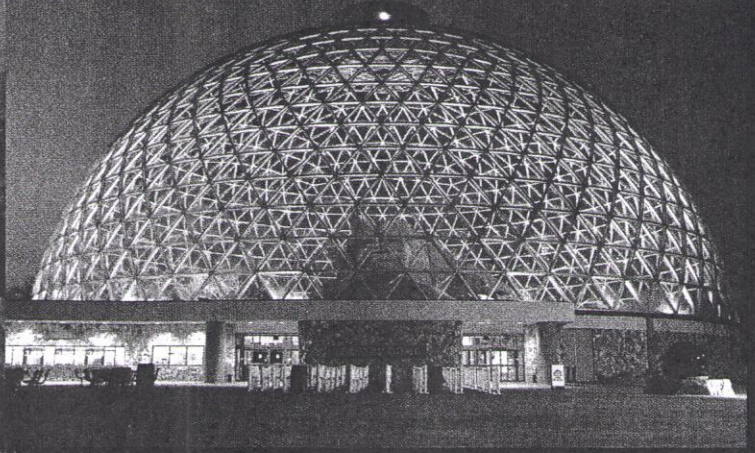
Society for the Study of Reproduction

Annual Meeting

Qwest Center Omaha
Omaha Nebraska

July 29–August 1, 2006

Hosted by the
University of Nebraska
Medical Center



BIOLOGY OF REPRODUCTION 2006 SPECIAL ISSUE

Platform Session 03. IMMUNOLOGY OF THE REPRODUCTIVE SYSTEM

Chair: Troy L Ott. Co-Chair: Koji Toyokawa.
Saturday, July 29, 2006

4:45:00 PM - 6:15:00 PM
Location: Qwest Center Omaha, 203

4:45 PM. 13. POLYMORPHISMS IN *PAAN-AG* PROMOTER INFLUENCES NF-κB BINDING AND TRANSCRIPTION ACTIVITY IN HEK293 CELLS. Daudi K Langat[1], Pedro J Morales[1], Charles OA Omwandho[2], Asgerally T Fazleabas[3] and Joan S Hunt[1]. [1]University of Kansas Medical Center, Kansas, KS; [2]University of Nairobi, Nairobi, Kenya; [3]University of Illinois at Chicago, Chicago, IL.

5:00 PM. 14. PIG CONCEPTUS INTERFERON (IFN) GAMMA MAY ABLATE UTERINE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I AND BETA-2-MICROGLOBULIN (B2) EXPRESSION AT THE MATERNAL-PLACENTAL INTERFACE. Margaret M Joyce, James R Burghardt, Robert C Burghardt, R Neil Hooper, Laurie A Jaeger and Greg A Johnson. Texas A&M University, College Station, TX.

5:15 PM. 15. EXAMINING THE IMMUNOPROTECTIVE PROPERTIES OF SERTOLI CELLS. Jannette M Dufour[1], Ray V. Rajotte[2], Michael D. Griswold[3], Doreen E. Dixon[2] and Gregory S. Korbutt[2]. [1]Texas Tech University Health Sciences Center, Lubbock, TX; [2]University of Alberta, Edmonton, AB, Canada; [3]Washington State University, Pullman, WA.

5:30 PM. 16. RELAXIN AND ESTROGEN REGULATE THE BONE REMODELING MARKERS, RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-kappa B LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG), IN RAT ADJUVANT-INDUCED ARTHRITIS. Teh-Yuan Ho[1], Karen Santora[2], Wenbo Yan[1], Denise Visco[2] and Carol Bagnell[1]. [1]Rutgers University, New Brunswick, NJ; [2]Merck & Co., Inc., Rahway, NJ.

5:45 PM. 17. EVIDENCE FOR OXYTOCIN RECEPTOR EXPRESSION IN BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS AND T LYMPHOCYTES. Kalidou Ndiaye and Joy L Pate. Ohio State University, Wooster, OH.

6:00 PM. 18. IDENTIFICATION AND EXPRESSION OF A HOMOLOGUE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN OVINE REPRODUCTIVE TISSUES. Federica Lopes[1], Grazyna Ptak[1], Alessandro Vannoni[2], Maria T del Vecchio[2], Alessandra Casciaro[2], Pasqualino Loi[1], Marcella Cintorino[2] and Felice Arcuri[2]. [1]University of Teramo, Teramo, Italy; [2]University of Siena, Siena, Italy.

Platform Session 04. NEUROENDOCRINOLOGY AND BEHAVIOR

Chair: Terry M. Nett. Co-Chair: Chad Forador.
Saturday, July 29, 2006

4:45:00 PM - 6:15:00 PM
Location: Qwest Center Omaha, 204

4:45 PM. 19. DIFFERING MODES OF CORTISOL-INDUCED SUPPRESSION OF PULSATILE LH SECRETION IN OVARIECTOMIZED VS. OVARY-INTACT EWES. Amy E Oakley[1], Kellie M Breen[1], Alan J Tilbrook[2], Elizabeth R Wagenmaker[1] and Fred J Karsch[1]. [1]University of Michigan, Ann Arbor, MI; [2]Monash University, Clayton, VIC, Australia.

5:00 PM. 20. PRENATAL TESTOSTERONE EXPOSURE PROGRAMS HYPERGONADOTROPISM AND PREPUBERTAL RESISTANCE TO ESTRADIOL NEGATIVE FEEDBACK IN OVARY-INTACT SHEEP TREATED POSTNATALLY WITH ESTRADIOL. Leslie M Jackson, Kathleen M Timmer and Douglas L Foster. University of Michigan, Ann Arbor, MI.

5:15 PM. 21. DEVELOPMENT AND USE OF TRANSGENIC ZEBRAFISH TO STUDY ELECTRICAL ACTIVITY FROM MIGRATING GnRH NEURONS IN THE INTACT EMBRYO. Nancy L Wayne[1], Matias Pandolfi[1] and David Kozlowski[2]. [1]David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Medical College of Georgia, Augusta, GA.

5:30 PM. 22. EFFECTS OF ESTRADIOL ON THE INDUCTION OF STRIATAL DOPAMINE RELEASE BY AMPHETAMINE IN OVARIECTOMIZED RATS. Paulus S Wang[1], Ching I Wu[1], Wynn HT Pan[2] and Jui Chih Su[1]. [1]Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221, Taiwan; [2]Department of Pharmacology, Shool of Medicine, National Yang-Ming University, Taipei 11221, Taiwan.

5:45 PM. 23. DEVELOPMENTAL PROGRAMMING: EXCESS PRENATAL EXPOSURE TO TESTOSTERONE COMPROMISES MATING AND PREGNANCY RATE. Eila K Roberts, Teresa L Steckler, Theresa M Lee and Vasantha Padmanabhan. University of Michigan, Ann Arbor, MI.

6:00 PM. 24. SUBFERTILITY IN FEMALE ESTROGEN RECEPTOR-b NULL MICE IS PARTIALLY DUE TO A SUBMAXIMAL LUTEINIZING HORMONE (LH) SURGE. Friederike L Jayes, John F Couse and Kenneth S Korach. National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC.

Platform Session 05. SEX DETERMINATION AND DEVELOPMENT

Chair: Robert Viger. Co-Chair: Kirsten Holthusen.
Saturday, July 29, 2006

4:45:00 PM - 6:15:00 PM
Location: Qwest Center Omaha, 205–206

4:45 PM. 25. DISSECTING THE CANONICAL WNT PATHWAY IN THE MÜLLERIAN DUCT: THE ROLE OF BETA-CATENIN AND EXPRESSION OF FRIZZLED RECEPTORS. Erica Deutscher and Humphrey Yao. University of Illinois, Urbana, IL.

5:00 PM. 26. ANDROGEN-INDEPENDENT EFFECTS OF INHIBIN BETA A ON EPIDIDYMIS FORMATION. Jessica T Tomaszewski, Avenel DE Joseph and Humphrey H-C Yao. University of Illinois, Urbana-Champaign, IL.

5:15 PM. 27. DECIPHERING THE ROLE OF SONIC HEDGEHOG AND INHIBIN BETA A IN WOLFFIAN DUCT DEVELOPMENT. Avenel Joseph, Jessica Tomaszewski and

PROGRAM
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incorporation of radioactive-labeled methionine as well as epididymal cell proliferation. We have detected by 2D electrophoresis the appearance of a secreted epididymal protein with a molecular weight of 100 kDa and a pI of 5.3 only when epididymal cells were co-incubated with spermatozoa. This protein was not found in culture condition without spermatozoa. The identification of such protein is actually in process by LC-QToF analysis. Microarrays spotted with 588 mouse cDNAs were used to examine the variation of gene expression under the co-culture condition. We have noticed after analysis that many genes in relation with cellular pathway were up-regulated by the presence of spermatozoa. Together, those results strongly suggest that spermatozoa can act as a paracrine factor on epididymal epithelial cells and induce gene expression and *de novo* protein synthesis and secretion. This novel finding provides new concept and working hypothesis to clarify how testicular factors, such as androgens and spermatozoa itself, initiate the activation and differentiation of epididymal epithelium during the sperm maturation process.

10. IDENTIFICATION OF A CpG-FREE METHYLATION BOUNDARY THAT SERVES AS AN INSULATOR BETWEEN ALTERNATIVE, SEX-SPECIFIC PROMOTERS. Sreenath Shanker, Manjeet K Rao and Miles F Wilkinson. MD Anderson Cancer Center, Houston, TX

Pem (Rhox5) is the founding member of the reproductive homeobox (Rhox) gene cluster that we recently identified on the mouse X chromosome. Rhox5's expression is restricted to specific cell types in the female and male reproductive tissues by two independently regulated promoters. Rhox5's distal promoter (Pd) is active in the ovary and placenta, whereas its proximal promoter (Pp) is expressed in the testis. To investigate whether DNA methylation plays a role in regulating Rhox5's expression pattern in vivo, we used bisulfite analysis to examine the methylation profile of its promoters in tissues. We found that the CpGs in the element essential for Pd transcription were unmethylated in ovary, whereas the CpGs in the Pp regulatory region were methylated, both consistent with the exclusive expression of the Pd in ovary. Conversly, in the testis, CpGs in the Pp were unmethylated and the Pd CpGs were methylated, corresponding with the testis-specific expression of the Pp. Analysis of the methylation status of CpGs in the intervening 1.5-kb region between the two promoters revealed the presence of a testis-specific DNA methylation boundary. In the testis, a 237-bp CpG free region seperates the unmethylated Pp from the methylated DNA upstream. We used an enhancer blocking assay to determine whether this boundary region contained a transcriptional insulator. We found that this 237-nt CpG-free region insulated the Rhox5 promoter against the activity of a heterologous enhancer (by 12–15 fold) in a position-dependent manner. It had no activity when placed upstream of the enhancer, demonstrating that it is not a general silencer. The strongest insulation (>75 fold) is obtained when we include a further 100bp at the 3' end of the 237-bp CpG free region. Further mapping studies reveal that most of the insulator activity is confined to a 50bp sequence. Studies are ongoing to further define a miminal element and to identify binding factors that mediate the activity of the Rhox5 insulator. To our knowledge, the Rhox5 insulator is the first insulator identified between alternative promoters from a single gene. We propose that it will serve as a useful model system to elucidate the function of insulator elements in controlling tissue-specific transcriptional regulation.

11. EXPRESSION OF HYPOXIA-INDUCIBLE FACTOR-1α IN ...AT LEYDIG CELLS. Preethi R Pirlamarla[1], Matthew P Hardy[2], Dale B Hales[3], Nadine Korah[4], Louis Hermo[4] and Michael A Palladino[1]. [1]Monmouth University, West Long Branch, NJ; [2]Population Council, Center for Biomedical Research, New York, NY; [3]University of Illinois at Chicago, Chicago, IL; [4]McGill University, Montreal, PQ, Canada

The transcription factor hypoxia inducible factor-1 (HIF-1) is a master regulator of oxygen homeostasis in many tissues. Active HIF-1 is comprised of a hypoxia-dependent α subunit and a constitutively expressed β subunit. Previously we have demonstrated that HIF-1 is present in the adult rat testis. We hypothesize that HIF-1 is involved in regulation of oxygen tension in the testis. The purpose of this study was to examine the effects of ischemia (I) and ischemia-reperfusion (I/R) on HIF-1α in the adult rat testis, to determine which cell type(s) in the testis express HIF-1α, and to begin to examine mechanisms that control HIF-1α activation in the testis. Unilateral testicular ischemia was created by 720° torsion ranging from 1–6 h followed by variable times of reperfusion. Nuclear proteins were isolated from ischemic and sham testes and analyzed by immunoblotting. Surprisingly, HIF-1α was abundant in normoxic testes and not significantly affected ($P<0.01$) by testis ischemia or I/R. Immunocytochemistry revealed that HIF-1α is primarily localized in Leydig cells of normoxic and hypoxic testes, and results confirmed by immunoblot analysis of proteins from purified populations of testis cell types. In most tissues, HIF-1α is ubiquitinated and degraded under normoxia and activated through oxygen-dependent stabilization of the α subunit during hypoxia. Immunoprecipitation demonstrated that HIF-1α from normoxic and hypoxic testes is primarily in a non-ubiquitinated form indicating activation even in the normoxic testis. In addition to hypoxia, HIF-1α is stabilized and activated by a range of stimuli including reactive oxygen species (ROS). To examine HIF activation pathways in the testis, freshly isolated Leydig cells, cells cultured at 5% or 21% O_2, and cells cultured with 250 μM H_2O_2 were subjected to protein isolation and immunoblotting. Freshly isolated Leydig cells produce an abundance of HIF-1α which significantly diminished ($P<0.05$) in 5% and 21% cultures. Treatment of Leydig cells with H_2O_2 as a source of ROS did not significantly ($P<0.05$) affect HIF-1α levels. In conclusion, these data indicate high levels of constitutively activated HIF-1α in normoxic Leydig cells. The role of HIF-1 in regulating oxygen homeostasis in the testis warrants further investigation. Supported by NIH grant HD046451 to MAP.

12. DEVELOPMENTAL AND GENE EXPRESSION PROFILING DURING SPERMATOGENESIS IN A CHEMICALLY-INDUCED GENETIC MOUSE MODEL OF INFERTILITY (*repro27*). Njotu L Agbor[1], Valentine A. Agbor[1], Lizhong Yang[2], Sheila Sweeney[3], Mary Ann Handel[3], Michael D Griswold[2] and Carol C Linder[1]. [1]New Mexico Highlands University, Las Vegas, NM; [2]Washington State University, Pullman, WA; [3]The Jackson Laboratory, Bar Harbor, ME

Male infertility accounts for about half of fertility problems worldwide and about 45% of these cases have unknown causes. However, it is hypothesized that the majority of these cases are due to genetic defects. The ReproGenomics Program at the Jackson Laboratory (http://reprogenomics.jax.org) has generated mouse models of infertility by chemical mutagenesis using ethylnitrosourea. The objective of this study was to phenotypically characterize one of these infertility mutants (C3Fe; B6-*repro27*) and to ultimately isolate the candidate gene responsible for its phenotype. Initial *repro27* analysis showed male specific infertility due to meiotic defects in spermatogenesis, very low testis weight, and abnormally shaped sperm heads without tails, low motility, and no IVF success. Preliminary mapping of *repro27* places the mutation on Chr 5 between 107.7 cM and 111.1 cM. This region contains 95 known genes (www.ensembl.org). Analysis was done in mice testis ages 2 - 10 wk of age. Histology analysis of PAS stained sections showed normal spermatogenesis in the control while in mutants, most germ cells undergoing meiosis failed to complete the process and die. The mean testis/body weight ratio at 2 wk was 0.25% in the control and 0.27% in the mutant whereas at 3 wk, it was 0.43% in the control and 0.33% in the mutant. This showed a significant reduction in the germ cell population in the mutant at 3 wk of age with no difference at 2 wk. Between 4 to 10 wk, mutant mice continued to show a significant reduction in testicular germ cell population and a delay the timing compared to controls. There was no significant difference in seminal vesicle weights in both genotypes of various age groups. RNA expression patterns were analyzed in 18 dpp mutant and controls using the GeneChip® Mouse Genome 430 2.0 Array, GeneChip® Operating Software (Affymetrix, Santa Clara CA), and the GeneSpring 7.2 software (Agilent Technologies, Redwood City, CA). Preliminary analysis indicates that 417 transcripts were expressed at least 2 fold higher in controls compared to mutants. On Chr 5, there were ~8 transcripts within the *repro27* candidate region differentially expressed in the control mice compared to mutants. We believe these data will be useful in identifying novel genes regulating male infertility.

13. POLYMORPHISMS IN *PAAN-AG* PROMOTER INFLUENCES NF-κB BINDING AND TRANSCRIPTION ACTIVITY IN HEK293 CELLS. Daudi K Langat[1], Pedro J Morales[1], Charles OA Omwandho[2], Asgerally T Fazleabas[3] and Joan S Hunt[1]. [1]University of Kansas Medical Center, Kansas, KS; [2]University of Nairobi, Nairobi, Kenya [3]University of Illinois at Chicago, Chicago, IL

The human leukocyte antigen-G (HLA-G), a protein highly expressed at the human maternal-fetal interface during pregnancy, is thought to be critical for the survival of the semi-allogenic fetus. Current evidence suggest that HLA-G programs immune cells at the maternal-fetal interface into immunosuppressive phenotypes, but definitive proof remains elusive since *in vivo* experiments in humans are not possible due to ethical concerns. In the search for an appropriate animal model, we have identified the olive baboon (*Papio anubis*) as a potential candidate. This primate expresses an HLA-G-like protein termed Paan-AG in the placenta. Preliminary data shows that *Paan-AG* gene shares many characteristics with *HLA-G*, including limited polymorphism, alternative splicing of the mRNA, and restricted tissue expression of the protein. Restricted tissue expression suggested that the two genes might share tissue-specific regulatory elements. We previously identified a number of putative regulatory elements in the proximal promoters of two *Paan-AG* alleles, 5'UTAG-1 (AG1) and 5'UTAG-2 (AG2). The objective of the current study was to assess binding of the transcription factor NF-κB to *Paan-AG* κB elements and determine the effects of binding on *Paan-AG* promoter activity. Both alleles contained two κB elements, κB1 and κB2. Binding was assessed using electrophoretic mobility shift assays and functional

activity using luciferase reporter assays. NF-κB bound both κB1 and κB2 elements in the AG1 allele. In contrast, only κB1 of the AG-2 allele bound to NF-κB; κB2 did not bind. The AG2 κB1 element bound NFκB with a stronger affinity compared to AG-1 κB1. Mutagenesis studies showed that the difference in binding was due to two nucleotide differences in the 3' end of κB1. The functional activity of the two alleles also differed; AG2 consistently showed higher luciferase activity compared to AG1. Mutating the last two nucleotides in the 3' end of κB1 resulted in an increase of luciferase activity to levels comparable to that of AG2. Overall, these results suggest that variations in the proximal promoter may influence transcription rates of *Paan-AG* as reported recently for *HLA-G*, and provide further evidence of the potential usefulness of the baboon as a model for *in vivo* HLA-G studies. Supported by NIH grant HD39878 (JSH)

14. PIG CONCEPTUS INTERFERON (IFN) GAMMA MAY ABLATE UTERINE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I AND BETA-2-MICROGLOBULIN (B2) EXPRESSION AT THE MATERNAL-PLACENTAL INTERFACE. Margaret M Joyce, James R Burghardt, Robert C Burghardt, R Neil Hooper, Laurie A Jaeger and Greg A Johnson. Texas A&M University, College Station, TX

During pregnancy, IFN-stimulated genes (ISGs) increase in the uteri of primates, rodents, ruminants and pigs. Two ISGs, MHC class I and B2, function in immune responses by discriminating self from non-self. Interestingly in sheep, MHC and B2 are silenced in uterine lumenal epithelium (LE), but increase in stroma in response to conceptus IFN tau. This silencing may be critical to prevent immune rejection of the conceptus. Since pig conceptuses also produce IFNs, we hypothesized that MHC, or swine leukocyte antigen class I genes (SLA), and B2 are modulated by IFNs in the uterus. Our first objective was to determine the expression of IFNs, SLA and B2 during the estrous cycle and early pregnancy. Pigs were hysterectomized on Day 5, 9, 12 or 15 of the estrous cycle, or Day 9, 10, 12, 13, 14, 15, 20, 25 or 30 of pregnancy. IFN gamma mRNA was localized to trophectoderm by Day 13 and maintained through Day 20, while protein was detected in peri-nuclear cytoplasmic vesicles throughout this time. RT-PCR confirmed the presence of classical SLA-1, –2, –3, and non-classical SLA-6, –7, –8 in pregnant uterus. Steady-state mRNAs for uterine SLA and B2 were higher during pregnancy compared to the estrous cycle. Paradoxically, while SLA and B2 proteins increased during pregnancy, both were silenced in the LE of pregnant but not cyclic uterus. Because pig conceptuses also produce estrogens for pregnancy recognition, our second objective was to determine the steroidal and/or cytokine regulation of SLA and B2 in the uterus. To evaluate the effect of estrogen, cyclic pigs were injected i.m. daily (Days 11–14) with estrogen or vehicle and hysterectomized on Day 15. To examine the effects of conceptus proteins, cyclic pigs were treated with steroid as above, implanted on Day 12 with mini-osmotic pumps that continuously delivered control serum protein to one horn and conceptus secretory proteins (CSP) to the other, and were hysterectomized on Day 16. Estrogen did not increase mRNA or silence LE expression of SLA and B2 proteins, however, CSP infusion down-regulated both SLA and B2 proteins in the LE. These results support the hypothesis that conceptus IFN gamma ablates key molecules involved in host defense and immune histocompatibility of transplanted tissues at the maternal-placental interface to ensure acceptance of the pig conceptus semi-allograft.

15. EXAMINING THE IMMUNOPROTECTIVE PROPERTIES OF SERTOLI CELLS. Jannette M Dufour[1], Ray V. Rajotte[2], Michael D. Griswold[3], Doreen E. Dixon[2] and Gregory S. Korbutt[2]. [1]Texas Tech University Health Sciences Center, Lubbock, TX; [2]University of Alberta, Edmonton, AB, Canada [3]Washington State University, Pullman, WA

Sertoli cells (SCs) possess unique immunoprotective properties, which allow them to prevent rejection of co-transplanted cellular grafts. However, not much is known about the mechanism involved. The objective of this study was to compare the immunoprotective ability of primary mouse SCs with a mouse Sertoli cell line (MSC-1) and to identify factors differentially expressed by these cells utilizing microarray analysis. Primary SCs or MSC-1 cells were co-transplanted with BALB/c islets underneath the kidney capsule of diabetic C3H mice (allogeneic). Blood glucose levels were measured and cell survival was analyzed by immunohistochemistry. When primary SCs were co-grafted, islet allograft survival was significantly prolonged (61.7 +/- 6.9 days; $p < 0.05$) compared with control mice that received allogeneic islets alone (31.3 +/- 3.3 days). In contrast, when MSC-1 cells were co-transplanted, islet allograft survival was not prolonged (24.6 +/- 4.4 days). Rejected MSC-1 cell co-grafts contained few islet cells and MSC-1 cells were surrounded by lymphocytes. Since MSC-1 cells were not immunoprotective, microarray analysis was used to compare the genes expressed by primary SCs with those expressed by MSC-1 cells. A total of 1,303 genes were differentially expressed, with 963 genes up-regulated in primary SCs and 340 genes up-regulated in MSC-1 cells. Genes up-regulated in primary SCs included the immunoprotective factors, clusterin and transforming growth factor beta1. While, proinflammatory and apoptotic markers were up-regulated in MSC-1 cells, which supports the transplant findings. Further study of the differences between primary mouse SCs and MSC-1 cells may identify novel factors important for testicular immune privilege and elucidate the mechanisms that allow SCs to prevent transplant rejection.

16. RELAXIN AND ESTROGEN REGULATE THE BONE REMODELING MARKERS, RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-kappa B LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG), IN RAT ADJUVANT-INDUCED ARTHRITIS. Teh-Yuan Ho[1], Karen Santora[2], Wenbo Yan[1], Denise Visco[2] and Carol Bagnell[1]. [1]Rutgers University, New Brunswick, NJ; [2]Merck & Co., Inc., Rahway, NJ

Rheumatoid arthritis is an autoimmune disease characterized by joint inflammation and destruction of soft tissue and bone. Symptoms of rheumatoid arthritis decline in pregnancy and in response to estrogen. Studies indicate that relaxin (RLX), a hormone of pregnancy, in combination with estrogen, reduces joint inflammation and soft tissue and bone damage in rats with adjuvant-induced arthritis, a model for rheumatoid arthritis. Osteoclasts are responsible for bone erosion in rheumatoid arthritis and their formation and activation are promoted by RANKL. Osteprotegerin, a decoy receptor for RANKL, antagonizes RANKL function. An important clinical goal is to develop therapies to lower the high RANKL/OPG ratio in bone eroding diseases, such as rheumatoid arthritis. The purpose of this study was to investigate whether RLX, alone or in combination with estrogen, regulates RANKL/OPG expression in a rat model of rheumatoid arthritis. Arthritis was induced in ovariectomized Lewis rats on day 0 by adjuvant injection and rats (n=8/group) were treated with estradiol valerate (E; 5 μg/sc in sesame oil/wk, starting day –10), porcine RLX (8 μg/sc in 1.0% benzopurpurin/day, starting day –3), E plus RLX, or vehicle. Healthy, non-injected rats were included as controls. On day 21, rats were sacrificed to collect blood and tissues. Plasma RANKL and OPG proteins were measured by enzyme-linked immunosorbent assay and total RNA from joint tissues was analyzed by real-time PCR. Treatment of arthritic rats with RLX alone decreased circulating RANKL ($P<0.05$) while E alone or in combination with RLX had no effect on the adjuvant-induced increase in RANKL protein. Treatment with E, but not RLX, increased systemic OPG ($P<0.05$), and combined treatment with E and RLX further increased circulating OPG ($P<0.05$) in comparison to E alone. Importantly, the RANKL/OPG protein ratio was lower in rats treated with E ($P<0.05$) or E in combination with RLX ($P<0.05$) when compared to arthritic controls. Real-time PCR analysis showed that the ratio of RANKL/OPG transcriptional activity in joints of rats treated with E and RLX decreased in comparison with adjuvant-injected animals ($P<0.05$). These data suggest a decrease in the RANKL/OPG ratio in response to RLX, in combination with E, could be important in reducing rheumatoid arthritis-induced bone loss.

17. EVIDENCE FOR OXYTOCIN RECEPTOR EXPRESSION IN BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS AND T LYMPHOCYTES. Kalidou Ndiaye and Joy L Pate. Ohio State University, Wooster, OH

Oxytocin is a posterior pituitary hormone whose primary action is to stimulate uterine contraction or milk ejection via oxytocin receptors (OTR). The corpus luteum (CL) also produces oxytocin which is involved in the regulation of pulsatile release of prostaglandin $F_2\alpha$ during luteolysis. Another role of oxytocin is proposed to be immunosuppressive to prevent full activation of proinflammatory responses during early and midcycle corpus luteum development. To verify the hypothesis that oxytocin produced in the CL of the cow may serve as a paracrine factor to regulate function of resident immune cells, it was first necessary to determine if bovine immune cells express OTR. Receptors for oxytocin have been identified and characterized in some tissues involved in immune function in the rat as well as in freshly prepared lymphocytes from human peripheral blood. Here we report the expression of OTR message in bovine peripheral blood mononuclear cells (PBMCs) and T lymphocytes (TC). In the present study, bovine PBMCs were isolated from whole blood of four different cows at different stages (day 3, 11, and 19) of the estrous cycle. TC were isolated and cultured in RPMI-1640 containing 10 % heat-inactivated fetal bovine serum, in the presence or absence of Concanavalin A (Con A, 1mg/ml) for 72 hours. Total RNA was extracted from fresh PBMCs and fresh TC along with cultured TC, and examined (2μg of total RNA) for OTR expression by real-time reverse transcription-polymerase chain reaction (RT-qPCR). The presence of OTR was observed in fresh PBMCs and TC at day 3, 11, and 19 of the estrous cycle. No difference was observed in OTR expression between total PBMCs and TC. However, OTR mRNA was greater in fresh cells compared with cultured cells with or without Con A. From these data, we conclude that OTR is expressed in bovine PBMCs and TC during the estrous cycle but OTR expression was dramatically reduced when cells were placed in culture. Furthermore, the expression of OTR in lymphocytes suggest that oxytocin