with pH to predict donkey parturition. Jennies had a high placental efficiency, as demonstrated by the high placental, dam, and foal ratios.

Keywords: Predicting parturition, foaling, periparturition, donkey, pH, electrolytes

Clinical and physiological ultrasonography of normal and abnormal donkey pregnancies

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Ultrasonography of the fetoplacental unit is carried out to detect abnormalities and to stage pregnancy. Transrectal and transabdominal ultrasonography have well-established physiological parameters and clinical applications in horses, but these techniques have not been characterized or established in donkeys. Season and sex of the foal are known to affect pregnancy length in horses but their effects are not known in donkeys. Pregnancy loss in horses is associated with abnormal progestin profiles but is not studied in donkeys. Major objective was to establish clinical and physiological ultrasonographic parameters of jennies carrying and delivering normal pregnancies and jennies undergoing premature delivery of stillborn foals. Additionally, effects of season and sex of the foal on gestational length (GL) were assessed. We hypothesized that season and sex of the foal affect GL, and pregnancy loss results in abnormal ultrasonography parameters and progestin profiles. Multiparous jennies (n = 140) ranging 4 - 16 years in age were enrolled by 120 days of pregnancy. Jennies were artificially inseminated with fresh semen during the spring, summer, and fall, in a single calendar year, all on 1 farm. All jennies were submitted to transrectal ultrasonography (Well. D, Medical Electronics Co., Shenzhen, China) coupled with a 7.5 MHz linear transducer at 15 day intervals until delivery. A subset of jennies (n = 50) had transabdominal ultrasonography (Well. D, Medical Electronics Co.) coupled with a 3.5 MHz sectorial convex transducer, also performed at 15 day intervals until delivery. Parameters assessed during each evaluation included combined thickness of uterus and placenta (CTUP) and fetal parameters (eyeball diameter, thorax, heartbeat, and aortic diameter). Serum samples were collected from each jenny during each evaluation for the determination of progesterone concentrations by RIA. Foals were weighed after birth. Data were assessed for normality with Shapiro-Wilk's test, and then ANOVA and Tukey's (aortic diameter, heartbeat, and thorax) or Kruskal-Wallis followed by Dunn's (eyeball and CTUP). Mixed models were used to assess the effects of season and interactions with foal sex and GL. Statistical significance was set at $p < 0.05$. The incidence of late pregnancy loss was 3.5% (5/140 jennies). The GL was 365.4 ± 10.4 days (range; 345 - 390 days) for jennies carrying and delivering normal pregnancies and was 345 ± 32.3 days (range; 290 - 352 days) for the group experiencing pregnancy loss. Spring bred jennies had the longest ($p < 0.05$) GL (375 ± 8.7 days), followed by summer bred (360 ± 32.3 days) and then fall bred (358.6 ± 5.8 days). Colts had longer GL than fillies (363 ± 10.2 versus 358.5 ± 9.3 days). There was no effect of GL on the foal's birth weight. There were significant associations between GL with eye orbit diameter (r = 0.70), fetal thorax (r = 0.80), fetal aortic diameter (0.60) and CTUP (r = 0.60). Fetal heartbeat (r = -0.9) was negatively correlated with GL. CTUP significantly increased from 150 days of pregnancy to term. Two jennies with premature deliveries had CTUP outside normal ranges and placental separation consistent with ascending placentitis; before abortion, these jennies also had an increase in progesterone concentrations in comparison to other jennies. The remaining 3 jennies undergoing premature delivery did not experience these changes. In conclusion, the study established clinical, physiological, and ultrasonographic parameters for donkey pregnancy. The incidence of late pregnancy loss was 3.5%. Spring-bred had the longest GL in jennies and colt-bearing pregnancies resulted in the longer GL than fillies; 40% of the abnormal pregnancies had abnormal CTUP, placental separation, and abnormal progesterone profiles.

Keywords: Fetoplacental unit ultrasonography, pregnancy loss, CTUP

No adverse effect of air exposure on stallion sperm motility after 48 hours of cooled storage

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Stallion semen may be collected, extended, and cooled for up to 48 hours prior to insemination. It is common practice to remove all the air from the package of extended semen prior to cooled storage. The aim of this pilot study was to assess the effects of air exposure on sperm motility parameters during 48 hours of cooled storage. We hypothesized that air exposure is associated with decreased sperm motility after 48 hours of cooled storage. A total of 12 ejaculates were collected (4 ejaculates from each of 3 stallions) using an artificial vagina. Semen was evaluated and diluted with a commercial extender (INRA 96, IMV Technologies, Maple Grove, MN) to a concentration of 25×10^6 progressively motile sperm per ml. The extended semen was aliquoted into 3 treatment groups. Group A: 40 ml of extended semen was placed into a 50-ml all-plastic syringe with all air removed (Henke-Ject®, Air Tite-Products Co., Inc., Virginia Beach, VA). Group B: 20 ml of extended semen was placed into a 50-ml syringe with all air removed. Group C: 20 ml of extended semen was placed into a 50-ml syringe along with 20 ml of air. The loaded syringes were placed into passive cooling containers (Equine Express II™ Cooled Semen Shipper™ boxes, Nasco, Fort Atkinson, WI) along with a frozen ice pack (PolarPack®, Sonoco, Hayward, CA). An aliquot (1 ml) of

semen was removed after 24 and 48 hours of cooled storage and warmed for 10 minutes at 37°C prior to evaluation of sperm motility parameters using a computer assisted sperm analysis unit (SpermVision®, Minitube of America, Inc., Verona, WI). Data are presented as a mean ± SD. A mixed model was fit to each response variable separately (SAS Institute, Carey, NC). Treatment (40 ml, 20 ml, or 20 ml plus air) was included as a fixed effect. Sample ID was included as a random effect to account for repeat observations on each sample. Tukey adjusted pairwise comparisons were also performed. Data were considered different at $p < 0.05$. Total sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 71.9 ± 14.3, 73.3 ± 13.3 and 76.3 ± 12.5 %, respectively. Total sperm motility values after 48 hours of cooled storage for groups A, B, and C were 65.6 ± 14.1, 65.8 ± 17.3 and 70.9 ± 12.8%, respectively. Progressive sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 66.7 ± 14.9, 67.8 ± 14.4 and 71.7 ± 14.3%, respectively. Finally, progressive sperm motility values for Groups A, B, and C after 48 hours of cooled storage were 60.3 ± 13.6, 60.8 ± 17.8 and 65.7 ± 14.3%, respectively. A difference ($p < 0.05$) in total and progressive motility was detected between Group A and Group C after 24 hours of cooled storage. There were no differences ($p > 0.05$) in total or progressive sperm motility values between aliquots of extended stallion semen in the presence or absence of air after 48 hours of cooled storage. These pilot data suggest that the necessity of removing all air during preparation of a cooled semen dose may not be as absolute as previously considered.

Keywords: Equine, cooled semen, air, sperm, motility

Comparison of nanoparticles and single-layer centrifugation for separation of dead from live stallion sperm

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Artificial insemination with fresh, cooled or frozen semen is commonly used in the equine breeding industry. Poor quality semen with a reduced number of live, motile sperm can lead to lower per cycle pregnancy rates. A study using boar sperm showed improved sperm motility when nanoparticles were used to separate dead from live sperm.[1] The objective was to determine if nanoparticles could separate dead from live stallion sperm. Our hypothesis was that iron-core nanoparticles bind to dead sperm and allow for subsequent separation from live sperm using a magnet. Experiment 1 compared 2 extenders (INRA 96 and TALP-E), 2 incubation temperatures (22 and 37°C) and 6 nanoparticle:sperm ratios (50, 100, 200, 400, 600, and 800 µl of nanoparticle working solution per 100 x 10^6 sperm) using magnetic nanoparticles (ST Genetics, Navasota, TX, US). A research model to mimic a poor-quality ejaculate was made by killing 50% of the sperm by submersion into liquid nitrogen. Experiment 2 compared sperm separation using single-layer centrifugation (SLC) with EquiPure™ (Nidacon International AB, Mölndal, Sweden) versus nanoparticle separation. In both experiments, total and progressive sperm motility, morphology, viability and acrosome status were evaluated. Statistical analysis was performed using one-way ANOVA (data presented as mean ± SD). Values were considered different at $p < 0.05$. Results of Experiment 1: Total and progressive sperm motility were not different between INRA 96 and TALP-E extenders or when incubated at either 22 or 37°C or when using 400 or 600 µl of nanoparticle solution per 100 x 10^9 sperm. Results for Experiment 2: Progressive sperm motility was higher ($p < 0.05$) after SLC (76 ± 9%) than after either nanoparticle treatment (59 ± 12%) or an untreated control (47 ± 5%). In addition, the percentage of viable and acrosome intact sperm was higher after SLC (61 ± 11%) than after nanoparticle treatment (43 ± 3%) or an untreated control (35 ± 3%). There was no statistical difference in sperm morphology among groups. In summary, under the current study conditions based on an induced sperm damage model, single-layer centrifugation performed better than nanoparticles for separating dead from live stallion sperm.

Keywords: Stallion, sperm, nanoparticles, single-layer centrifugation

Reference

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Comparing serum progesterone measurements by a point-of-care analyzer with a chemiluminescent immunoassay in bitch breeding management of the bitch

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Accurate serum progesterone measurements for timing bitches during breeding management is critical for reproductive practice. By monitoring the rise in progesterone during estrus, it is possible to predict the date of ovulation and the peak window of fertility, which is especially important as artificial insemination has become routine to facilitate breeding of animals that are geographically or temporally separated. Although progesterone is a highly conserved molecule across species, laboratory methods for measuring serum progesterone concentrations in the dog vary in accuracy and precision. To measure serum progesterone, chemiluminescent immunoassay (CLIA) has replaced radioimmunoassay as the current standard in the bitch, due to its high correlation and increased practicality. In January 2019, a colorimetric point-of-care (POC) immunoassay was released as an in-clinic diagnostic for quantitative canine