

was guided through the cervix and the uterus insufflated with room air. In 5 mares, a 600  $\mu\text{m}$  laser fiber was advanced through the biopsy channel, and using a diode laser (Dornier Medilas D, Dornier MedTech America, Inc., Kenneaw, GA), set at 20 W, 2 - 6 direct contact pulses of 3 - 5 seconds in duration were delivered at the oviductal papilla. The endoscope was then guided up the contralateral uterine horn and the process repeated. Total energy delivered ranged from 700 to 1500 J. In 2 control mares, the oviductal papillae were visualized, but no laser ablation was performed. All mares received 5 mg dinoprost IM, and a uterine lavage was performed using 1 - 3 liters of lactated ringer's solution within 4 hours postprocedure. Transrectal ultrasonography was performed every 2 - 3 days until a 35 mm follicle was detected, and then mares were bred using a semen sample from a fertile stallion with a minimum of  $500 \times 10^9$  progressively motile sperm. Mares were bred every 48 hours until ovulation, and pregnancy status was determined by transrectal ultrasonography 14 days post-ovulation. After examination, mares were treated intramuscularly with 5 mg of dinoprost to induce luteolysis and were rebred on 2 - 4 consecutive estrous cycles. Control mares conceived on 6 out of 9 cycles (67% pregnancy rate). Pregnancy rate was lower ( $p = 0.003$ ) in the treatment group (5%, 1 out of 20 estrous cycles). The first mare that was laser ablated conceived on the 4<sup>th</sup> cycle, and repeat hysteroscopy determined that the left oviduct was not effectively ablated. The procedure was repeated, and the mare reenrolled in the breeding trial. The third mare enrolled in the study developed fever and tachycardia 6 hours after hysteroscopy and was diagnosed with peritonitis via abdominocentesis. The mare was treated with broad spectrum antibiotics and recovered uneventfully. The mare that developed postprocedure complications was believed to be due to equipment difficulties early in the development of the procedure, and the 3 final mares included in the trial had no inflammation on peritoneal fluid evaluation after oviductal ablation, and no change in physical exam parameters. In conclusion, when the laser ablation of the oviductal papillae was appropriately performed, scar tissue formation effectively prevented pregnancy for a minimum of 4 months postprocedure. Laser ablation of the oviductal papillae is a promising technique for permanent sterilization of the mare and a follow-up long-term fertility study is warranted.

**Keywords:** Antisperm antibodies, epididymis, blood-epididymis barrier, epididymitis

### Mammary gland electrolytes and pH to detect impending parturition in jennies

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Assisted foaling is ideal for maximum foal survival, as it allows for early intervention to cope with simple dystocia or referral to appropriate tertiary veterinary care facilities. Unfortunately, most jennies foal unattended, and a high incidence of donkey

foal mortality is a common problem in the breeding industry. The prediction of parturition is critical to ensure assisted delivery. Because equids have a prolonged pregnancy and foal during the night, continuous foaling monitoring is not feasible on small farms. Thus, serial assessment of mammary gland electrolytes and pH are used to circumvent this issue to detect impending parturition in mares but not in jennies. Major objective was to determine the usefulness of serial assessment of mammary gland electrolytes and correspondent pH to detect impending parturition in jennies. In addition, the relationships between maternal, fetal membranes, and foal birth weight were investigated. We hypothesized that serial assessment of mammary gland pH predicts foaling in jennies. Multiparous jennies ( $n = 37$ ) were monitored daily starting from 350 to 355 days of pregnancy until foaling. The pH of mammary gland secretions was assessed daily with a hand-held device (LAQUA Twin pH Meter, Horiba, Irvine, CA). Aliquots of mammary secretions were frozen daily and then assessed retrospectively for electrolyte concentrations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ ) (Beckman Coulter, Switzerland) starting 5 days before foaling. Electrolyte concentrations and pH values were analyzed with a mixed-effect model. Student's t-test was performed to compare dependent variables (pregnancy length, birth weight, placental weight, and umbilical cord length) according to foal sex as an independent variable. Sensitivity, specificity, and negative predictive value (NPV) and positive predictive values (PPV) were evaluated using a cut-off value for  $\text{pH} \leq 6.4$  and  $\text{Ca}^{2+} > 10 \text{ mmol/l}$ . Most foalings (91.9%) were during the night. The overall pregnancy length was  $374 \pm 8.7$  days (range 357 - 390 days). There were no differences ( $p > 0.05$ ) in pregnancy length for colts ( $374 \pm 2.1$  range 357-385 days) and fillies ( $373 \pm 2.3$  range 358 - 390 days). Colts and fillies were 61.8 and 38.2%, respectively. Fetal membranes weighed  $3.4 \pm 0.1$  kg (range 1.9 - 4.7 kg). Foals at birth weighed  $31.1 \pm 2.5$  kg (range 26.5 - 37.5 kg), with no differences ( $p > 0.05$ ) in birth weights for colts ( $31.1 \pm 2$ ; range 26.5 - 37.5 kg) and fillies ( $30.8 \pm 2.2$ ; range 26.5 - 34 kg). The ratio of foal birth weight with the dam's bodyweight was 9.7%, and the ratio with fetal membranes was 11%. There was a significant reduction in  $\text{Na}^+$  and an increase in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$  concentrations leading to foaling. The pH of mammary secretions glands decreased during the 5 days preceding parturition. Additionally, 2 distinct profiles for pH reduction were recorded, with 32% of the jennies displaying a fast reduction in pH values (profile 1) and 65% presenting a slow reduction in pH (profile 2) from the mammary gland secretions; 3% foaled with high and alkaline pH ( $\text{pH} = 7.5$ ). The pH had a 90% sensitivity for foaling within 24 hours, whereas the specificity was 70%, and the PPV and NPV values were 40 and 97%, respectively. Of interest,  $\text{Ca}^{2+}$  ( $> 10 \text{ mmol/l}$ ) had a sensitivity and specificity of 71 and 85%, respectively, whereas the PPV and NPV were 72 and 84%, respectively. In conclusion, daily measurements of the pH of mammary gland secretion can predict foaling in jennies, whereas  $\text{Ca}^{2+}$  is a useful marker to determine when parturition will not occur. Therefore,  $\text{Ca}^{2+}$  needs to be associated

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 progesterin 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 progesterin 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  $365.4 \pm 10.4$  days (range; 345 - 390 days) for jennies carrying and delivering normal pregnancies and was  $345 \pm 32.3$  days (range; 290 - 352 days) for the group experiencing pregnancy

loss. Spring bred jennies had the longest ( $p < 0.05$ ) GL ( $375 \pm 8.7$  days), followed by summer bred ( $360 \pm 32.3$  days) and then fall bred ( $358.6 \pm 5.8$  days). Colts had longer GL than fillies ( $363 \pm 10.2$  versus  $358.5 \pm 9.3$  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  $25 \times 10^6$  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