COMPETITION SESSION

Epididymal sperm granulomas are associated with antisperm antibodies in frozen-thawed donkey semen

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Prior to ejaculation, sperm is stored in the epididymis for a variable time; the blood-epididymis barrier regulates exchanges of nourishment and hormones while maintaining sperm isolation. The innate and adaptive immune system intervenes when sperm is extravasated in the interstitium. Although several conditions are known to affect the integrity of the blood-epididymis barrier (i.e. trauma, toxicants, parasites, and infections) in domesticated mammals, epididymal sperm granulomas and antisperm antibodies are a rare finding and have never been described in donkeys. This study aimed to describe and compare semen parameters (pre- and post-freezing) and antisperm antibodies of donkeys with epididymal sperm granuloma (Granuloma) and healthy controls (Control). Feral donkeys (n = 10) castrated in a concurrent study were enrolled. Three donkeys had unilateral granulomas, 2 donkeys had bilateral granulomas, whereas the remaining 5 were grossly normal. The granulomas were either single or multiple, firm, well-circumscribed, tan to red, and 1 - 5 mm in size. Upon incision, abundant, thick, tan to white-yellow fluid was recovered. Histopathology revealed epithelioid macrophages, multinucleated giant cells, and abundant sperm cell fragments with mineralized cellular debris. Semen was harvested for cryopreservation through retrograde flushing of the cauda epididymis. Sperm concentration and motility parameters (total motility, TM; progressive motility, PM) were assessed with an automated sperm analyzer; plasma membrane integrity (PMI), and mitochondrial membrane potential (HMMP) were assessed with flow cytometry pre- and post-freezing. Postfreezing semen was assessed through flow cytometry for the presence of antisperm antibodies (IgG and IgA). Statistical analysis was performed with the Wilcoxon matched-pairs signed-rank test. Significance was set at $p < 0.05$. The total sperm yield did not differ ($p > 0.05$) between groups (Control 11.0 ± 2.0, Granuloma 9.0 ± 0.4 x 10^9). TM did not change ($p > 0.05$) after freezing in the Granuloma group (TM prefreezing 29 ± 6%, postfreezing 18 ± 3%). After freezing, PM and PMI of donkeys with sperm granuloma were lower ($p < 0.05$) than healthy ones (PM Control 15 ± 2%, Granuloma 7 ± 2%; PMI Control 51 ± 4%, Granuloma 36 ±5 %). Pre- and post-freezing HMMP did not differ ($p > 0.05$) among groups. Three of the 5 donkeys with granuloma had a percentage of IgG- and IgA-bound sperm above the maximum value observed in control donkeys. Mean percentage of IgG- and IgA-bound sperm did not differ ($p > 0.05$) among groups (IgG-bound Control 2 ± 0.4%, Granuloma 16 ± 10%; IgA-bound Control 0.1 ± 0.1%, Granuloma 0.5 ± 0.4%). In conclusion, sperm granulomas only marginally affected sperm quality and resulted in IgG and IgA antisperm antibodies binding to sperm. It remains to be determined if sperm granuloma and antisperm antibodies affect fertility in donkeys.

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

Laser ablation of the equine oviductal papilla as a novel contraceptive technique

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Reproductive control of wild horse populations has frustrated the bureau of land management for years. GnRH and Zona Pellucida vaccines have a limited duration of efficacy, intrauterine devices are unpredictably retained, and attempts to ovariectomize mares were met with public outcry in 2018. The development of a nonsurgical permanent sterilization technique has the potential to revolutionize management of wild horses and burros on public lands. The objectives of this study were to develop a safe and efficient sterilization technique, and to demonstrate technique effectiveness in reproductively healthy mares. We hypothesized that laser ablation of the oviductal papillae would be an effective method of permanent sterilization. Seven light breed reproductively healthy mares (5 - 21 years) were enrolled in the study after pregnancy confirmation at 14 days. Mares were given prostaglandin to induce abortion and within 14 days were sedated with xylazine hydrochloride for hysteroscopy. Examination of the endometrium and oviductal papillae was accomplished using a 103 cm flexible endoscope (Olympus GIF-160 Gastroscope, Center Valley, PA) attached to an Olympus EVIS EXERA CV-160 video processor. The endoscope

was guided through the cervix and the uterus insufflated with room air. In 5 mares, a 600 µm laser fiber was advanced through the biopsy channel, and using a diode laser (Dornier Medilas D, Dornier MedTech America, Inc., Kenneasaw, 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 x 10^9 progressively motile sperm. Mares were bred every 48 hours until ovulation, and pregnancy status was determined by transrectal ultrasonography 14 days postovulation. 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 4th 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 se-cretions 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 (Ca^{2+}, Mg^{2+}, K^+, and 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 pH ≤ 6.4 and Ca^{2+} >10 mmol/l. Most foalings (91.9%) were during the night. The overall pregnancy length was 374 ± 8.7 days (range 357 - 390 days). There were no differences ($p > 0.05$) in pregnacy length for colts (374 ± 2.1 range 357-385 days) and fillies (373 ± 2.3 range 358 - 390 days). Colts and fillies were 61.8 and 38.2%, respectively. Fetal membranes weighed 3.4 ± 0.1 kg (range 1.9 - 4.7 kg). Foals at birth weighed 31.1 ± 2.5 kg (range 26.5 - 37.5 kg), with no differences ($p > 0.05$) in birth weights for colts (31.1 ± 2; range 26.5 - 37.5 kg) and fillies (30.8 ± 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 Na^+ and an increase in Ca^{2+}, Mg^{2+}, and 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 (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, Ca^{2+} (> 10 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 Ca^{2+} is a useful marker to determine when parturition will not occur. Therefore, Ca^{2+} needs to be associated