Temporal associations of B-mode, power doppler, and ovarian steroid changes of the periovulatory follicle and corpus luteum during luteogenesis and luteolysis in jennies

Humberto Magalhaes,[a,b] Jose Dell-Aqua Jr,[b] Igor Canisso[a]

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

Assessing the relationships between B-mode and power doppler ultrasonography of the periovulatory donkey follicle and respective corpus luteum (CL) could prove useful in clinical practice to predict impending ovulation and determining CL viability. This study's objectives were to assess the associations between B-mode and power doppler ultrasonography and ovarian steroids of the periovulatory follicle and respective CL during luteogenesis and luteolysis in jennies. We hypothesized that information on changes in the granulosa are useful to detect impending ovulation and on blood flow are useful to differentiate an active versus inactive CL. One inter-ovulatory interval between 2 subsequent ovulations of 12 jennies (144 ± 22.5 kg; height 95.5 ± 113 cm) was used. Jennies were teased daily to a mature jack. B-mode ultrasonography was carried out until the detection of a periovulatory follicle (≥ 28 mm, endometrial edema, and estrous signs). Thereafter, jennies were monitored every 4 hours by B-mode and power doppler transrectal ultrasonography. Once presumed signs of impending ovulation (thickened and irregular follicular wall, hyper-echogenicity of granulosa layer in transrectal ultrasonography and softened follicle in transrectal palpation) were detected, jennies were reexamined at 1 hour intervals until ovulation. After ovulation, the CL was examined daily until the completion of luteolysis (progesterone < 1.5 ng/ml). Plasma estradiol and progesterone concentrations were assessed daily with chemiluminescence assays (Immulite 1000, Siemens, US). Data were analyzed using RM-ANOVA followed by Tukey`s test (steroid concentrations, follicle and corpus luteum parame-ters), Friedman test adjusted by Dunn's test (edema score and behavior), and Pearson's coeffi-cient correlations (thickness and echogenicity of the granulosa layer). Mouth-clapping, a species-specific estrous sign, was the first and the last sign to be detected (± 24 hours postovulation). The diameter of the ovulatory follicle was 34.6 ± 3.3 mm (31 - 38 mm). The echogenicity and the thickness of the granulosa layer increased ($p < 0.05$) from 36 to 1 hour before ovulation in 70% of jennies; strong correlations between thickness ($r = 0.70$), granulosa echogenicity ($r = 0.80$), and impend-ing ovulation were noted. Follicular wall blood flow increased ($p < 0.05$) from 72 to 24 hours before ovulation and estra-diol concentrations declined from 42 pg/ml at 72 hours to 31.6 pg/ml at 24 hours before ovulation. Vascularization of periovulatory follicle decreased ($p < 0.05$) from 62% (36 hours before ovulation) to 37% (1 hour before ovulation); 75% of the jennies had a homogenous CL echogenicity with a white hyperechogenic central lacuna. The maximum CL size represented 76% of the periovulatory follicle diameter. Vascularization of the CL and progesterone concentrations had a gradual rise, reaching the peak at 11 and 10 days after the ovulation, respectively ($p < 0.05$). Luteal echo-genicity increased ($p < 0.05$) 4 days after luteolysis as a consequence of corpus albicans formation). Vascularization of the CL started to decline ($p < 0.05$) 3 days before luteolysis and progesterone concentrations had a sharp reduction ($p < 0.05$) for 4 days before luteolysis. In conclusion, the structural changes of the periovulatory follicle detected on B-mode can be used to detec impending ovulation in donkeys; however, B-mode ultrasonography cannot be used to assess CL functionality. Conversely, power doppler can be used to differentiate a functional versus nonfunctional CL in jennies.

Keywords: Periovulatory period, luteogenesis, luteolysis, steroid

Suitability of noncycling recipient mares for in vitro produced equine embryos

Charles Scoggin, Etta Bradecamp, Jamie Kaczor, Erin Lohbeck, Crystal Howard, Holly Hersey, Alaina Broach

Rood and Riddle Equine Hospital, Lexington, KY

Suitable cycling recipient mares are not always available at an equine embryo transfer station (ETS). Noncycling recipients primed with estrogen followed by progesterone before transfer of in vivo embryos can have similar pregnancy rates as those that are cycling. However, in vitro produced (IVP) embryos may be less hardy and require a more precise window of synchrony with recipient mares compared to in vivo embryos.[1] To our knowledge, no data exist on the suitability of noncycling recipient mares when transferring IVP embryos. A retrospective study was conducted whereby data from a single ETS during the 2020 breeding season were analyzed and pregnancy rates were compared between cycling and noncycling recipients receiving IVP embryos. All embryos were derived from commercial donors and transferred at the ETS. Cycling recipients (CRs) were examined via transrectal ultrasonography until emergence of a dominant follicle, then evaluated daily until ovulation and subsequently received an IVP embryo 4 days postovulation. Noncycling recipients (NCRs) were determined to be anestrus or transitional by a lack of luteal tissue and the presence of small follicles based on transrectal ultrasonography and serum progesterone concentrations. Initially, NCRs were treated intramuscularly with 10 mg estradiol-17β in oil (E_2) on the first day, 6.6 mg on the second day, and 3.3 mg E_2 on the third day. The day after the last E_2 treatment, NCRs were treated intramuscularly with 200 mg progesterone in oil (P_4); once a day for 3 consecutive days. On the fourth day (at transfer), NCRs received intramuscularly 500 mg of P_4 and were supplemented with P_4 at least until their first pregnancy examination. Data were analyzed using Chi-Square or Fisher's Exact tests, and values considered significant at $p < 0.05$. During the study, 78 IVP embryos were shipped to the ETS. Of these, 60.3% (41/78) were transferred into CRs and 39.7% (31/78) were transferred into NCRs. Overall transfer rate for IVP embryos was 52.6% (41/78). Day 42 pregnancy and embryo loss rates were 30.8% (24/78) and

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

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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