2008 First Annual Rocky Mountain Reproductive Sciences Symposium

University of Colorado Health Sciences Center

University of Wyoming — New Thinking

Colorado State University

University of Northern Colorado

University of Nebraska Lincoln

NM State University

Saturday, May 3
10:00 AM — 6:00 PM
228-230 Lory Student Center
Colorado State University
Fort Collins, Colorado

The First Annual Rocky Mountain Reproductive Sciences (RMRS) Symposium will be held on May 3, 2008 at the Colorado State University (CSU) Lory Student Center. The intent of this Symposium is to foster regional interests in the various aspects of reproductive sciences and to provide a forum for interaction and exchange of ideas. Each year there will be a Basic and Clinical Science Keynote Lecture on a given topic in reproduction, thereby providing a "Bench to Bedside" thematic focus. The 2008 RMRS Symposium will start with introductory remarks from Dr. Thomas (Tod) Hansen, Director of the Animal Reproduction and Biotechnology Laboratory, CSU, followed by Student Platform presentations, and two Keynote Lectures. The Keynote Lectures for this symposium were organized by Dr. Russ Anthony who holds academic appointments in the Department of Biomedical Sciences at CSU and in the Department of Pediatrics at the University of Colorado Health Sciences Center (UCHSC). Dr. Giacomo Meschia, MD, UCHSC will present "*Blood flow to the pregnant uterus: Its relation to fetal and placental oxygen consumption.*" Dr. Fred Battaglia, MD, UCHSC will present *"Clinical studies of perfusion and transport in human pregnancies."* The Symposium will conclude with a Poster Session, refreshments, and broadcast of the Kentucky Derby.

Scientists from CSU, UCHSC, University of Northern Colorado, University of Wyoming and the University of Nebraska-Lincoln and New Mexico State University, in addition to local MDs and DVMs, are participating. Twenty-nine abstracts have been submitted; eight have been selected for the Student Platform Session and the remaining twenty-one will be presented during the Poster Session.

Drs. Jerry Bouma (CSU), Jason Bruemmer (CSU), Russ Anthony (CSU-UCHSC) and Thomas Hansen (2008, Chair) serve on the 2008 RMRS Program Committee. For more information please visit: http://www.cvmbs.colostate.edu/bms/arbl/rmrs.htm.

Table of Contents

PROGRAM

9:45 am **Welcome and Introduction – Dr. Tod Hansen**

10:00 am – Noon **Student Platform Sessions – Jerry Bouma and Jason Bruemmer, Session Co-Chairs**

10:00-10:15 1. **Scott Purcell**, Colorado State University
Periattachment factor is required for conceptus development in sheep. S.H. Purcell, J.D. Cantlon, G.E. Seidel, R.V. Anthony.

10:15-10:30 2. **Rebecca Bott**, Colorado State University
Uterine vein infusions of interferon-tau act directly on the corpus luteum through inducing ISG15 expression. R.C. Bott, R.L. Ashley, LE. Henkes, J.E. Bruemmer, G.D. Niswender, T.E. Spencer, F.W. Bazer, R.V. Anthony, T.R. Hansen.

10:30-10:45 3. **Aida Ulloa**, Colorado State University
G-Protein coupled receptor-stimulated calcium entry in human myometrial cells is attenuated by knockdown of TRP-C4. A. Ulloa, M. Zhong, Y.-S. Kim, J. Cantlon, C. Clay, C.-Y. Ku, B.M. Sanborn.

10:45-11:00 4. **Christianne Magee**, Colorado State University
Evaluation of kisspeptin in the estrous mare. C. Magee, C.D. Foradori, J.E. Bruemmer, C. Corning, T.A. Farmerie, P.M. McCue, R.J. Handa, C.M. Clay.

11:00-11:15 5. **Jenny Powers**, Colorado State University
Effects of GnRH immunization on reproduction and behavior in Rocky Mountain elk. J.G. Powers, D.L. Baker, M.M. Conner, A.H. Lothridge, T.L. Davis, T.M. Nett.

11:15-11:30 6. **Amber Wolf**, Colorado State University
Membrane compartmentalization of luteinizing hormone receptors during receptor signaling and desensitization. A.L. Wolf, P.W. Winter, B.G. Barisas, D.A. Roess.

11:30-11:45 7. **Lucia Dohnal**, University of Colorado Health Sciences Center
Early gestation utero-placental hemodynamics in an ovine model of fetal growth restriction and desensitization. L. Dohnal, J.S. Barry, H.L. Galan, R.B. Wilkening, R.V. Anthony.

11:45-noon 8. **R.R. Cockrum**, University of Wyoming
Expression of appetite regulatory genes, receptors and hormones in obese dams and their fetuses. R.R. Cockrum, K.M. Cammack, K.J. Austin, S.P. Ford, B.W. Hess, G. E. Moss, B.M. Alexander.

Noon – 1:00 pm **Lunch** ($10.00 registration covers buffet lunch)

1:00 – 3:00 pm **Keynote Speakers – Russ Anthony, Session Chair**

1:00-2:00 **Giacomo Meschia**, MD, UCHSC
Blood flow to the pregnant uterus: Its relation to fetal and placental oxygen consumption

2:00-3:00 **Fred Battaglia**, MD, UCHSC
Clinical studies of perfusion and transport in human pregnancies

3:00 – 6:00 pm **Poster Session –** Posters, refreshments, cash bar and broadcast of Kentucky Derby

TESTICULAR BIOLOGY

9. Effects of vascular endothelial growth factor (VEGF) isoforms on rat testis composition and germ cell numbers
S.G. Kruse, R.G. Slattery, D.T. Clopton, A.S. Cupp

10. Altered expression of genes involved in testicular descent in testes from Alaska Sitka black-tailed deer
J.C. da Silveira, G.J. Bouma, M.E. Legare, R.P. Amann, D.N.R. Veeramachaneni

11. Effect of RU486 on development of testicular steroidogenesis and ram sexual behavior
P. Singh, K.J. Austin, V.A. Uthlaut, B.M. Alexander

REPRODUCTIVE ENDOCRINOLOGY AND SIGNALLING

12. Estradiol conjugated to bovine serum albumin (E2BSA) decreases secretion of LH without modifying secretion of GnRH in ovariectomized (OVX) ewes
J.A. Arreguin-Arevalo, E. Wagenmaker, A. Oakley, F. Karsch, T. Nett

13. Functional involvement of the plasma membrane PKA/AKAP interaction in signaling events in uterine smooth muscle
D. Murtazina, C.Y. Ku, M. Zhong, A. Ulloa and B.M. Sanborn

14. Involvement of signal-regulated calcium entry in store refilling in myometrial cells
D. Chung, C.-Y. Ku, B.M. Sanborn

15. Differential gene expression for IGF-I in endometrium of ewes fasted during the luteal phase of the estrus cycle
A.B. Breton, K.J. Austin, E.A. Van Kirk, G.E. Moss, B.M. Alexander

GAMETE BIOLOGY AND FUNCTION

16. Differences in resumption of oocyte maturation in young and old mares
L.F. Campos-Chillon, C.M. Clay, J.L. Altermatt, G.J. Bouma, E.M. Carnevale

17. The effect of angiotensin II on bovine oocyte nuclear maturation is mediated by PGE_2 and $PGF_{2\alpha}$
M.H. Barreta, J.F.C. Oliveira, A.Q. Antoniazzi, R. Ferreira, L.R. Sandri, P.B.D. Gonçalves

PREGNANCY AND DEVELOPMENT

18. Examination of periattachment factor (PRR15) in the human placenta
S.H. Purcell, J.D. Cantlon, V.D. Winn, R.V. Anthony

19. Isg15 is a molecular sentinel that functions to assist mothers in coping with environmental stressors imposed on pregnancy
R.L. Ashley, L.E. Henkes, R.V. Anthony, K.C. McBroom, J.K. Pru, T.R. Hansen

20. Implications for the type-I interferon pathway in intrauterine growth restriction
M.L. Shoemaker, K.J. Austin, H. Van Campen, H. Bielefeldt-Ohmann, L. Rempel, N.P. Smirnova, H. Han, D.J. Montgomery, R.V. Anthony, A. Van Olphen, J.A. Clapper, T.R. Hansen

21. The SUMO pathway in relation to fetal ovarian development
B. Fromme, G.J. Bouma

22. Genetic control of fetal ovarian development
E. Loomis, G.J. Bouma

23. MicroRNA's in ovine reproduction and development
K.J. Torley, R.V. Anthony, G.J. Bouma

24. Impact of prenatal hypoxia on offspring growth and cardiac development
J. Rhodes, K.C. McBroom, A.J. Chicco, R.V. Anthony

25. Effects of overfeeding adolescent ewe lambs on progeny growth
G.J. Eckerle, R.V. Anthony, R.K. Peel

ASSISTED REPRODUCTION

26. Cryopreservation of large equine embryos
J.P. Barfield, P.M. McCue, E.L. Squires, G.E. Seidel, Jr.

27. Efficacy of medroxyprogesterone acetate in suppression of estrous behavior and follicular activity in cycling mares
E.K. Gee, P.M. McCue, C.A. DeLuca, J.L. Stylski

28. Effect of short-term exposure of hydrogen peroxide on stallion sperm motility and DNA fragmentation
L. Hovda, P.D. Burns, B. Curtis, A. Albers, L. Herickhoff

STUDENT PLATFORM SESSION ABSTRACTS

1. Uterine vein infusions of interferon-tau act directly on the corpus luteum through inducing ISG15 expression

Rebecca C. Bott, Ryan L. Ashley, Luiz E. Henkes, Jason E. Bruemmer, Gordon D. Niswender, Thomas E. Spencer, Fuller W. Bazer, Russell V. Anthony and Thomas R. Hansen

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

The ruminant conceptus releases interferon-τ (IFN) that acts in a paracrine mechanism on the endometrium to alter release of prostaglandin F2alpha (PGF), indirectly protecting the corpus luteum (CL). Recently, we have shown that IFN is released into the uterine vein and may have systemic endocrine action on the corpus luteum. Therefore, we hypothesized that interferon-stimulated genes (ISGs) would prevent the anti-steroidogenic actions of PGF on the CL. To test this hypothesis, three specific aims were developed using western range ewes of reproductive age that were assigned to treatment on Day 9-11 of the estrous cycle. The first aim examined if an injection of IFN (200 ng) into the ovarian artery (n = 2 ewes/treatment) would elicit ISGs in the CL when compared to controls (BSA injection). Corpora lutea were collected 12 h after injection of IFN. Injection of 200 ng IFN into the ovarian artery tended to increase ISG15 in CL compared to BSA controls ($P<0.06$). The second aim examined if uterine vein infusion of IFN increase expression of ISG15 in the CL of non-pregnant ewes. Alzet mini-osmotic pumps (200 µl volume) were surgically implanted in Day 9-11 ewes to deliver 200 ng IFN (n=6) or BSA (n=5) into the uterine vein over a 24 h period (~ 8 µl/h) and then CL were removed. ISG15 mRNA increased 94-fold over BSA controls ($P<0.02$) and 12-fold over mRNA concentrations following ovarian artery injection of IFN ($P<0.01$). This level of induction of ISG15 was similar in ipsilateral and contralateral CL to uterine vein infusion of IFN, indicating that IFN had a systemic action although we can not rule out a possible local uterine vein-ovarian artery countercurrent action at this time. Western blot analysis confirmed that ISG15 and conjugation to proteins (isgylation) increased in the CL in response to uterine vein infusion of IFN. The third aim of the study was to determine if IFN had direct anti-luteolytic effects on the CL. Osmotic pumps containing either 200ng IFN (n=6) or BSA (n=5) were surgically inserted. Twelve hours after surgery three ewes from each treatment group were injected with a sub-luteolytic dose of PGF (5mg) to determine if IFN prevented a decline in progesterone production. Blood samples were collected every other hour for an additional 12 h, at which time CL were collected. Progesterone concentrations did not differ 12 hours post treatment in animals treated with IFN vs BSA. Progesterone did decrease with BSA plus PGF treatment compared to BSA alone ($P<0.04$). There was a tendency for lower progesterone in animals treated with BSA plus PGF compared to those receiving IFN alone ($P=0.053$). Interestingly, there was no difference in progesterone when comparing IFN groups with or without PGF ($P=0.2$). ISG15 mRNA expression in CL of IFN treated ewes that were challenged with PGF tended ($P <0.08$) to be decreased compared to IFN treated animals without PGF. Luteal ISG15 was elevated in animals receiving IFN plus PGF compared to those that had received BSA plus PGF. Isgylation also increased in CL from ewes treated with IFN plus PGF compared to BSA with or without PGF ($P <0.01$). It is concluded that IFN from the uterine vein acts to induce ISG responses in the CL

that may prevent the anti-steroidogenic actions of PGF. This research was funded by the National Research Initiative Competitive Grant no. 2006-35203-17258, USDA Cooperative State Research, Education, and Extension Service and the Colorado Agriculture Experiment Station.

2. **Periattachment factor is required for conceptus development in sheep**

S.H. Purcell, J.D. Cantlon, G.E. Seidel and R.V. Anthony

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Periattachment factor (PF) is a nuclear protein, believed to be acting as a coregulator of transcription, that is expressed in the elongating ruminant conceptus. Previously, we determined that sheep PF (oPF) mRNA becomes detectable at day 13, before increasing to peak concentrations at day 15-16, and then declining through day 30 of gestation. This pattern of expression corresponds with the time of rapid conceptus elongation and attachment to the endometrium. Furthermore, we have immunolocalized oPF to the nucleus of day 15 conceptus trophectoderm and trophendoderm cells. Therefore, it was our hypothesis that oPF acts in regulating trophoblast proliferation and/or migration. Our current objective was to ablate oPF expression in sheep conceptuses by *in vivo* RNA interference to determine its role and importance during conceptus development. Superovulated ewes were naturally mated and embryos were collected by hysterectomy and uterine flushing on day 8 post mating. Upon collection, zona-free blastocysts were infected with a lentivirus (pLL3.7-shRNA-3) expressing a short-hairpin (sh) RNA designed to target PF mRNA degradation, with a lentivirus expressing a shRNA which contained three mismatched nucleotides (pLL3.7-shRNA-MM) or with a lentivirus (pLL3.7) expressing no shRNA. Following a 6 hour infection period, the blastocysts were surgically-transferred into the uterus of synchronized recipient ewes. On day 15 of gestation, relative to the time of blastocyst donor-ewe mating, recipient ewes were euthanized and conceptuses were collected. Sixteen out of the 17 pLL3.7 infected conceptuses recovered had elongated normally by day 15. Seventeen out of the 19 pLL3.7-shRNA-MM infected conceptuses had elongated normally by day 15, whereas none of the conceptuses infected with pLL3.7-shRNA-3 elongated, and most of these underwent embryonic demise. These results indicate that oPF is required for conceptus elongation and survival, and it will be interesting to determine for which genes that oPF acts as a transcriptional coregulator, as well as determining how the transcription and activation of oPF is regulated. This project was supported by National Research Initiative Competitive Grant no. 2005-35203-15885 from the USDA Cooperative State Research, Education, and Extension Service.

3. **G-Protein coupled receptor-stimulated calcium entry in human myometrial cells is attenuated by knockdown of TRPC4**

Aida Ulloa[1,2], Miao Zhong[1], Yoon-Sun Kim[1], Jeremy Cantlon[1], Colin Clay[1], Chun-Ying Ku[1], and Barbara M. Sanborn[1,2]

Department of Biomedical Sciences[1] and Cell and Molecular Biology Program[2], Colorado State University, Fort Collins, CO

Canonical transient receptor potential (TRPC) proteins may play a role in regulating changes in intracellular calcium ([Ca2+]i) and thus contractility. Human myometrium expresses TRPC4, TRPC1 and TRPC6 mRNAs in greatest abundance relative to other TRPCs. Specific contributions of TRPC4 to signal-regulated calcium entry (SRCE) were assessed in PHM1 and primary human myometrial (UtSMC) cells using RNA interference (RNAi). Four shRNAs elicited 60-90% knockdown of a TRPC4 target construct in the psiCHECK-2 reporter system and TC4sh1 was selected for construction of an adenoviral vector that infected PHM1 cells with 90% efficiency. Adenovirus expressing TC4sh1 induced both mRNA and protein TRPC4 knockdown, whereas infection with empty vector had no effect and expression of other TRPCs were unchanged. PHM1 cells were treated with 100nM oxytocin to examine effects on receptor-stimulated Ca entry, 100nM thapsigargin for store-operated Ca entry, and 100mM OAG for diacylglycerol-stimulated Ca entry, measured as increases in [Ca2+]i using Fura-2. Cells infected with vector expressing TC4sh1 exhibited attenuated oxytocin-mediated SRCE, but there was no effect on thapsigargin- or OAG-stimulated calcium entry. Furthermore, the SRCE elicited by two additional G-protein coupled receptor (GPCR) stimulants, ATP and PGF2□, were also attenuated by TC4sh1 vector. Interestingly, TRPC4a overexpression induces no significant changes in response to any of the SRCE stimulants tested. Similar results were obtained in primary UtSMC. These data show that adenoviral constructs expressing TC4sh1 induce a specific knockdown in TRPC4 mRNA and protein expression. In myometrial cells, such knockdown specifically induces a decrease in GPCR-stimulated increases in [Ca2+]i, but not in thapsigargin or OAG-stimulated increases in [Ca2+]i. These data indicate that Ca entry in response to cellular signals can be specifically affected by attenuation of a specific TRPC in myometrial smooth muscle cells. Supported by NIHF3-HD051037, NIHT32-HD0703, March of Dimes #6-FY05-77 and HD38970.

4. **Evaluation of kisspeptin in the estrous mare**

C Magee, CD Foradori, JE Bruemmer, C Corning, TA Farmerie, PM McCue, RJ Handa, and CM Clay

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Kisspeptin and its receptor, GPR54, are crucial elements in the onset of pubertal development and may play a role in seasonal estrus and induction of ovulation via a mechanism of signaling GnRH release at the hypothalamus. The purpose of this series of experiments was to evaluate the role of kisspeptide and GPR54 in the seasonally estrous mare. To elucidate a physiologic role for kisspeptide in the mare, LH response profiles to kisspeptide administration using three doses of rat decapeptide (1.0 µg, 0.5 mg, 1.0 mg KiSS-10[a], iv) were compared to that of native GnRH (25 µg LHRH[b], iv) in diestrus mares (n=12 per treatment). Our equine LH (eLH) radioimmunoassay data from dose response trials suggest that the mare has a threshold response to iv rat KiSS-10 administration between 1.0 µg and 0.5 mg ($P < 0.0001$, ANOVA), such that iv 0.5 mg and 1.0 mg of KiSS-10 elicits a mean eLH fold response similar to that of 25 µg LHRH ($P=0.56$, t-test). To determine if a single intravenous injection of the decapeptide formulation was sufficient to induce ovulation in the estrous mare, 1.0 mg KiSS-10 was compared to a known ovulation inducing agent (2,500 IU hCG[c], iv), and a negative control (1.0 ml saline, iv). No significant difference ($P > 0.3$, t-test) was observed in the time (mean hours ± standard error) from treatment to ovulation in the saline (n=11, 64.5 ± 9.5 hr) and kisspeptide (n=11, 69.1 ± 10.1 hr) mares. When compared with kisspeptide treated mares, hCG treated mares consistently ovulated within a predicted and significantly shorter (n=12, 41.4 ± 0.5 hr) period of time ($P < 0.005$, t-test). Using dual-labeling immunohistochemistry, KiSS fiber and GnRH neuron contacts were identified in the pre-optic area (POA), anterior hypothalamic area (AHA), and medial basal hypothalamus (MBH) of six diestrus mares. Contacts were quantified as a percent of immunoreactive GnRH neurons (GnRH-ir) with immunoreactive KiSS (KiSS-ir) fibers in close association. The mean percent of GnRH-ir neurons with KiSS-ir fiber contact for each region was not significantly ($P > 0.12$, ANOVA) different between the examined areas (n= number of mares with intact regions, mean percent contacts ± standard error; POA: n=3, 42.3 ± 10.7; AHA: n=3, 15.3 ± 8.7; MBH: n=6, 31.8 ± 5.1). The total percentage of contacts between GnRH-ir neurons and KiSS-ir fibers was 33.7%. In conclusion, we have demonstrated that the estrous mare can respond to exogenous administration of kisspeptide by releasing LH. Upon further investigation of the decapeptide and its receptor, a dose regimen of kisspeptide sufficient to induce ovulation will be determined. Evaluation of the role of kisspeptin in the hypothalamic-pituitary-gonadal axis and differential expression of KiSS-ir and GnRH-ir fibers with season or sex steroid administration would enable future application of kisspeptide for ovulation induction in the estrous mare or management of seasonal transition. Funded by the Preservation for Equine Genetics Foundation at Colorado State University. Keywords: kisspeptin, equine, gonadotropin, ovulation, radioimmunoassay, immunocytochemistry

5. **Effects of GnRH immunization on reproduction and behavior in rocky mountain elk**

Jenny G. Powers[1], Dan L. Baker[1], Mary M. Conner[2], Anneke H. Lothridge[1], Tracy L. Davis[1,3], Terry M. Nett[1]

[1]Colorado State University, Animal Reproduction and Biotechnology Laboratory, Fort Collins, CO. [2] Utah State University, Department of Wildland Resources, Logan, UT. [3]University of Idaho, Department of Animal and Veterinary Science, Moscow, ID

There is an increasing need in protected environments, for non-lethal methods of managing overabundant wild ungulates. Immunocontraception using gonadotropin releasing hormone (GnRH) vaccination is one approach. This study evaluated the safety and efficacy of GnRH immunization in captive female elk.

Seventeen captive pregnant female elk, in mid gestation, were randomly assigned to either a GnRH vaccine treatment group (n = 10) or sham vaccine control group (n = 7). Treated females were hand injected with 1500μg GnRH-Blue Protein/ AdjuVac™ vaccine (GonaCon™). Control females were similarly immunized with Blue Protein/ AdjuVac™ alone. Blood was collected throughout gestation to measure serum GnRH antibody and progesterone concentrations. Calf birth rates, survival rates, and neonatal growth rates were compared between groups. Females were evaluated for evidence of systemic physiologic changes and injection site inflammation. During the 2006 breeding season, intensive reproductive behavior observations were recorded and assessed for differences in general herding, pre-copulatory, and copulatory behaviors. Pregnancy diagnoses were completed in January 2007 and 2008. During and after the 2006 breeding season, ultrasound examination was used to measure ovarian structures.

We found no differences in serum progesterone concentrations and calving rates between groups. Likewise, there were no differences in neonatal survival or growth rates. There was no indication of systemic changes in organ function 3 months post-vaccination. However, 3 animals developed severe purulent abscesses 15-20 months post-vaccination. Ultrasound examination of ovarian structures, between December 2006 and April 2007, revealed small to medium size follicles in treated animals, where as ovaries in control elk contained a wide range of follicular sizes including apparently dominant follicles as well as occasional corpora lutea.

Seven of 7 (100%) of control females became pregnant 9 and 21 months post vaccination (95% C.I., 0.708-1.0). One of 10 (10%) treated cows was pregnant in January 2007 (95% C.I., 0.011-0.381) and 2 of 8 (25%) were pregnant in January 2008 (95% C.I., 0.056-0.592). Anti-GnRH antibody titer did not consistently correlate with pregnancy status. Reproductive behavior measurements, revealed no differences in general breeding/herding behaviors. Male pre-copulatory behaviors directed towards treated females were nearly twice those directed towards control females (P = 0.07), while differences in female pre-copulatory behavior rates were not statistically different (P = 0.5). Copulatory behaviors were too infrequent to detect a difference between treatment groups.

We conclude that active immunization of female elk during mid-gestation using a single dose of GonaCon does not affect success of the current pregnancy, significantly reduces pregnancy rates the following 2 breeding seasons, and induces minimal changes in social breeding behaviors; however, individual breeding behaviors may be altered. Ongoing studies are investigating the duration of vaccine efficacy in treated females, prevalence of vaccine related injection site reactions, and potential long-term reproductive effects in developing calves born to treated females. Funded by Morris Animal Foundation (D05ZO-303) to J.G. Powers. Keywords: GnRH vaccine, immunocontraception, elk, fertility control.

6. **Membrane compartmentalization of luteinizing hormone receptors during receptor signaling and desensitization**

Amber L. Wolf[1], Peter W. Winter[2], B. George Barisas[3] and Deborah A. Roess[1]

[1]Department of Biomedical Sciences, [2]Cell and Molecular Biology Program and [3]Department of Chemistry, Colorado State University, Fort Collins, CO

Luteinizing hormone receptors (LHR) are G protein-coupled membrane proteins with important functions in reproduction. We have recently shown that rat and human LHR, upon binding of hCG, translocate into plasma membrane compartments of low buoyant density (rafts). This translocation depends upon formation of a functional hormone-receptor complex. Moreover, LHR remain in rafts for long times while receptors are desensitized to further hormone signals. To explore ligand-receptor structures necessary for raft translocation, we have used single particle tracking methods to monitor the lateral diffusion of individual LH receptors and the size of plasma membrane compartments accessed by the receptor on viable cells. Following treatment of cells with 0.01nM hCG, LHR are distributed in two groups. Approximately 65% of the receptors are confined in small compartments with a diameter of 69 ± 38 nm while the remaining receptors exhibit unconfined lateral diffusion in large 230 ± 79 nm compartments typical of untreated LHR. Palmitoylation of the LHR C-terminus appears to be important for raft localization and for confinement in small membrane compartments. LHR mutants lacking potential palmitoylation sites at position 621 and 622 in the C-terminus do not translocate into membrane rafts. These receptors remain in large compartments and their rapid diffusion coefficients are not affected by hCG treatment. The cytoskeleton also appears to play a role in restricting hormone-treated receptors within small compartments: LHR on cells treated with the microfilament disruptor cytochalasin D exhibit fast lateral diffusion within large compartments both before and after exposure to 100 nM hCG. Possible membrane models to explain this behavior include compartmentalization by cytoskeletally-anchored proteins that serve as protein fences. The role of small compartments in receptor desensitization will also be discussed as will the use of quantum dots for single particle tracking of individual receptors confined by compartments defined by fluorescent actin filaments. This work was supported, in part, by CHE-0628260 (N.S.F.), AG030230 (N.I.H.) and RR023156 (N.I.H.). Keywords: luteinizing hormone, LH receptor, receptor desensitization

7. **Early gestation utero-placental hemodynamics in an ovine model of fetal growth restriction**

Lucia Dohnal[1], James S Barry[1], Henry L Galan[1], Randall B Wilkening[1] and Russell V Anthony[1]

[1]Perinatal Research Center, University of Colorado Health Sciences Center, Aurora, CO, 80045.

Objective: Fetal growth restricted (FGR) pregnancies, during late gestation, exhibit altered placental hemodynamics, and reduced capacity for O_2 and nutrient transfer. It was our objective to examine utero-placental hemodynamics and O_2 uptake during early gestation in an ovine model of FGR. **Methods**: Singleton-bearing ewes were instrumented with uterine artery flow probes, uterine venous and femoral artery catheters before being placed into a high-ambient temperature (FGR; n=9) or normothermic (CON; n=6) environment at 40 days of gestation (dGA). Maternal arterial and venous blood, uterine artery flow, heart rate, arterial pressure and respiration rate was collected until 55 dGA, at which time umbilical venous blood, fetal weight, placental weight and tissue were harvested. Data reported here were analyzed by Students T-test. **Results:** Maternal respiration rate (153.3±5.4 vs 91.6±9.2 breaths/min) and arterial PO_2 (91.0±1.2 vs 85.8±1.0 mmHg) were increased ($P \leq 0.01$), whereas maternal heart rate (74.3±1.83 vs 88.8±0.03 beats/min), blood pressure (83.7±1.3 vs 94.0±3.2 mmHg) and arterial PCO_2 (30.2±1.1 vs 35.6±0.9 mmHg) were reduced ($P \leq 0.01$) in FGR pregnancies. At 55 dGA, fetal weight was not different ($P \geq 0.10$), but placental (total placentome) weight (85.5±15.1 vs 146.7±27.0 g) was reduced ($P \leq 0.05$) in FGR pregnancies. While uterine artery (pregnant horn) flow (115.5±13.4 vs 178.4±54.3 ml/min) tended ($P=0.064$) to be reduced in FGR pregnancies, relative uterine artery flow (4.6±0.5 vs 5.8±0.5 ml/min/g fetus; 157.6±25.8 vs 128.1±17.3 ml/min/100g placenta) was not different ($P \geq 0.10$). Uterine O_2 uptake (mmol/min), relative uterine O_2 uptake (ml/min/g fetus or ml/min/100g placenta) and uterine O_2 extraction (%) were not different ($P \geq 0.10$) between FGR and CON pregnancies. At 55 dGA, umbilical vein PO_2 (mmHg), O_2 content (mM) and O_2 capacity (mM) were also not different between FGR and CON pregnancies. **Conclusions:** Reduction in absolute uterine artery flow (ml/min) did not impact utero-placental O_2 uptake or transfer to the umbilical vein, and may have resulted from reductions in maternal cardiac output. Relative uterine artery flow was not reduced, suggesting that uterine blood flow and delivery of O_2 to the conceptus does not mediate the placental growth restriction initiated during early gestation which leads to severe FGR in these pregnancies.
Supported by NIH R01 HD43089 to R.V.A.

8. **Increased expression of phospho-mTOR, phospho-p70, phospho-AKT and phospho-ERK in an ovine model of fetal growth restriction**

Juan A Arroyo, Brad Ziebell, and Henry L Galan

Obstetrics and Gynecology, University of Colorado and Health Sciences Center, Denver, CO, USA

OBJECTIVE: Both phosphorylated (p) mTOR and p70 are known to be involved in protein synthesis and are regulated by physiological conditions such as fetal growth restriction (FGR). In a hyperthermic (HT) ovine model of FGR we hypothesize that mTOR, p70, 4EBP1, ERK and AKT will be phosphorylated (activated) in the placentae of 130 age (dGA) animals.
STUDY DESIGN: 4 ewes were exposed to HT conditions for 55 or 80 days to induce IUGR and 4 were placed in ambient conditions at each gestational age studied. At necropsy (95 dGA; mid gestation or 130 dGA; near-term), placentomes were separated into the maternal (caruncle) and fetal (cotyledon) components and frozen for Western blot analysis with antibodies against (p) mTOR, mTOR, (p) p70, p70, (p) 4EBP1, 4EBP1, (p) ERK, ERK, (p)AKT and AKT.
RESULTS: Compared to control animals**,** FGR animals had smaller fetuses (2914±201g v. 1718±433g; $p=0.03$) and smaller placentae (349±21g v. 169±22g; $p=0.03$) at 130 dGA. FGR cotyledon showed an increase in p-MTOR (1.8-fold; $p=0.01$), p-p70 (1.8-fold; $p<0.008$), p-ERK (1.4-fold; $p<0.008$) and p-AKT (2.6-fold; $p<0.02$). In contrast, caruncle (maternal) did not show any changes for the mTOR pathway.
CONCLUSION: In FGR ovine pregnancies, the fetal placental tissues (cotyledons) showed upregulation of the mTOR pathway for protein synthesis via phosphorylation of the p70 but not 4EBP1 while this was not seen in the maternal (caruncle) tissues. In addition neither the cotyledon or caruncle tissues at mid-gestation (95 dGA) showed changes in these endpoints, which is prior to the exponential fetal growth that starts at mid-gestation (Supported by NIH grant R01 HL071990-01A1).

POSTER SESSION ABSTRACTS

TESTICULAR BIOLOGY

9. Effects of vascular endothelial growth factor (VEGF) isoforms on rat testis composition and germ cell numbers

Shantille G Kruse, Racheal G Slattery, Debra T Clopton and Andrea S Cupp

Department of Animal Science, University of Nebraska-Lincoln, Lincoln NE 68583-0908

Vascular Endothelial Growth Factor (VEGF) is a paracrine growth factor responsible for blood vessel development (neovascularization) as well as endothelial cell migration in many organs including the developing gonad. Multiple isoforms of VEGF are generated from alternative splicing and two of these isoforms are: 1) VEGF164 and 2) VEGF164b. VEGF164b is an anti-angiogenic isoform, which inhibits VEGF164 mediated angiogenesis. In the testis, our laboratory has demonstrated that angiogenic isoforms create a chemoattractive gradient to entice endothelial cell migration from the adjacent mesonephros to allow for sex-specific blood vessel formation and development of seminiferous cords which enclose developing germ cells. We also determined that the receptor for VEGF, Kinase Domain Region (KDR) receptor was expressed in developing germ cells. The objectives of the current experiment were to determine the effect of VEGF isoforms on: 1) testis composition (seminiferous cord area versus interstitial area); and 2) Germ cell numbers. Male rat pups were injected on postnatal day 0 (P0), P1, and P2 with one of five treatments: VEGF 164 (0.5 mg, n= 6), VEGF 164b (0.5 mg; n=6), Anti-VEGF Axxxb (1 mg; n=6) IgG Control (1 mg; n=5), or PBS Control (0.5 mg; n=3). Pups were euthanized at day P8 and testis tissue was collected, fixed in bouins, embedded, and sectioned. Hematoxylin and eosin sections from each group were utilized to determine seminiferous cord area and interstitial area with Scion image. The males treated with VEGF164b had significantly less seminiferous cord area (1309477 $\pm$ 26870 vs 1375279 pixels/area), and more interstitial area (332123 $\pm$ 26870 vs 266321 pixels/area) compared to PBS control ($P = 0.05$). However, germ cell numbers per area were not significantly different ($P > 0.05$). Therefore, VEGF164b may reduce seminiferous cord area through alterations in germ cell stage or maturation which are not reflected in germ cell numbers. Further analysis of germ cell viability will give us more information about the effects of VEGF164b on spermatogenesis.

Keywords: VEGF, testis, germ cell, seminiferous cord area

10, **Altered expression of genes involved in testicular descent in testes from Alaska Sitka black-tailed deer**

Juliano C. da Silveira[1], Gerrit J. Bouma[1], Marie E. Legare[2], Rupert P. Amann[1], D.N. Rao Veeramachaneni[1]

[1]Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, [2]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523

We found that 74% of male Sitka black-tailed deer (SBTD), hunted during 1999-2007 on the Aliulik Peninsula (major affected area; southern Kodiak Island), were without scrotal testes (bilateral cryptorchid; BCO). Most male SBTD on northern Kodiak and Afognak Islands were unaffected and had scrotal testes (non-cryptorchid; NCO). Analyses of mitochondrial and microsatellite DNA revealed that SBTD on the Aliulik Peninsula are not genetically isolated (Anim Conserv, In Press), ruling out a genetic (inbreeding) cause for BCO. We reasoned that if an estrogenic endocrine disruptor was affecting testicular descent in male fetuses gestating on the Aliulik Peninsula, this might be evidenced by examining gene expression in adult testes. Testicular tissue from hunter-killed deer was placed into RNA*later,* in the field during fall 2005-2007. Primers for transcripts in SBTD were designed from sequences in GenBank, and RT-PCR products were sequenced to confirm specificity. Real time RT-PCR analyses examined expression of 4 genes important for testis descent [*Insl3, Lgr8 (Great), ERα,* and *AR*] in testes from SBTD residing on Afognak Island (n = 6 NCO) or the affected area (n = 17 NCO, 40 BCO). Expression of all 4 genes was higher in NCO from the affected area than NCO in unaffected area; *Insl3* ~19 fold (P = 0.10), *Great* ~11 fold (P = 0.12), *AR* ~5 fold (P = 0.20), and *ERα* ~ 4 fold (P<0.02). In the affected area, expression of *Great* and *ERα* was ~14 and ~8 fold, respectively, higher in BCO compared to NCO testes (P-values of 0.09 and 0.12, respectively). We conclude that testes in adult SBTD in the affected area displayed greater expression of certain genes than testes from deer in an unaffected area. Cause of this difference is unknown, but might be localized exposure to endocrine agents in browse, water, or atmosphere. Funded by NIEHS R21 ES-014607-01A1 and ARBL Morphological Services.

Keywords: Cryptorchidism, endocrine disruption, testicular descent genes

11. **Effect of RU486 on development of testicular steroidogenesis and ram sexual behavior**

Priyanka Singh, Kathryn J. Austin, Valerie A. Uthlaut, Brenda M. Alexander

Department of Animal Science, University of Wyoming, Laramie, WY

Progesterone influences the development and expression of male sexual behavior in rodents and may be important for the expression of sexual behavior in rams. Masculinization and/or defeminization of the central nervous system in sheep occurs between d 60 and 70 of pregnancy. A second phase of testosterone-responsive sexual development occurs at 6 to 8 weeks of age in ram lambs. To determine if progesterone may influence adult sexual behavior during this developmental period, twin born male lambs (n = 10) were used in this study. One of each twin was treated with 10 mg of the progesterone receptor antagonist RU486 (n = 5), and his co-sibling was treated with an equal volume of control solution (n = 5) twice daily from 4 to 8 wk of age. Sexual behavior and serum concentrations of testosterone were evaluated at 9 mo of age. Lamb BW were similar (P = 0.4) at the end of RU486 treatment (8 wk of age), and did not differ (P = 0.15) during behavior testing at 9 mo of age. Change of weight, however, tended (P = 0.08) to be greater in RU486 treated ram lambs. Testes were measured by a scrotal tape at the end of the treatment period and during behavior testing at 9 mo of age. Although testes size ($P \geq 0.4$) and BW (P = 0.15) did not differ, serum concentrations of testosterone were decreased (P = 0.06) in rams treated with RU486. Sexual behavior was evaluated for 30 min at three different times by placing the rams with two estrous ewes. Behavior was classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick, vocalization, and nudge) and consummatory (mount attempt, mount, and ejaculation) behavior. Expression of investigatory behavior was decreased (P = 0.03) at the first exposure to estrous ewes in RU486 treated rams, but not ($P \geq 0.4$) in subsequent tests. Consummatory behavior was similar ($P \geq 0.24$) among treatment groups at all observations. Blocking the progesterone receptor at 6 – 8 wk of age may influence steroidogenesis in the yearling ram, but an influence on the expression of sexual behavior remains to be determined.

Keywords: Progesterone, RU486

POSTER SESSION ABSTRACTS

REPRODUCTIVE ENDOCRINOLOGY AND SIGNALLING

12. Estradiol conjugated to bovine serum albumin (E2BSA) decreases secretion of LH without modifying secretion of GnRH in ovariectomized (OVX) ewes

J Alejandro Arreguin-Arevalo[1], Elizabeth Wagenmaker[2], Amy Oakley[2], Fred Karsch[2], Terry Nett[1]

Colorado State University, Fort Collins, CO[1]; University of Michigan, Ann Arbor, MI[2]

We previously demonstrated that free estradiol (E2) or E2 conjugated to BSA (E2BSA) induced a rapid decrease in secretion of LH (luteinizing hormone) in OVX ewes and that this effect could be recapitulated in primary cultures of ovine pituitary cells, suggesting a direct action on the pituitary. Here we tested the possibility that the decrease in LH secretion induced by E2BSA could also be mediated by changes in the pulsatile pattern of GnRH release. OVX ewes (n = 5) were fitted with an apparatus for collection of pituitary portal blood. Approximately 2 weeks after surgery pituitary portal and jugular blood samples were collected through a remote automated sampling system at 10-min intervals during 4 h before and 4 h after administration of E2BSA (12 mg IM). Plasma concentrations of LH and GnRH were assayed by RIA. In 4 of 5 ewes, LH pulses were not detected within 68 ± 10 min after E2BSA. In the remaining ewe, LH decreased steadily during the post-E2BSA period. Compared to pre-treatment period, E2BSA decreased mean plasma concentrations of LH ($P < 0.02$; 7.2 ± 0.81 vs. 5.7 ± 0.58 ng/ml) and the number of pulses of LH ($P < 0.01$; 6.0 ± 0.32 vs. 3.0 ± 0.45 pulses); however, no changes were detected in the amplitude of pulses of LH (2.36 ± 0.41 vs. 1.68 ± 0.48 ng/ml). In contrast to LH, E2BSA did not alter the number of pulses of GnRH (8.4 ± 0.51 vs. 7.2 ± 0.40 pulses), or the amplitude of pulses of GnRH (0.47 ± 0.12 vs. 0.45 ± 0.12 pg/min). We conclude that the decrease in LH secretion induced by E2BSA is mediated by a direct action on the pituitary gland by decreasing the responsiveness of the pituitary to GnRH. The apparent lack of effect of E2BSA on the secretory pattern of GnRH supports the idea that E2 conjugated to BSA does not gain access to the brain to have a hypothalamic effect. Supported by USDA-NRI grant 2005-35203-15376 and NIH-HD grant 30773.

Keywords: GnRH, LH, E2BSA, ewes

13. **Functional involvement of the plasma membrane PKA/AKAP interaction in signaling events in uterine smooth muscle**

D. Murtazina, C.Y. Ku, M. Zhong, A. Ulloa and B.M. Sanborn

Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523

In rat uterine smooth muscle (myometrium), a fall in plasma membrane protein kinase A (PKA) without a fall in AKAP150 is associated with loss of the ability of cAMP to inhibit contractant (oxytocin)-stimulated phospholipase C activation (Dodge et al, Mol. Endo 12: 1977, 1999). The effect of cAMP was reversed by SHt31, an AKAP interaction inhibitor. Progesterone treatment prolonged rat pregnancy and attenuated the decline in myometrial plasma membrane PKA (Ku et al, BOR 67:605, 2002). The antiprogestins RU486 and onapristone induce a fall in rat myometrial plasma membrane PKA with no change in membrane AKAP150 at mid-pregnancy. In the latter case, the fall in membrane PKA accompanies the onset of electrical and contractile activity prior to the onset of labor (R. Garfield). Phospholipase Cβ3-Ser^{1105} in the oxytocin signaling cascade is a PKA target. Phosphorylation of PLC β3-Ser^{1105} occurs in myometrial cells in response to uterine relaxants and is attenuated by PKA inhibitors. An AKAP5 shRNA construct produces 90% suppression of a psiCHECK-2 luciferase reporter in AD293 cells. Because myometrial cells are difficult to transfect, we inserted this construct into an adenoviral vector, achieving ~100% infection. To date, AKAP5 mRNA and protein knockdown have ranged 65% and 55%, respectively. Phenotype is being explored. These data point to a role for progesterone in regulating the myometrial plasma membrane PKA/AKAP interaction and to the potential importance of this interaction in negative crosstalk between relaxant and contractant pathways in the myometrium during pregnancy. Supported by HD09618.

14. **Involvement of signal-regulated calcium entry in store refilling in myometrial cells**

Daesuk Chung, Chun-Ying Ku, and Barbara M. Sanborn

Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Increases in intracellular Ca ([Ca2+]i) regulate contraction in uterine smooth muscle and different types of Ca channels contribute to the increase in [Ca2+]i. The purpose of this study was to determine if signal-regulated Ca entry (SRCE), which increases [Ca2+]i, plays a role in endoplasmic reticulum (ER) Ca refilling. The simultaneous measurement of changes in Ca, in cytoplasm and ER was accomplished using Fura-2 to measure changes in [Ca2+]i and Mag-fluo-4 to measure changes in ER Ca ([Ca2+]L). Oxytocin (100 nM) elicited a transient increase in [Ca2+]i in PHM1 and a sustained decrease in ER Ca in the absence of extracellular Ca. Thapsigargin (100 nM), which irreversibly inhibits Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA), increased [Ca2+]i and produced a greater Ca store depletion than oxytocin. The addition of 1 mM extracellular Ca after thapsigargin resulted in an increase in [Ca2+]i but only a small increase in [Ca2+]L. CPA (10 μM), a reversible SERCA inhibitor, showed similar changes in [Ca2+]i and [Ca2+]L except that wash-out of CPA before the addition of 1 mM extracellular Ca resulted in complete ER store refilling. These data are consistent with the premise that Fura-2 and Mag-fluo-4 are simultaneously measuring changes in [Ca2+]i and [Ca2+]L, respectively. We then used Ca channel inhibitors to assess the contribution of different types of Ca channels to ER store refilling. Oxytocin-induced SRCE and ER refilling were not inhibited by nifedipine, a L-type channel blocker. Mibefradil, a T-type channel blocker, also did not affect oxytocin-induced SRCE and ER refilling. Two inhibitors for SRCE (SKF96365 or gadolinium), inhibited oxytocin-induced SRCE and ER refilling in a concentration-dependent manner. These data suggest that there is a close correlation between the oxytocin-induced increase of [Ca2+]i and ER Ca depletion and between SRCE and ER refilling. The data also provide evidence for a possible role for Ca entry through SRCE/receptor-operated channels in myometrial ER store refilling. Supported by HD38970.

Keywords: signal-regulated calcium entry, store refilling, myometrial cells

15. **Differential gene expression for IGF-I in endometrium of ewes fasted during the luteal phase of the estrus cycle**

Ashley B. Breton, Kathleen J. Austin, Edward A. Van Kirk, Gary E. Moss, and Brenda M. Alexander

Department of Animal Science, University of Wyoming, Laramie, Wyoming

Short-term fasting during the luteal phase of the estrous cycle perturbs circulating concentrations of progesterone and estradiol causing a delay in the onset of the surge release of LH. Although ovulation rate is not affected by fasting in this model, numbers of lambs born are decreased. Fasting may affect fertility by decreasing ova quality and/or altering the endometrium/oviduct environment. The objective of this study was to elucidate differences in gene expression in the endometrium near the time of expected ovulation in fasted and fed ewes. Estrous cycles were synchronized using $PGF_2\alpha$. Control ewes were given ad libitum access to grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through d 11 of the estrous cycle (d 0 = first day of estrus). On d 12 all ewes were treated with $PGF_2\alpha$, and fasted ewes were returned to ad libitum feed. Endometrial tissue was collected from fasted and fed ewes during the expected periovulatory period (72 hr following $PGF_2\alpha$ and realimentation of fasted ewes). At 72 hr, ewes were euthanized and sections of endometrium were dissected and snap frozen for RNA analysis. Semi-qualitative Real Time PCR was used to examine gene expression within the endometrium of ewes which had ovulated. Data was analyzed using GLM procedures of SAS. Gene expression for IGF-I was up-regulated ($P < 0.01$) in the endometrium of fasted compared to fed ewes. Gene expression for IGF-II, IGFBP1, IGFBP3, IGFBP6, estrogen receptor α, estrogen receptor β, and progesterone receptor did not differ ($P \geq 0.4$) among fasted and fed ewes. Although estrogen can stimulate IGF-I synthesis, serum concentrations of estrogen did not differ ($P = 0.7$) between groups at 0, 24, 48, and 72 hours following realimentation. Fasting during the luteal phase of the estrous cycle preceding proestrus influences uterine expression of IGF-I which may alter peri-implantation embryo survival.

Keywords: Fasting, Endometrium, Sheep, IGF

POSTER SESSION ABSTRACTS

GAMETE BIOLOGY AND FUNCTION

16. Differences in resumption of oocyte maturation in young and old mares

L.F. Campos-Chillon, C.M. Clay, J.L. Altermatt, G.J. Bouma, and E.M. Carnevale

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523

The decline in fertility of aged mares is linked with declining oocyte quality. Oocyte viability is dependant on the ability of oocytes to remain in meiotic arrest until the initiation of maturation. We hypothesize that aging is associated with quantitative and temporal differences in meiotic arrest and resumption in oocytes, ultimately resulting in a dissociation of oocyte and follicular maturation. The objectives of this study were to determine temporal differences in the mRNA content of amphiregulin and epiregulin in granulosa cells; PDE4 in cumulus and granulosa cells; and PDE3A, GPR3, GDF9 and BMP15 in oocytes during in vivo maturation in young (3-12 yr) versus old (>20 yr) mares. Oocytes and follicular cells were collected by transvaginal follicular aspirations. Maturation was induced in estrous mares with a follicle > 30 mm by injection of 750 μg of recombinant equine LH. Aspirations were attempted at 0, 6, 9, and 12h after LH. Six oocytes and follicular cell samples from each age group and time point were collected and stored immediately after aspiration. Total RNA was isolated from single denuded oocytes and lysed cumulus and granulosa cells. A fraction of the total lysate was used to determine cell numbers from DNA copy number of the equine CGβ subunit gene. DsRED RNA was added to each RNA isolate to serve as an exogenous standard. Quantitative RT-PCR was performed from cDNA with equine primer pairs. Copy numbers were calculated with an intra assay standard curve of plasmid containing the specific gene and corrected with the exogenous DsRED RNA and cell number. For each gene mean mRNA copy number for each time point and each age group were compared by ANOVA and Tukey's hsd. Expression of PDE4D in cumulus cells was similar between young and old mares and time points. However, PDE4D peaked ($p<0.05$) at 6 h in granulosa cells from young, but not old mares. Amphiregulin expression in granulosa cells of young mares peaked ($p<0.05$) at 9 h and did not increase in the old mares. Epiregulin expression in granulosa cells peaked ($p<0.05$) at 9 h and 6h in young and old mares, respectively. The pattern of expression of PDE3A for oocytes of young and old mares was similar with an increase ($p<0.05$) at 9 h. There was an interaction ($p<0.05$) in the expression of GPR3 for age x time. Expression peaked at 9 and 6 h in young and old mares, respectively. Pattern of expression of GDF9 was similar between young and old mares except for a decrease ($p<0.05$) in expression in old mares at 9 h. There was an interaction ($p<0.05$) in the expression of BMP15 for age x time. Expression in young mares peaked at 9h while in old mares peaked at 6 h and decreased at 9h. These results suggest that key gene expression patterns involved in oocyte and follicular maturation cascades were asynchronous for young versus old mares and could explain some aspects of the age-associated decline in fertility.

17. **The effect of angiotensin II on bovine oocyte nuclear maturation is mediated by PGE_2 and $PGF_{2\alpha}$**

Marcos H Barreta[1], João Francisco C Oliveira[1], Alfredo Q Antoniazzi[1], Rogério Ferreira[1], Luciano R Sandri[1], Paulo Bayard D Gonçalves[1]

[1]Laboratório de Biotecnologia e Reprodução Animal, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

In mammals, it is well know that resumption of meiosis occurs after the preovulatory LH surge and results in germinal vesicle breakdown (GVBD), initiating the so-called oocyte maturation. However, the pathway by which this gonadotrophin acts is not completely clear. We have recently demonstrated that AngII plays an important role on the onset of ovulation in cattle, potentially acting as an intrafollicular LH mediator. We also observed that AngII prevents the inhibitory effect of follicular cells during bovine oocyte nuclear maturation *in vitro*. These results suggest that AngII plays a role in LH-induced resumption of meiosis in the bovine oocyte. The aims of this study were to verify the role of AngII in LH-induced meiosis resumption and test the hypothesis that prostaglandins E2 and F2α participates of AngII-induced meiosis resumption in bovine oocytes. In the first experiment, seven cows were superovulated with FSH and follicles larger than 12 mm in diameter were subjected to an intrafollicular injection of saralasin or saline. Follicles from the right ovary (n=17) where intrafollicular injected with saralasin (10□M) and follicles from the left ovary (n=17) were treated with saline (control group). A preovulatory LH surge was induced by im injection of a GnRH agonist (gonadorelin 100μg im) following the intrafollicular injections. Fifteen hours later, the animals were ovariectomized and the oocytes were recovered to evaluate the stage of meiotic maturation. All oocytes (n=12) were at germinal vesicle stage (GV) 15 hours after GnRH agonist injection in the saralasin group while in the control group (n=13) the oocytes were at the GVBD (30.8%) or Metaphase I (MI; 69.2%; $P<0.001$) stage. In the second experiment, oocytes were co-cultured with follicular hemisections during 15 hours, to evaluate the role of prostaglandins mediating the effect of AngII on meiotic resumption. The inhibitory effects caused by follicular cells on oocyte nuclear maturation was prevented by adding 100pM of AngII to the culture medium (26.6% MI without AngII vs. 77.5% MI with AngII; $P<0.001$). However, when a nonselective cyclooxygenase (COX) inhibitor (10μM of indomethacin) was present in the culture system with AngII and follicular hemisections, oocytes reached MI in a percentage (13.4%) significantly lower than without indomethacin ($P<0.001$). Furthermore, when 1μM of PGE_2 or $PGF_{2\alpha}$ was added to the co-culture system with follicular cells, oocyte nuclear maturation rate followed the same pattern as the high maturation rate observed in the presence of AngII (PGE_2 77.4%, $PGF_{2\alpha}$ 70.0% and AngII 75.0% of MI). The results of the present study demonstrate for the first time that the LH-induced meiosis resumption in bovine oocytes requests AngII. Using an *in vitro* co-culture system of the bovine oocytes and follicular hemisections was demonstrated that the AngII-induced meiosis resumption in bovine oocytes is dependent of the cyclooxygenase production by follicular cells. With this same *in vitro* system we did demonstrate that the prostaglandins E2 and F2α participate of the AngII-induced meiosis resumption in bovine oocytes. Supported by CAPES and CNPq, Brazil.

Keywords: angiotensin II, oocyte nuclear maturation, prostaglandins

POSTER SESSION ABSTRACTS

PREGNANCY AND DEVELOPMENT

18. Examination of periattachment factor (PRR15) in the human placenta

Scott H. Purcell, Jeremy D. Cantlon, Virginia D. Winn, Russell V. Anthony

Colorado State University, Fort Collins, CO; University of Colorado Health Sciences Center, Aurora, CO

Background: Periattachment factor (PF) is a nuclear protein first described in the bovine conceptus. Our research in sheep has shown PF mRNA concentration peaks when the conceptus is undergoing elongation and initial apposition to the endometrium, and that PF is a nuclear protein localized to the trophoblast. *In silico* analysis identified a human homolog, hPRR15. **Objective:** The objective of this experiment was to determine if PF was expressed in the human placenta, and to develop short-hairpin (sh) RNAs for hPF to begin investigating its function. **Materials and Methods:** Immunohistochemistry was performed on paraffin embedded first and second trimester human placental samples. Placental sections were immuno-stained using rabbit polyclonal anti-ovine PF or anti-human cytokeratin-7. Cytotrophoblasts from first trimester pregnancies (n=5) were subjected to an *in vitro* invasion assay and RNA was harvested following 0, 3, and 12 h. Quantitative RT-PCR was performed on these samples with intron-spanning primers for hPF, and normalized on hS15 mRNA concentrations. Based on the human PF sequence, four putative shRNA constructs were generated and cloned into a lentiviral expression vector. BeWo human choriocarcinoma cells were treated with one of four shRNA constructs or an empty vector for 72 h and then RNA was harvested from cells for analysis by quantitative RT-PCR. **Results:** Periattachment factor was present in the nuclei of both first and second trimester cytotrophoblasts. hPF mRNA concentration increased as invasion occurred from 0, 3, to 12 h in all samples; while hypoxia decreased expression at 18 h of invasion compared to 18 h under normoxic conditions. The four lentiviral vectors expressing shRNA against hPF resulted in hPF mRNA concentrations at 2, 18, 83 and 97% of hPF mRNA concentration with the control vector. **Conclusion:** The presence of PF in the human placenta and the increase in PF mRNA during cytotrophoblast invasion may indicate this gene plays a role during implantation. We have developed shRNAs against PF that result in greater than 98% mRNA knockdown and will be using these to begin to elucidate the function of PF in the human placenta, specifically during the invasion process. This project was supported by National Research Initiative Competitive Grant no. 2005-35203-15885 from the USDA Cooperative State Research, Education, and Extension Service.

19. **Isg15 is a molecular sentinel that functions to assist mothers in coping with environmental stressors imposed on pregnancy**

R.L. Ashley[1], L.E. Henkes[1], R.V. Anthony[1], K.C. McBroom[1], J.K. Pru[2], T.R. Hansen[1]

[1]Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO
[2]Vincent Center for Reproductive Biology, Vincent Obstetrics and Gynecology Service, Massachusetts General Hospital, and Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA

The ubiquitin homolog, Isg15 is up-regulated in the endometrium in response to pregnancy in humans, baboons, ruminants, pigs, and mice. Isg15 is produced in response to type I interferon (IFN), becomes covalently attached to intracellular proteins (isgylation) and regulates numerous intracellular responses. The purposes of this study were to: 1) solidify a functional role for Isg15 during pregnancy using *Isg15* mutant mice; 2) identify cytokines that regulate Isg15 expression during decidualization; and 3) identify pathways negatively impacted by Isg15 deficiency *in vivo*. We previously reported up to 70% embryo mortality in *Isg15* knockout (KO) female mice when mated to either wild type (WT) or KO males. More recently, and in response to low relative humidity (<30%), the average litter size was reduced 36 % in *Isg15* KO females compared to WT. Embryo mortality was also increased 1.5 fold in KO females kept under hypoxic conditions. Our findings using two different model systems indicate that Isg15 functions to assist mothers in coping with stressors imposed on pregnancy by the environment. IL-1β initiates murine and human decidualization responses. It was next hypothesized that IL-1β induces isgylation in cultured mouse decidual explants and in human uterine fibroblast (HuF) cells. Culture of mouse decidual explants (7.5 dpc) or HuF cells with 10 ng/mL IL-1β induced an increase in *Isg15* mRNA. In parallel, IL-1β up-regulated expression of enzymes (*Herc5, Ubch8*) that coordinate the covalent addition of Isg15 to target proteins, as well as the gene that encodes the de-isglyation enzyme *UBP43* in HuF cells. In a final series of experiments qRT-PCR was used to validate expression of select genes identified in our previous microarray analysis. Here ~500 genes were differentially expressed in *Isg15* KO versus WT deciduas on 7.5 dpc. We confirmed that *Ifi202b*, an anti-apoptotic and cell-survival gene is up-regulated and that *Adam8* and *Adam12* are down-regulated in decidual tissue isolated from *Isg15* KO. Adam12 is a disintegrin that functions primarily in adhesion. However, Adam12 also releases IGF1 from IGFBP3 and is inversely correlated with pre-eclampsia and intra-uterine growth restriction in humans. In summary, *Isg15* KO mothers are less able to cope with environmental stressors such as low humidity and hypoxia. Mutant *Isg15* mice have been invaluable to our search for factors that regulate Isg15 expression during pregnancy, and in identifying pathways that are targets of Isg15 function. The authors thank Dr. A.T. Fazleabas for providing the HuF cells. Supported by NIH HD32475.

20. **Implications for the type-I interferon pathway in intrauterine growth restriction**

Megan L Shoemaker[1], Kathy J Austin[2], Hana Van Campen[1], Helle Bielefeldt-Ohmann[1], Lea Rempel[2], Natalia P Smirnova[1], Hyungchul Han[2], Donald J Montgomery[4], Russell V Anthony[1], Alberto Van Olphen[2], Jeffery A Clapper[3] and Thomas R Hansen[1,2]

[1]Dept. of Biomedical Sciences and Dept. of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO; [2]Dept. of Animal Sciences and Dept. of Veterinary Science, University of Wyoming, Laramie, WY; [3]Dept. of Animal and Range Services, South Dakota State University, Brookings, SD; [4]College of Public Health, University of South Florida, Tampa, FL.

Infection with noncytopathic bovine viral diarrhea virus (ncpBVDV) during early bovine pregnancy (<150d gestation) results in fetal immunotolerance, persistent infection (PI) and intrauterine growth restriction (IUGR). In contrast, infection after the development of adaptive immune competence (>150d gestation or postnatal) results in a transient infection (TI). We have previously reported an IUGR in PI fetuses presenting as decreased body weight and ponderal index. A growth defect can be the result of many factors, including placental insufficiency and nutrient restriction, however it was hypothesized that the IUGR seen in BVDV PI fetuses may be an immunopathological effect caused by the persisting virus. Our two part experimental design examined the relationship between BVDV and its PI host. In Experiment 1, blood cell mRNA was collected from PI steers (n=2; confirmed by virus isolation), or uninfected control steers (n=3) and used to identify differentially expressed genes using microarray (Affymetrix) and qtRT-PCR approaches. In Experiment 2, BVDV naive pregnant heifers (n=6 per group) were not infected (control) or infected with ncpBVDV on d.75 or d.175 of pregnancy creating PI and TI fetuses, respectively. Fetuses were collected by c-section on d.190; infection was confirmed by ELISA and qtRT-PCR. Histology of placental tissue revealed no placentitis or pathology, and glucose and lactate levels in fetal serum were normal. Microarray analysis revealed 294 genes that were differentially regulated in PI vs. controls ($p<0.05$, >1.5 fold). qtRT-PCR of steer and fetal blood revealed a significant upregulation of activators and products of the antiviral type-I interferon (IFN-I) pathway. As IFN-I can act as a growth-suppressive cytokine, a long-term upregulation may contribute to the IUGR seen in persistent BVDV infection and in other viral infections observed during pregnancy. National Research Initiative Competitive Grant no. 2005-2006-03907 from the USDA Cooperative State Research, Education, and Extension Service.

21. **The SUMO pathway in relation to fetal ovarian development**

Brittany Fromme and Jerry Bouma

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1683

An important question in developmental biology is how does the ovary become an ovary? We know that the ovary develops in the absence of a Y chromosome, but the exact way this happens is still unclear. In mice the ovary differentiates adjacent to the mesonephros between embryonic day 10.5 and embryonic day 13. Proliferation of primordial germ cells occur until embryonic day 13 after which they enter meiosis. SUMO (small ubiquitin-like modifiers) genes encode proteins with important functions in post transcriptional modification. Recently, the three major SUMO proteins (SUMO1, 2, 3) were found to be involved in the process of meiosis in yeast, especially in prophase 1 and metaphase 1. We hypothesize that SUMO proteins play a role in fetal ovarian development by regulating the process of meiosis. Using real time PCR techniques we examined the expression of 20 genes involved in the SUMOylation pathway in fetal ovarian tissue. Tissues were collected from embryonic days 11.5 to 17. Preliminary analysis revealed dynamic expression of many SUMO genes during fetal ovarian development. There were also several genes not expressed in ovarian fetal tissue. We conclude that SUMO genes are expressed during fetal ovarian development and play an important role in regulating meiosis during mammalian ovarian development.

Keywords: ovary, meiosis, embryonic day 13, primordial germ cell

22. **Genetic control of fetal ovarian development**

Emilie Loomis, Gerrit J. Bouma

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1683

Human gonadal sex reversal is characterized by the development of ovarian tissue in XY and development of testicular tissue in XX individuals. The underlying cause of sex reversal in humans is unknown in most cases. The mammalian Y-linked testis determining-gene *SRY* was discovered in 1990, and additional genes have been identified that play an important role in gonadal sex determination, called gonadal sex determining genes (GSD). The majority of genes identified so far, however, either play a role in establishment of bipotential gonadal primordia, and/or development of testes in XY individuals. Little is known about what regulates fetal ovarian development. GSD genes are expressed in fetal gonadal somatic support cells. Expression of GSD genes causes somatic support cells to develop into either granulosa cells (XX) or Sertoli cells (XY) in the ovary and testis, respectively. To obtain a better understanding of the genes that are involved in fetal ovarian development, we used a unique transgenic mouse to isolate these somatic support cells from undifferentiated and early-differentiated XX and XY fetal gonads, and determined their gene expression profiles. Our data reveals a number of transcription factors that are preferentially expressed in isolated somatic support cells from fetal ovaries compared to fetal testes. Furthermore, we used real time RT-PCR analysis to examine the expression of 6 transcription factors, *Fem1b, Lbx2, Lmo7, Msx1, Sept9,* and *Vgll2* in fetal ovaries. Data revealed that expression of these transcription factors is dynamic during fetal ovarian development, with most transcription factors being down-regulated. These results suggest an important role for 6 transcription factors in fetal ovarian development in mammals.

23. **MicroRNA's in ovine reproduction and development**

KJ Torley, RV Anthony, GJ Bouma

Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratories, Colorado State University, Fort Collins, CO, 80523-1683

Even with the identification of several sex determining genes, the underlying cause of many cases of human abnormal gonad development are unexplained. In addition, the regulation of gene expression during gonad development remains elusive.. The sheep is a good model to study human gonad development, as there are many similarities in fetal gonadal expression patterns of sex determining genes between humans and sheep. MicroRNAs (miRNAs) have been shown to regulate gene expression in tissues throughout the body and most likely have a role in mammalian gonadal development. MiRNAs are small non-coding RNAs, ~22 nucleotides in length. Through modification by Drosha and Dicer and assembly within a ribonucleoprotein complex called RNA-induced silencing complex (RISC), miRNAs regulate gene expression and function at the post-transcriptional level. It is the hypothesis of this study that conserved miRNAs are expressed in fetal sheep ovaries and testes, and regulate gene expression. Expression profiling of 128 selected miRNAs was conducted using real time PCR. A total of 24 miRNAs were identified as significant differentially expressed between fetal ovaries and testes at a gestational age of 42 days while 43 were found to have differential expression at a gestational age of 75 days. Using a bioinformatics approach, a summation of the predicted targets for these miRNAs yielded thousands of possible target genes, including known sex determining genes. Results from this study provide important new insight into the complex process of fetal gonadal development and its regulation by miRNAs. Funded by the College Research Council.

Keywords: Sheep, Fetal Gonad, Gene Regulation, Non-Protein Coding RNA

24. **Impact of prenatal hypoxia on offspring growth and cardiac development**

Jann Rhodes, PhD[1,2], Katherine C McBroom, BS[1], Adam J Chicco, PhD[3] and Russell V Anthony, PhD[1,2]

[1]Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523; [2]Pediatrics, University of Colorado Health Sciences Center, Aurora, CO, 80045; [3]Integrative Physiology, University of Colorado, Boulder, CO, 80309

Objective: Hypoxia as a result of placental insufficiency, maternal smoking, or residence at high altitude, impacts fetal growth and development. In a preliminary study, we observed a significant decrease in birth weight, subsequent compensatory postnatal growth, and an increase in relative right ventricular (RV) weight at postnatal (pn) day 30 in female offspring of rats exposed to hypoxia (14,000 ft; ~11.6% PO_2) from days 12 thru 15 of gestation (dga). Thus, our objective was to further elucidate the impact of prenatal hypoxia on fetal growth and postnatal development. **Methods:** Pregnant dams (Hx, n=15) were hypoxic from 12-15 dga with additional control dams either fed *ad libitum* (AL, n=10), pair-fed with the Hx dams throughout gestation (PG, n=12), or only pair-fed during the window of hypoxia (PH, n=12). Female offspring from Hx, PG, and PH dams were cross-fostered onto additional AL dams (n=8/litter) by 48 h after birth. **Results:** At birth, there was no difference in litter size; however, body weight (bw) of the Hx, PG, and PH pups was lower ($P<0.05$) than that of AL pups, and Hx pups were lighter ($P<0.05$) than PH pups. Weight of Hx offspring remained lower ($P<0.05$) than AL pups until the termination of the study at pn120, while the PG and PH pups reached weights comparable to the AL offspring by pn90. Relative to bw, heart weight and left ventricular/septal (LVS) weight was not different among groups; however, right ventricular weight (RV/bw) was greater ($P<0.05$) in the Hx offspring at pn120 as was RV/LVS ($P<0.05$). Cardiac function was evaluated by echocardiography at pn174. RV wall thickness was 47% greater ($P<0.05$) in Hx pups as compared to AL pups, confirming the significantly higher relative RV weight observed at necropsy. PEP, PEP/AT, and PEP/ET were 36%, 14%, and 31% higher respectively in the Hx offspring relative to the AL offspring. LV end diastolic and end systolic diameters were smaller ($P<0.05$) in Hx and PH offspring relative to the AL group. Myosin heavy chain (MHC) α and β mRNA concentrations in the RV were evaluated by qRT-PCR, and the MHC β/α mRNA ratio was greater ($P<0.05$) in the Hx pups. **Conclusion:** Prenatal hypoxia from 12-15 dGA impacted both fetal and postnatal growth, altered postnatal heart development and function, with the primary impact being on the RV. Supported by NIH HD48902.

25. **Effects of overfeeding adolescent ewe lambs on progeny growth**

G.J. Eckerle[1], R.V. Anthony[2], R.K. Peel[1]

[1]Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523 USA
[2]Department of Biomedical Sciences, Colorado State University. Fort Collins, CO 80523 USA

Adolescent lambs, impregnated by embryo transfer and fed *ad lib* throughout gestation, are reported to produce growth restricted offspring. It was our objective to determine if *ad lib* feeding of peripubertal ewes following natural mating at the second observable estrus, impacted birthweight and postnatal growth characteristics of the offspring. Two replicate studies were completed, in which singleton-bearing ewe lambs were fed a complete gestational diet (11.4 MJ metabolic energy/ kg DM) at a rate which met NRC gestational age requirements (MN; n=8 year 1, n=7 year 2) or were fed *ad lib* (15% refusal rate) throughout gestation (HN; n=6 year 1, n=7 year 2). There was no effect (P≥0.10) of gestational intake on lamb birthweight (year 1: 5.0±0.23 vs. 4.9 ±0.20 kg; year 2: 5.01±0.42 vs. 5.68±0.40 kg; MN vs. HN respectively), lamb abdominal circumference or crown-rump length. However, during year 2, neonatal death loss was increased in HN pregnancies (0% vs. 57%; MN vs. HN), due to increased (P≤0.01) dystocia (1.0±0.0 vs. 3.5±0.7 score; 1=no assistance to 5=cesarean section). During year 1, lambs were weaned at 80 days of age and fed *ad lib* (11.4 MJ metabolic energy/kg DM) until 140 days of age. Neither preweaning (0.32±0.018 vs. 0.33±0.015 kg/d, MN vs. HN) nor overall (0.28±0.009 vs. 0.29±0.010 kg/d; MN vs. HN) growth rate were effected (P≥0.10) by gestational intake. During year 2, lambs remained with their dams until they were weaned at 60d., and were fed *ad lib* until reaching the target slaughter weight (59 kg). Similar to year 1, no differences (P≥0.10) were observed in growth rate, target weight age, or in carcass characteristics (rib eye area, back fat thickness and percent kidney pelvic and heart fat) collected at slaughter. Collectively, our results indicate that *ad lib* feeding adolescent ewe lambs, which conceived to natural service, does not impact fetal or postnatal growth rate of the progeny, but may result in increased dystocia rate and neonatal mortality.

Keywords: peripubertal lambs, gestational overfeeding, progeny, growth rates

POSTER SESSION ABSTRACTS

ASSISTED REPRODUCTION

26. Cryopreservation of large equine embryos

Jennifer P. Barfield, Patrick M. McCue, Edward L. Squires, George E. Seidel, Jr.

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Cryopreservation of equine embryos greater than 400 μm in diameter has resulted in low rates of survival using cryopreservation protocols that work well for smaller equine embryos and embryos of other mammals. Experiments were designed to test the potential benefit of incorporating a dehydration step by exposure of embryos to a non-penetrating sugar (galactose) prior to initiating the standard cryopreservation protocol. In 2007, 46 day 7-8, grade 1 equine embryos ≥ 400 μm in diameter were collected and subjected to one of the following treatments: (A) 2-min incubation in 0.6 M galactose, 10 min incubation in 1.5 M glycerol, slow freeze (n=21); (B) 10 min incubation in 1.5 M glycerol, slow freeze (n=15); (C) 2 min incubation in 0.6 M galactose, 10 min in 1.5 M glycerol, followed by exposure to thaw solutions, then culture medium (50:50 DMEM-F12 medium + 10% FCS; toxicity control, n=5); (D) transferred directly to culture medium (control, n=5). Embryos were blocked into three groups based on measured diameter at time of collection: 400-599, 600-799, and 800-1350 μm. Embryos were cooled to -6°C, straws seeded, and further cooled at 0.5°C/min to -32°C at which point they were plunged into liquid nitrogen. Embryos were thawed by exposure to air for 8 sec followed by submersion in a 37°C water bath for 30 sec and the following: (1) 8 min in 0.6 M glycerol + 0.6 M galactose, (2) 8 min in 0.3 M glycerol + 0.6 M galactose, (3) 8 min in 0.6 M galactose. Five embryos in treatment A and 5 embryos in treatment B were thawed and cultured to determine if embryos survived the cryopreservation process. Treatment C served as a toxicity control. Cultured embryos from all treatments across all blocks were given a quality score (QS; 1=excellent, 2=good, 3=fair, 4=poor, 5=degenerate) after 24 and 48 h of culture; After 24 h in vitro 4 of 5 embryos from treatment A had a QS > 3, 2 of 5 from treatment B, 5 of 5 from treatment C, and 4 of 5 from treatment D. After 48 h, 4 or 5 embryos from treatment A, 1 of 5 from treatment B, 5 of 5 from treatment C, and 4 of 5 from treatment D had QS > 3. Subsequent embryos were assigned to treatment A or B and reserved for embryo transfer (n=26). These embryos were matched in sets of two for size and treatment, thawed, and immediately transferred in pairs to 13 synchronized recipients. Of 16 embryos from treatment A, 6 were 400-599 μm, 6 were 600-799 μm, and 4 were 800+ μm. Of 10 embryos from treatment B, 4 were 400-599 μm, 2 were 600-799 μm, and 4 were 800+ μm. Pregnancies were detected in two recipient mares via transrectal ultrasonography including detection of fetal heartbeats on day 25. One mare received two 400 μm embryos from treatment A and the other mare received one 400 μm and one 425 μm embryo from treatment B. No pregnancy resulted from the transfer of embryos >425 μm. There was no advantage of incorporating a 2 min dehydration step into the cryopreservation protocol for large equine embryos.

Keywords: equine embryos, cryopreservation

27. **Efficacy of medroxyprogesterone acetate in suppression of estrous behavior and follicular activity in cycling mares**

E.K. Gee[1], P.M. McCue[1], C.A. DeLuca[1], J.L. Stylski[1]

[1]Equine Reproduction Laboratory, Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Various progestins are used in performance mares to pharmacologically suppress estrous behavior, including injectable medroxyprogesterone acetate (MPA). However, there have been no scientific studies to evaluate the efficacy of MPA in suppressing estrus in the mare. The purpose of our study was to evaluate the effects of MPA on follicular activity and estrous behavior. Eighteen cycling Quarter Horse-type mares were randomly assigned to one of three treatment groups: MPA (Wedgewood Pharmacy, Swedesboro, NJ, USA), saline or altrenogest (Regu-Mate®, Intervet, Millsboro, DE, USA). Treatments began on day 7 post-ovulation. Mares in the MPA treatment group (n = 6) were treated with 1600 mg MPA intramuscularly (IM) initially, then 400 mg once per week for 5 weeks. Saline treated mares (n = 6) were treated with saline IM once per week for 6 weeks. Altrenogest treated mares (n = 6) received 10 ml altrenogest (22 mg) orally each day for 48 days. For the treatment period and 18 days after treatment ceased, mares were teased daily by an experienced stallion and were categorized as displaying signs characteristic of estrous or diestrous behavior. Transrectal ultrasound examinations were performed three times weekly, or daily beginning when a 35 mm follicle was identified until ovulation. Blood samples were collected weekly and serum frozen for analysis of progesterone, and from days 10 to 19 for analysis of luteinizing hormone (LH). Assessors of teasing behavior, ultrasonographic follicular activity and serum progesterone and LH were blinded to the treatment each mare received. Data were analyzed by Chi-squared comparisons and analysis of variance with pair wise comparisons using SAS v9.1.3.

Mares treated with saline vs. MPA showed no significant differences in duration of diestrous behavior (mean ± S.D., 14 ± 2 and 16 ± 4 days, respectively) or estrous behavior (8 ± 2 and 7 ± 1 day, respectively) for the duration of the study. No altrenogest treated mares showed signs of estrous behavior during the treatment period and four resumed estrous behavior 6 to 18 days after cessation of treatment. All mares in the saline and MPA treatment groups showed normal follicular development and ovulations for the duration of the study. No mares in the altrenogest treatment group ovulated during the treatment period; four of these mares resumed normal follicular development after treatment ceased. Least squares means showed significant differences in the interovulatory interval of altrenogest vs. saline treated mares and altrenogest vs. MPA treated mares ($P < 0.0001$ and $P < 0.0001$, respectively), but no significant differences in interovulatory interval between saline vs. MPA mares ($P = 0.7$). Serum progesterone and LH profiles showed agreement with the follicular and behavioral activity for all mares. In conclusion, MPA is not effective in suppression of estrous behavior or follicular activity in normal cycling mares and consequently its use for this purpose is not recommended.

Acknowledgement: United States Equestrian Federation, Inc. provided support for this project.

Keywords: Equine; Estrus suppression; Estrous behavior; Medroxyprogesterone acetate

28. **Effect of short-term exposure of hydrogen peroxide on stallion sperm motility and DNA fragmentation**

Lindsay Hovda[1], Patrick D. Burns[1], Beth Curtis[1], Amy Albers[1], and Lisa Herickhoff[2]

[1]School of Biological Sciences, University of Northern Colorado, Greeley, CO 80639 and
[2]Membrane Protection Technologies Inc., Fort Collins, CO 80524

The objectives of this study were to 1) examine the effect of short-term exposure of H_2O_2 on stallion sperm motility and DNA fragmentation, and 2) evaluate two commercially available assays for measuring DNA fragmentation in stallion sperm cells. Single ejaculates were collected from four stallions and concentration of sperm cells in each ejaculate was determined spectrophotometrically. Semen was diluted with three parts E-Z Mixin-CST equine semen extender to one part semen and immediately transported to the laboratory. In the laboratory, ejaculates were split into two treatment groups. In treatment 1, semen was extended to a final concentration of 25 x 10^6 motile cells/ml with E-Z Mixin-CST and in treatment 2, semen was extended to a final concentration of 25 x 10^6 motile cells/ml E-Z Mixin-CST containing 300 μM H_2O_2. Samples were placed in a passive cooling device and cooled to 5°C. Motility and DNA fragmentation were determined immediately before cooling (0 h) and at 24 h post cooling. Motility was determined using a computer assisted analysis system. Single cell gel electrophoresis (Comet) and TdT-mediated-dUTP nick end labeling (TUNEL) assays were used to evaluate DNA fragmentation. The addition of H_2O_2 to the extender had no effect on either total or progressive motility ($P>0.05$). Neither Comet parameters (Comet length, tail length, and tail moment) nor percentage of oxidative damaged cells detected by TUNEL differed between 0 h and those cells stored for 24 h in the absence of H_2O_2 ($P>0.05$). However, the addition of H_2O_2 to the extender and stored for 24 h resulted in greater total Comet length, tail length, and tail moment as well as percentage of oxidative damaged sperm cells detected by TUNEL when compared to 0 h ($P<0.05$). Motility was not correlated with either percentage of oxidative damaged cells detected by TUNEL or Comet parameters ($P >0.05$). In conclusion, both the Comet and TUNEL assays detected considerable amount of DNA fragmentation in stallion sperm cells exposed to H_2O_2. Furthermore, we show that a significant amount of DNA damage can occur in the presence of H_2O_2 without having an appreciable affect on motility.

Keywords: Stallion Sperm Motility

ATTENDEES

Brenda Alexander, University of Wyoming, balex@uwyo.edu

Russ Anthony, Colorado State University / University of Colorado Health Sciences Center, russ.anthony@colostate.edu

Alfredo Antoniazzi, Colorado State University, alfredo@colostate.edu

Alejandro Arreguin-Arevalo, Colorado State University, inifap@lamar.colostate.edu

Juan Arroyo, University of Colorado Health Sciences Center, juan.arroyo@uchsc.edu

Ryan Ashley, Colorado State University, ryashley22@yahoo.com

Kevin Bachus, Rocky Mountain Center for Reproductive Medicine, kevin@royalwolf.org

Tiffany Bohlender, University of Nebraska-Lincoln, marvelgirl15@msn.com

Rebecca Bott, Colorado State University, rebecca.bott@colostate.edu

Gerrit Bouma, Colorado State University, gerrit.bouma@colostate.edu

Richard Bowen, Colorado State University, richard.bowen@colostate.edu

Ashley Breton, University of Wyoming, ashnel471@aol.com

Jason Bruemmer, Colorado State University, jason.bruemmer@colostate.edu

Greg Burns, South Mesa Veterinary Hospital, reprovet@comcast.net

Fernando Campos-Chillon, Colorado State University, lfcampos@lamar.colostate.edu

Jeremy Cantlon, Colorado State University, jeremiah.cantlon@colostate.edu

Daesuk Chung, Colorado State University, dchung@lamar.colostate.edu

Rebecca Cockrum, University of Wyoming, rcockrum@uwyo.edu

Andrea Cupp, University of Nebraska-Lincoln, acupp2@unl.edu

Lucia Dohnal, University of Colorado Health Sciences Center, lucia.dohnal@uchsc.edu

Greg Eckerle, Colorado State University

Brittany Fromme, Colorado State University, brittany.fromme@colostate.edu

Erica Gee, Colorado State University, ekgee@mail.colostate.edu

Laura Godsman, University of Northern Colorado, laura.godsman@gmail.com

Claire Goodwin, Colorado State University, claire@holly.colostate.edu

Hyungchul Han, Colorado State University, hyungchul.han@colostate.edu

Tod Hansen, Colorado State University, thomas.hansen@colostate.edu

Dean Hawkins, New Mexico State University, dhawkins@nmsu.edu

Kim Hayes, Poudre Valley Health Systems, hayes4120@comcast.net

Luiz Henkes, Colorado State University, luiz.henkes@colostate.edu

Lindsay Hovda, University of Northern Colorado, lindsay.hovda@unco.edu

Amanda Krull, Colorado State University, alkrull@colostate.edu

Shantille Kruse, University of Nebraska-Lincoln, shantillekruse@hotmail.com

Emilie Loomis, Colorado State University, emilie.loomis@colostate.edu

Christianne Magee, Colorado State University, christianne.magee@colostate.edu

Pat McCue, Colorado State University, pmccue@colostate.edu

Gary Moss, University of Wyoming, gm@uwyo.edu

Dilyara Murtazina, Colorado State University, dilyara.murtazina@colostate.edu

Terry Nett, Colorado State University, terry.nett@colostate.edu

Gordon Niswender, Colorado State University, gordon.niswender@colostate.edu

Jenny Powers, Colorado State University, jennygpowers@yahoo.com

Scott Purcell, Colorado State University, scott.purcell@colostate.edu

Jann Rhodes, University of Colorado Health Sciences Center, jann.rhodes@uchsc.edu

Deborah Roess, Colorado State University, daroess@lamar.colostate.edu

Barbara Sanborn, Colorado State University, barbara.sanborn@colostate.edu

George Seidel, Colorado State University, gseidel@colostatee.edu

Megan Shoemaker, Colorado State University, mshoe@colostate.edu

Juliano da Silveira, Colorado State University, juliano.da_silveira@colostate.edu

Priyanka Singh, University of Wyoming, psinght@uwyo.edu

Racheal Slattery, University of Nebraska-Lincoln, rslattel@bigred.unl.edu

Sahatchai Tangtrongsup, Colorado State University, tsahat@colostate.edu

Katie Torley, Colorado State University, katie.torley@colostate.edu

Renata Cristina Uliani, Colorado State University, renatinhauliani@hotmail.com

Aida Ulloa, Colorado State University, aida.ulloa@colostate.edu

Rao Veeramachaneni, Colorado State University, d_n_rao.veeramachaneni@colostate.edu

Richard Wheeler, Poudre River Veterinary Clinic, vetwheeler@msn.com

Amber Wolf, Colorado State University, amber.wolf@colostate.edu

ACKNOWLEDGEMENTS

The organizers thank the Animal Reproduction and Biotechnology Laboratory and the Department of Biomedical Sciences, Colorado State University for funds that were used to help support this symposium. The organizers also thank Ms. Sallie Varner for assistance in preparing this program book and Ms. Brenda Martin for coordinating local arrangements.