34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Cysteine-rich Secretory Protein-3 (CRISP-3) Expression in the Reproductive Tract of Prepubertal and Mature Stallions**
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Abstract: In the horse, the seminal plasma protein Cysteine-rich Secretory Protein-3 (CRISP-3) has been shown to be located in the ampulla of the vas deferens with a more moderate amount expressed in the seminal vesicles. In humans and rodents, this protein has been described as an androgen-dependent protein, but androgen dependency has not been identified in the horse. The objectives of this study were to a) confirm the localization of equine CRISP-3 in the stallion reproductive tract, and b) determine if expression of CRISP-3 increases after puberty. We hypothesized that expression would be localized primarily to the ampulla of the ductus deferens with a more moderate expression in the seminal vesicles, and that expression of CRISP-3 would increase after puberty. Reproductive tissues were collected postmortem from three prepubertal colts (< six months) and six mature stallions (> 3 years). Fixed tissues and tissues in RNALater were collected from the ampulla, seminal vesicles, bulbourethral gland, prostate gland, testis, as well as the cauda, corpus, and caput aspects of the epididymis. qRT-PCR and immunohistochemistry was performed on the tissues. A mixed linear additive model was used to compare mRNA expression between age groups, and significance was set to $P<0.05$. mRNA expression of CRISP-3 was found primarily in the ampulla of the ductus deferens with more moderate expression in the seminal vesicles, and expression of CRISP-3 was higher in the mature stallion when compared to the prepubertal colt for the ampulla ($P<0.0001$) and seminal vesicles ($P=0.0013$). IHC confirmed that CRISP-3 was expressed primarily in the ampulla of the ductus deferens, and to a lesser degree in the seminal vesicles, although variability was seen amongst stallions. In conclusion, equine CRISP-3 is primarily expressed in the ampulla of and to a lesser degree, in the seminal vesicles. Mature stallions have significantly higher expression in the ampulla suggesting that it may be regulated by steroid hormones whose synthesis increases after puberty.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Automated detection of estrus using multiple commercial precision dairy farming technologies in synchronized dairy cows**
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Abstract: The objective of this study was to evaluate precision dairy farming technologies (PDFT) for estrous detection. Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus) beginning 45-85 DIM. Resumption of ovarian cyclicity at enrollment, presence of a corpus luteum (CL) on the day of the final PGF2α injection (designated experimental day 0), regression of the CL by day 5, and presence of a new CL on day 11 were verified by transrectal ultrasonography. Cows were observed for estrous behaviors for 30 min, 4X per day, on days 2 to 5. Blood samples were collected on days -2, -1, 0, 1, 2, 5, 7, 9, and 11 to quantify progesterone to verify luteal regression and ovulation. Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Eighteen cows followed this pattern. Cows that failed to follow the pattern served as negative controls (n=6). Detection of estrus by PDFTs, an estrous behavioral scoring system as defined by Van Eerdenburg et al. (1996), and by visual observation of standing estrus were compared to the gold standard (Table 1). The PDFT used: AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel) measured activity, rest time, and rest bouts; GEA CowScout Leg Tag (GEA Farm Technologies GmbH, Bönen, Germany) measured activity; Animart Track a Cow (Animart Inc., Beaver Dam, WI and ENGS, Rosh Pina, Israel) measured activity, lying behavior, and feeding behavior; and Agis SensoOr (Agis Automatisering, Harmelen, Netherlands) measured ear movement, feeding time, rumination time, and ear skin temperature. Sensitivity for AfiAct Pedometer Plus, CowScout, Track a Cow, and SensoOr was 89%, 83%, 78% and 78% respectively. Specificity was 100% for all PDFT. Only 56% of cows that ovulated were observed in standing estrus by visual estrous detection. All systems tested are capable of detecting estrus more effectively than visual observation.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**The expression and regulation of chemokine CXC motif receptor 4 (CXCR4) in the human ovary during the periovulatory period.**
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Abstract: Ovulation is a complex process requiring coordinate action of various factors induced by the LH surge in preovulatory follicles. This process includes prostaglandin secretion, chemokine and cytokine production, and leukocyte infiltration. A chemokine CXC motif receptor 4 (CXCR4) is known to exert potent chemotactic activity for immune cells by binding to its ligand, stromal cell-derived factor-1 (SDF-1). Previous studies have documented the increase in CXCR4 expression in periovulatory ovaries of mouse and horse, but little is known about the expression and regulation of CXCR4 in the human ovary. In the present study, we hypothesized that CXCR4 expression is induced by the LH surge in the human periovulatory follicles and plays a role in the ovulatory process. To characterize the expression of CXCR4 in the human ovary, patients undergoing laparoscopic tubal sterilization were recruited and their follicular growth was monitored by ultrasonography. Patients with normal cycles were placed into 4 groups (preovulatory, early ovulatory, late ovulatory, and post ovulatory). The preovulatory stage was defined as an ovary containing a growing dominant follicle (14-18 mm) prior to the LH surge. The early, late, and post ovulatory stages were defined at 12h-18h, 18h-34h, and 44-70h after rhCG administration, respectively. Granulosa and theca cells were isolated from dominant follicles surgically retrieved. Real-time PCR revealed that levels of CXCR4 mRNA were increased in granulosa cells obtained at the late ovulatory stage. Theca levels of CXCR4 mRNA were not changed prior to ovulation, but the level was decreased in post ovulatory follicles. Similarly, strong immunopositive staining for CXCR4 protein was localized to granulosa and theca cells of late ovulatory and post ovulatory follicles. To determine the regulation of CXCR4 expression, human granulosa-lutein cells (hGLCs) were obtained from women undergoing in vitro fertilization (IVF). The cells were cultured in media alone for 7 d prior to hCG. The level of CXCR4 mRNA was transiently increased by hCG, peaking at 12h. hCG also increased the level of CXCR4 protein. Next, we determined whether progesterone (P4) regulates CXCR4 expression by treating hGLCs with RU486 (PGR antagonist) with or without hCG. The hCG-induced CXCR4 mRNA expression was inhibited by RU486. Lastly, to investigate the potential function of CXCR4, hGLCs were treated with SDF-1 and AMD3100 (CXCR4 specific antagonist). hCG stimulated P4 production, but the stimulatory effect of hCG was inhibited by SDF-1, but not ADM3100. n summary, the present study demonstrated for the first time that the LH surge increases the expression of CXCR4 in human periovulatory follicles and this increase depends on P4/PGR. The activation of CXCR4 by SDF-1 may play a role in modulating P4 production in human granulosa cells.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Chemokine Ligand 20: A signal for leukocyte recruitment during human ovulation?**
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Abstract: Ovulation is one of the cornerstones of female fertility. Disruption of the ovulatory process results in infertility which affects approximately 10% of couples. Utilizing a unique model where the dominant follicle is collected across the periovulatory period in women, we have identified a leukocyte chemoattractant, chemokine ligand 20 (CCL20) in the human ovary. CCL20 mRNA is massively induced after an in vivo hCG stimulus in granulosa (>10,000 fold) and theca (>4,000 fold) cells collected during the early ovulatory (12-18h) and late ovulatory (18-34h) periods post hCG. As the LH surge sets in motion an inflammatory reaction characterized by an influx of leukocytes and CCL20 is known to recruit leukocytes in other systems, the composition of ovarian leukocytes (CD45+) containing the CCL20 receptor CCR6 was determined immediately prior to ovulation. CD45+/CCR6+ cells were primarily natural killer-cells (41%) along with B-cells (12%), T-cells (11%), neutrophils (10%), and monocytes (9%). Importantly, exogenous CCL20 stimulated ovarian leukocyte migration 59% within 90 minutes. Due to the difficulties in obtaining human follicles, an in vitro model was developed using granulosa-lutein cells (GLC) to explore the CCL20 regulation. CCL20 expression increased 40-fold within 6h after hCG, was regulated by the EGF pathway, and was positively correlated with progesterone production. These results demonstrate that hCG dramatically increases CCL20 expression in the human ovary, that ovarian leukocytes contain the CCL20 receptor, and that CCL20 stimulates leukocyte migration. Our findings raise the prospect that CCL20 may aid in the final ovulatory events and contribute to fertility in women.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Effects of Low Peripheral Progesterone Concentrations on the Equine Endometrial Transcriptome**
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Abstract: Progesterone (P4) changes gene expression in the endometrium and it is essential for maintenance of pregnancy in the horse. However, there is little information available concerning changes in the endometrial transcriptome associated with low P4 concentrations. The objectives were to evaluate changes in the endometrial transcriptome at Day 12 of the estrous cycle between mares with normal versus low P4. Mares (n=6) were randomly assigned to control or treatment cycles (125 mg of cloprostenol IM on Days 0-3) in a switchback design. P4 concentrations were measured daily via ELISA. Endometrial biopsies were collected at Day 12 postovulation and RNA was sequenced generating 100-bp paired-end reads. The sequences were mapped using CLC Genomics Workbench software to the equine reference genome (EquCab2.0) and Ensembl's consensus gene models (www.ensembl.org). Gene expression analyses were performed using an empirical analysis of DGE. Gene functions and pathways were analyzed using Ingenuity® Pathway Analysis on the differentially expressed genes. For the control cycle, P4 was 94.7±4.3 ng□day□mL-1±SEM versus 19.6±1.0 in treated cycles (p<0.0001). Differential expression of 623 gene loci (p<0.05 FDR adjusted) was identified. Of these, 381 were up-regulated and 242 were down-regulated in the low P4 samples. Some of the functions and pathways significantly altered included cholesterol biosynthesis and lipid metabolism and activation of estrogen mediated S-phase entry, cyclins and cell cycle: G1/S checkpoint regulation, oxidative phosphorylation, and apoptosis signaling. In conclusion, lower concentrations of progesterone during the early and mid luteal phase resulted in substantial levels of differential gene expression and significant changes for several cell signaling pathways in the equine endometrium.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Proteomic Analysis of the Cervical Mucus Plug in the Late Pregnant Mare**
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Abstract: The cervical mucus plug provides the first line of defense against bacterial and viral infections in pregnant mammals; however, very little is known about its composition. For this study, protein was isolated from four cervical mucus plugs from healthy mares at 10 months gestation, then analyzed by LC-MS/MS with Orbitrap analysis. Results were compared against the NCBI Equus caballus database. Proteins with a score greater than 20 combined with two independently confirmed high-confidence peptides were considered. Cervical mucus was also fixed and evaluated histologically. Overall, 78 distinct proteins from the equine genome were detected in equine cervical mucus during late gestation, primarily deriving from the innate and acquired immune systems. The most abundant protein was lactotransferrin, followed closely by immunoglobulins. Two clades of serine peptidase inhibitors were highly abundant, including alpha-1 antitrypsin and uterine serpins. Other abundant proteins involved in the immune response include complement, pantheinase, secretoglobin, serotransferrin and chitotriosidase. The most highly prevalent mucin in each of the four mares was mucin-5B, previously shown to be abundant during the luteal phase as well. Several endometrial proteins were detected as well. Histologic evaluation revealed distinct cellular and acellular regions, with pyknotic nuclei highly prevalent in the cellular regions. Together, these results suggest that the cervical mucus plug in pregnant mares is composed of both cervical and endometrial proteins. The cervical mucus plug is far more than a simple physical barrier, and draws in a number of components from both the innate and acquired immune systems to provide a formidable first line of defense against pathogens. (The authors would like to acknowledge Igor Canisso for his assistance in obtaining cervical mucus plug samples for this study.)

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Effects of Feeding a Yeast-Based Supplement Containing Selenized Yeast, Vitamin E and a DHA-rich Microalgae on Sperm Motion Characteristics**
Author(s):	L. D. Goedde, Dept of Veterinary Science, U of Kentucky K. M. Brennan, Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech Inc. B. A. Ball, Dept of Veterinary Science, U of Kentucky L. M. Lawrence, Dept of Animal Sciences, U of Kentucky M. H. Troedsson, Dept of Veterinary Science, U of Kentucky E. L. Squires, Dept of Veterinary Science, U of Kentucky

Abstract: The use of cooled and frozen stallion semen has become quite popular. However, there are some stallions that have sperm that are quite susceptible to cold shock. Thus, there is a need for a product that will alter sperm so that they can withstand the stress of cooling and freezing and thus improve pregnancy rates. Studies have shown that a diet high in omega-3 (n-3) fatty acids can improve the motility of cooled and frozen/thawed sperm. Many of the omega 3 fatty acid products for stallions have low levels of docosahexaenioc acid (DHA) and are based on fish oil, which may have reduced palatability. The objectives of this study were to determine if a DHA-rich microalgae meal would enhance the motility of fresh and cooled stallion sperm. Twelve stallions, 3 to 12 y old were used. Semen was collected every other day for two wks (July) and sperm motion parameters (total and progressive motility) were determined by computer assisted motility sperm analysis (CASA) on the last three ejaculates. These ejaculates were cooled to 5C (Equitainer, Hamilton Thorne) and held for 48 hr. Stallions were then paired based on CASA values for fresh and cooled semen, age of stallion, sperm output and body condition. Stallions were fed one of two dietary treatments for 60 d: A basal diet, Control, 0.4% BW as concentrate and 1.8% BW as grass hay, and DHA, basal diet plus 160 g of a yeast-based supplement containing selenized yeast, vitamin E and a DHA-rich microalgae (Schizochytrium limacinum CCAP 4087/2; Alltech Inc., Nicholasville, KY) to provide 2mg Se, 1000IU vitamin E and 15g DHA. Consumption of the supplement was accepted within a few days of feeding. Beginning on day 46, stallions were collected every other day until day 60. Sperm motion parameters were assessed with CASA. Data were averaged for the last 3 collections of the pre-treatment and post-treatment and log transformed. A paired T test was used to compare pre and post-treatment values within control and treated stallions. There was an increase ($P< 0.05$) in total motility for DHA stallions at 0 (63.6, 68.4), 24 (51.6, 58.4), and 48 (45.9, 52.1), hr of cooling from pre-treatment to post-treatment, whereas means for controls were similar between pre and post-treatment samples. Also, progressive motility increased ($P< 0.05$) in treated stallions between pre and post-treatment at 0 (59.2, 64.8), 24 (46.1, 52.7) and 48 (40.5, 47.3) hr but not in controls. Based on these data, this dietary supplement was effective in improving motility of stallion sperm after 60 days of feeding.

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34th Annual Symposium in Reproductive Science and Women's Health
Oral Presentation Abstracts

Abstract Title:	**Determination of Peripheral Progestin Concentrations in the Late Pregnant Mare Based Upon Immunoassay and Liquid Chromatography - Tandem Mass Spectrometry**
Author(s):	M. Wynn, Department of Veterinary Science, U of Kentucky E. Legacki, Department of Animal Science, U of California Davis A. Conley, Department of Veternary Medicine, U of California Davis S. Loux, Department of Veterinary Science, U of Kentucky A. Esteller-Vico, Department of Veterinary Science, U of Kentucky T. Curry Jr., Department of Obstetrics and Genecology, U of Kentucky S. Stanley, Department of Veternary Medicine, U of California Davis E. Squires, Department of Veterinary Science, U of Kentucky M. Troedsson, Department of Veterinary Science, U of Kentucky B. Ball, Department of Veterinary Science, U of Kentucky

Abstract: Maintenance of pregnancy in late gestation in the mare is dependent upon several progestins originating from the feto-placental unit. Currently, both progesterone (P4) and 5α-dihydroprogesterone (5α-DHP) are known bioactive progestins in the mare. Peripheral progestin concentrations are used to evaluate problems during late pregnancy in mares; however, the interpretation of immunoassay results is complicated by variable cross reactivities of antibodies to other progestins and by high concentrations of several progestins during late gestation. The objective of this study was to compare progestin concentrations as determined by four immunoassays with the concentrations of P4 and 5α-DHP determined by LC-MS/MS in four mares between gestational age day 298 and parturition. Samples from every fourth day starting at day 298, and daily for five days prior to parturition were selected and assayed across the following platforms: Immulite 1000, miniVIDAS, and two pre-validated ELISAs using antibodies R4859 and CL 425. Data were analyzed by regression analysis of the immunoassay progestin concentration against both P4 and 5α-DHP as determined by LC-MS/MS. Regression analysis for P4: Immulite =2.4+3.4xP4; miniVIDAS=21.8+19.7xP4; antibody R4859=44.3+63.7xP4; antibody CL 425=73.6+156.7xP4. Regression analysis for 5α-DHP: Immulite =1.3+0.07xDHP; miniVIDAS=13.4+0.47xDHP; antibody R4859=3.6+1.78xDHP; antibody CL 425=-20.4+4.26xDHP. Hormone concentrations found in the immunoassays significantly correlated with the values determined by LC-MS/MS, displaying a similar pattern over time. However, individual samples varied across the immunoassays. These date confirm progestin determination by immunoassay during late gestation varies depending upon the assay used and the apparent cross reactivity to other progestins present in high concentrations during late gestation in the mare. More widespread application of LC-MS/MS for determination of progestins in the late pregnant mare will enhance the interpretation of progestin concentrations.

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