

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

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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  $\pm$  SD) ( $30.7 \pm 15$

pg/ml and  $17 \pm 6$  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

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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  $50 \times 10^6$  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