41.5% (17/78), respectively. There were no differences between CRs and NCRs in rates of initial transfer (55.3 versus 48.4%, p = 0.5485), pregnancy at day 42 (34.0 versus 25.8%, p = 0.1367) or embryo loss (38.5% versus 46.7%, p = 0.7449) rates. Results suggest that NCRs treated with E_2 and P_4 prior to transfer may be suitable recipients for IVP embryos.

Keywords: Embryo transfer, IVP embryos, recipients

Reference

1.Cuervo-Arango J, Claes AN, Stout TAE: In vitro-produced horse embryos exhibit a very narrow window of acceptable recipient mare uterine synchrony compared with in vivo-derived embryos. Reprod Fertil Dev 2019;31:1904-1911.

Serum prostaglandin E metabolite in diestrous and pregnant mares

Mariana Diel de Amorim,[a] Michael Byron,[a] Claire Card[b]

[a]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY; [b]Department of Large Animal Clinical Sciences; Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

In the nonpregnant mare, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is luteolytic, and in early pregnancy prostaglandin E_2 is reported to be luteotrophic. However, there is a lack of information on circulating concentrations of prostaglandin metabolite (PGEM) during diestrus and pregnancy. We hypothesized that circulating concentrations of PGEM increase in pregnant mares during the expected time of pregnancy recognition day (D) D13 - D15 compared to diestrous mares. Our objective was to compare daily/hourly plasma PGEM concentrations and secretion profiles in pregnant and diestrous cycles using a randomized cross over design (n = 4 mares), with 1 cycle in between study periods. Transrectal ultrasonography was used to detect estrus, day of ovulation (D0), and pregnancy. Mares were bred to a fertile stallion during estrus. Blood was sampled on D0, 4, 8, 12, 18, and 20. A jugular catheter was used to obtain hourly blood samples from D13 through D16. Blood was placed in chilled EDTA tubes and immediately centrifuged at 4 °C. Plasma was separated, placed in cryovials, frozen in liquid nitrogen, and stored at -80 °C until assayed. PGEM was measured using commercial enzyme-linked immunosorbent assays (ELISA) (Cayman Chemical, Ann Arbor, MI) validated in our laboratory according to the manufacturer's instructions. Progesterone concentrations were determined every 6 hours from D13 to D16 (Siemen's Immulite, Los Angeles, CA). Both assays had an intra- and inter-assay coefficient of variation (CV's) of < 15.8%. Statistical analysis was performed on JMP® Pro 15 at $p < 0.05$ using Wilcoxon tests to compare differences between plasma PGEM in diestrus and pregnant cycles. Differences between days and times were compared individually by Student's *t*-test. One mare failed to become pregnant. Diestrous mares had higher ($p < 0.0001$) overall plasma PGEM concentrations from D0 to 20 compared to pregnant mares (mean ± SD) (30.7 ± 15 pg/ml and 17 ± 6 pg/ml, respectively) PGEM concentrations were also higher ($p < 0.0002$) in diestrous mares compared to pregnant mares for D13, 14, 15 and 16. The PGEM secretion profile was substantially different than that previously reported for PGFM. Pregnant mares had small peaks of PGEM that were different ($p < 0.05$) from diestrous mares on D13 and D14. This study is novel and demonstrated that plasma PGEM concentrations in diestrous mares are higher than in pregnant counterparts. However, a larger number of estrous cycles has to be studied to characterize the PGEM profile during early pregnancy. Further investigation of PGFM and PGEM in pregnancy is warranted to understand the importance of circulating concentrations and if the ratio or pattern of PGE:PGF may be altered during the expected period of luteolysis and maternal recognition of pregnancy.

Keywords: Equine, pregnancy, progesterone, prostaglandin

Cholesterol-loaded cyclodextrin improves cooling and fertility of donkey semen

Lorenzo Segabinazzi,[a,b] Veronica Scheeren,[b] Camila Freitas-Dell'Aqua,[b] Jose Dell'Aqua Jr,[b] Frederico Papa,[b] Marco Alvarenga,[b] Igor Canisso

[a]Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL; [b]Department of Animal Reproduction, São Paulo State University, Botucatu, Brazil

The donkey and mule show industry is an ever-growing industry. High-performance mules drive the stud fee values and increase their demand as a sire to breed mares and jennies. Cooled-shipped semen is the primary approach used by the industry. Skim milk-based (SKM) extenders are most used to cool and ship equid semen. However, donkey semen does not tolerate cooling with a SKM extender unless the seminal plasma is removed by centrifugation or 2% egg yolk is included as an additional cholesterol source. Neither approach is practical in ambulatory conditions; thus, alternatives must be identified. Inclusion of cholesterol-loaded cyclodextrin (CLC) in freezing extender improves post-thaw semen quality of donkeys; however, CLC has not been tested for cooling donkey semen. This study's objective was to compare semen parameters and fertility of cooled donkey semen extended in a commercially available SKM with and without CLC (SKM-CLC). We hypothesized that CLC enhances semen cooling and fertility of donkey semen. In the first experiment, 35 ejaculates from 7 mature jacks were split into SKM (BotuSemen, Botupharma) and SKM-CLC (BotuSpecial, Botupharma) groups and extended at 50 x 10^6 sperm/ml. After extension, samples were stored in a passive semen cooling container (BotuFlex, Botupharma) at 5 °C for 48 hours. Total motility (TM), progressive motility (PM), and percentage of sperm with rapid motility (RAP) were assessed with CASA (I.V.O.S. 12, Hamilton Thorne, Beverly, MA). Plasma membrane integrity (PMI), and mitochondrial membrane potential (MMP) were assessed with the combination of Yo-Pro® and MitoStatusRed with flow cytometry (LSR-Fortessa, Becton Dickinson, Mountain View, CA). Semen was assessed

before (time 0), 24, and 48 hours after cooling. In the second experiment, 2 estrous cycles of 15 mares were used for fertility assessment. Mares were examined every other day by transrectal ultrasonography (SonoScape A6®, China). Once a preovulatory follicle was detected (i.e. ≥ 35 mm in the presence of endometrial edema > 1, 0 absent and 3 max), ovulation was induced with 250 µg of histrelin acetate. At induction, semen from 1 jack was collected (n = 28), extended in either SKM or SKM-CLC, and cooled for 24 hours. Mares were randomly and equally assigned in a crossover for breeding with either extender 24 hours after induction of ovulation. Thereafter, mares were examined daily to detect intrauterine fluid accumulation and ovulation. Mares received oxytocin (20 units) to prevent intrauterine fluid accumulation. Pregnancy diagnosis was carried out on day 15 day after ovulation and mares received dinoprost (5 mg) intramuscularly to induce estrus. Data were analyzed with GraphPad Pris 8.0.1. (GraphPad, San Diego, CA). Semen parameters were analyzed with a Mixed model and Tukey's as posthoc. Pregnancy diagnosis was assessed with Fisher's Exact test. Significance was set at $p \leq 0.05$. There were no differences ($p > 0.05$) in TM, PM, RAP, PMI, and MMP for semen extended in either extender at time 0. There was a reduction in TM, PM, RAP, PMI, and MMP over time across groups; however, semen extended with SKM-CLC had superior ($p < 0.05$) TM, PM, RAP, PMI, and MMP than semen extended in SKM at 24 and 48 hours postcooling. Mares bred with semen extended in SKM had lower ($p < 0.05$) conception rate (13%, 2/15 cycles) than mares bred with SKM-CLC (47%, 7/15 cycles). Incorporating CLC to SKM extender improved semen parameters and fertility of cooled donkey semen

Keywords: Donkey, semen cryopreservation, extenders, chilling, cholesterol

Luteinizing hormone receptor activation stimulates endothelial adhesion of neoplastic canine T-lymphocytes

Alexa Dietz,[a] Michelle Kutzler[b]

[a]College of Engineering, Oregon State University, OR; [b]Department of Animal and Rangeland Sciences, College of Agricultural Sciences, Oregon State University, OR

Previous research from our laboratory has demonstrated that luteinizing hormone receptors (LHR) are expressed in neoplastic lymphocytes in canine lymph nodes and that activation of the LHR with human chorionic gonadotropin (hCG) increases LHR gene expression and cell proliferation in isolated neoplastic T-lymphocytes. The objective of the current study was to determine if hCG activation of LHR in neoplastic T-lymphocytes increase their adhesion to an endothelial cell monolayer. The hypothesis was that increasing hCG concentration induces a dose-dependent increase in neoplastic T-lymphocyte adhesion. Canine aortic endothelial cells (#Cn304-05, Cell Applications, Inc.) were cultured to form a monolayer. Endothelial cells were activated with tumor necrosis factor-alpha for 12 hours. Immortalized T-cell lines isolated from 3 dogs (CLC, EMA, CLK) with multi-centric lymphoma were cultured for 72 hours with increasing concentrations of hCG (from 4 - 4,000 IU/ml). Neoplastic T-lymphocytes were then fluorescently labeled (CytoSelect LeukoTracker, Cell Biolabs, Inc.) and added to the endothelial monolayer. After a 2-hour incubation, non-adherent cells were removed by washing. Images of adherent cells were digitally captured (#QIC-F-M-12-C, QImaging) at 400 x magnification using fluorescent microscopy (#DM4000B, Leica Microsystems). Adherent cells were then quantified on a fluorescence plate reader (Synergy 2, Biotek) using 50% gain. Four replicates of each T-cell line were used for each assay and the assays were repeated 3 times. Results (mean ± SEM) were expressed as a fold of baseline and compared between different hCG concentrations using a one-way analysis of variance (GraphPad Prism). Significance was defined as $p < 0.05$. Activation of LHR in neoplastic lymphocytes increased cell adhesion in a dose-dependent manner in all 3 cell lines (CLC: $p = 0.030$; EMA: $p = 0.016$; CLK: $p = 0.004$). Increases in hCG concentrations stimulated more neoplastic T-lymphocyte adhesion (Figure). This is the first study to demonstrate that activation of LHR in neoplastic canine lymphocytes increases endothelial cell adhesion. These results could explain why gonadectomized dogs with elevated circulating LH concentrations develop lymphoma at higher rates than intact dogs.

Keywords: Cancer, dog, human chorionic gonadotropin, lymphoma

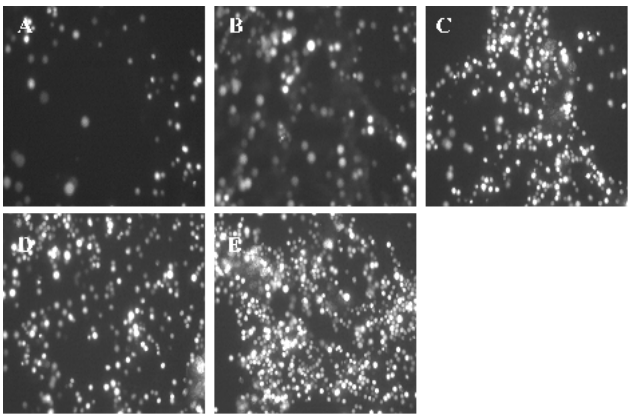

Figure. Neoplastic T-lymphocyte adhesion A: hCG 0 IU/ml; B: hCG 4 IU/ml; C: hCG 40 IU/ml; D: hCG 400 IU/ml; E: hCG 4000 IU/ml.