and stimulated menstruation on days 2-4 of the next cycle. Endometrium was collected on cycle day 3 (menstruation), days 7 and 14 (proliferative phase), day 17 (early secretory phase; E2 +3 days of P), day 21 (mid secretory phase; E2 +7 days of P), and day 28 (late secretory phase; E2 +14 days of P). In 3 animals, E2 was withdrawn at the onset of the secretory phase and samples were collected after 14 days of P treatment alone. Samples were frozen in liquid nitrogen for RNA isolation and TaqMan Real-time PCR analysis of MMP-26. Similar samples were embedded in Tissue-Tek OCT (Miles Laboratories), frozen in liquid propane, cryosectioned, and subjected to *in situ* hybridization (ISH) with macaque-specific [³⁵S]-labeled riboprobes to MMP-26. Samples were also fixed in 4% paraformaldehyde, embedded in paraffin and analyzed by immunocytochemistry (ICC) for MMP-26 with goat polyclonal anti-MMP-26 IgG or with monoclonal antibodies to estrogen receptor alpha (ESR1) and progesterone receptor (PGR). Real-time PCR confirmed that MMP-26 transcript was minimal during the mid and late proliferative phase (n=9) and then increased >50 fold (P<0.001; n=4) by the mid secretory phase of the cycle. MMP-26 mRNA levels significantly declined by >10 fold (P<0.001) on day 14 of P treatment. Mean MMP-26 levels further declined to baseline during menses (P<0.01; n=8). ISH revealed that these striking changes in MMP-26 mRNA were localized solely to the endometrial glands. Immunocytochemistry showed that MMP-26 staining was absent in the glandular epithelium during proliferative phase. By day 3 of P treatment (early secretory phase), MMP-26 staining was strong in glands of the functionalis zone. MMP-26 staining was maximal on day 7 of P treatment and then declined in the late secretory phase. At this time MMP-26 was minimal by ICC and ISH in 2 of 3 animals (n=6 total). Secretory phase glands that retained MMP-26 immunoreactivity also retained staining for ESR1 and PGR. Animals with E2 removed at the onset of the secretory phase showed no immunoreactivity for ESR1, PGR, or MMP-26. We conclude that both E2 and P action is required for expression of MMP-26, and that down-regulation of glandular ESR1 by extended P action results in suppression of MMP-26 in the late secretory phase. Supported by NIH grants HD18185 and RR000163 and the NIH Fogarty International Center grant TW/HD-00668 (P. Michael Conn P.I.).

134. Anti-Proliferative Effects of Evodiamine and Rutaecarpine on Human Ovarian Cancer Cell Line SKOV3. Ching-Han Yu, Ru-Cui Lin, and Paulus S. Wang. Chung Shan Medical University, Taichung, Taiwan, Republic of China; National Yang-Ming University, Taipei, Taiwan, Republic of China

Ovarian cancer is the fifth most common cancer among women in the United States, and it causes more deaths than any other type of female gynaecological malignancies. The human HER-2/*neu*-overexpressing ovarian cancer cells are resistant to chemotherapeutic agent (Taxol). Therefore, there are almost no efficacious therapeutic modalities for malignant ovarian cancer. It is important to develop some new effective therapies. Evodiamine and rutaecarpine are quinazolinocarboline alkaloids extracted from a kind of Chinese herb named Wu-Chu-Yu and has been demonstrated to be effective in preventing the growth of a variety of cancer cells, including downregulating the estrogen receptor of breast cancer. In the present study, the mechanism by which evodiamine and rutaecarpine inhibited the HER-2/*neu*-overexpressing ovarian cancer cell line SKOV3 was examined. Based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cell proliferation rate was reduced dose-dependently by evodiamine and rutaecarpine. According to the flow cytometric analysis, evodiamine treatment resulted in G2/M arrest in SKOV3 cells, but not rutaecarpine. Furthermore, by using the annexin V assay, evodiamine- and rutaecarpine-induced apoptosis was observed at 48 hours and extended to 72 hours. These results suggested that evodiamine and rutaecarpine inhibited the growth and induced the cell apoptosis of the HER-2/*neu*-overexpressing