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CLINICAL CASE SESSION

Proteomic analysis of sperm with impaired acrosomal exocytosis from a subfertile stallion

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Stallion subfertility due to impaired acrosomal exocytosis has been reported in Thoroughbred stallions that carry the genotype A/A-A/A for 2 SNPs in exon 5 of the FKBP6 gene (ECA13). These animals are subfertile despite having otherwise normal sperm quality and good breeding management. Mass spectrometry-based technologies are a powerful tool to investigate the potential causes of unexplained subfertility in the stallion and might further improve our understanding of the molecular processes that take place during fertilization. Herein, we describe a preliminary experiment conducted to compare the sperm proteome from a fertile stallion (percycle pregnancy rate = 60%) and a subfertile stallion that carries the susceptibility genotype for IAE (percycle pregnancy rate = 30%). Fresh semen from each stallion was processed to induce spontaneous acrosomal exocytosis (AE) using a lactate-only-containing modified Whitten's medium (Lac-MW). At 0, 4, and 6 hours of incubation, sperm aliquots were analyzed for sperm viability (VIAB) and the rate of AE in viable sperm (AE/VIAB) via flow cytometry (FITC-PSA and fixable live/dead red stain). Also, at each period, the sperm proteomes from each stallion were analyzed via data-independent acquisition mass spectrometry. Student's t-test was used to assess differences between experimental groups. During incubation in Lac-MW, VIAB was similar ($p > 0.05$) between both stallions and was not affected by incubation time. AE/VIAB increased ($p < 0.05$) over time (0 hour: 3%, 4 hours: 32%, and 6 hours: 56%) for the fertile stallion, but not ($p > 0.05$) for the subfertile stallion (0 hour: 3%, 4 hours: 5%, and 6 hours: 5%). Mass spectrometry analysis detected a total of 2,252 proteins in sperm (false discovery rate < 1.0%). Of these, 144 proteins exhibited differences in relative quantity between the fertile and subfertile stallion (log2 fold change; $p < 0.01$). Data analysis using the PANTHER protein class annotation system revealed that most of the proteins with lower abundance (▼) in the subfertile stallion belonged to the calcium-binding protein ($p = 3.36 \times 10^{-5}$), and the metabolite interconversion enzyme ($p = 1.11 \times 10^{-7}$) groups, whereas proteins with higher abundance (▲) included those of the chaperonin ($p = 8.25 \times 10^{-4}$), protease ($p = 5.21 \times 10^{-4}$) and histone ($p = 3.22 \times 10^{-11}$) groups. Among these, proteins of interest that were identified and are known to be related to sperm capacitation/acrosomal exocytosis and stallion fertility included: ADAM7 (▼), Annexin-A2, -A4, and -A5 (▼), Calpain-5 (▼), Kallikrein-1E2(▼), and CRISP-2 (▼). Current experiments are focused on determining the potential relation between the FKBP6 gene genotype and the changes observed on the sperm proteome of more IAE-affected stallions.

Keywords: Stallion subfertility, acrosomal exocytosis, FKBP6, proteome

Thoraco-omphalopagus conjoined twins in a Standardbred mare

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An eight-year-old Standardbred mare presented to The Ohio State University College of Veterinary Medicine in dystocia of 30 minutes duration. No breeding records were provided at presentation. Pregnancy had been confirmed in this mare at 14 days postovulation with 1 embryonic vesicle visible. On vaginal palpation, the fetus was in posterior presentation, dorsosacral position with hindlimbs extended and there were no signs of fetal viability. General anesthesia was induced to allow for better manipulation of the fetus for extraction. During controlled vaginal delivery, 2 hind limbs were identified and a fetotomy was performed at the level of the tibiotarsal joint. Following removal of 2 hind limbs, another hind limb was identified on palpation and removed via fetotomy at the level of mid-femur. Following removal of 3 hind limbs, 2 distinct pelvises were identified on palpation. Retropulsion of 1 pelvis was attempted, however no progress could be made to separate the suspected twins. Caesarean section was recommended, but humane euthanasia was elected due to financial constraints. Postmortem examination revealed a thoraco-omphalopagus conjoined twin following a ventral midline approach to the uterus. Prior to necropsy of the conjoined twin, a computed tomography scan was performed. The calvarium of the conjoined twin was fused at the level of the facial crest caudal to the orbit. Two independent vertebral columns with separate spinal cords were present connecting at a single sternum. One thoracic limb was present on

either side of the joined sternum. There was another fused thoracic limb that formed a bipedal hoof below the metacarpophalangeal joint with the metacarpal bones fused. There were 2 prepuces and 2 anuses present with meconium present in both anuses. Two tails were also present. There was an umbilical hernia present with small intestines protruding through the hernia. Respiratory and digestive tracts were fused at the larynx with a single trachea and esophagus. There was a single pair of lungs along with a single heart with 2 descending aortas. The single esophagus entered into a single stomach. The jejunum was divided into 2 ~ 90 cm oral to the ileocecocolic junctions. Caudal to that point, there were 2 ilea, ceca, and large intestines present with fecal balls in both small colons. Thoraco-omphalopagus conjoined twins have been reported in other species, including humans. To our knowledge, this is the first reported case of thoraco-omphalopagus conjoined twins in the horse.

Keywords: Mare, dystocia, twins, thoraco-omphalopagus

Anaphylactic reaction following intrauterine administration of misoprostol in a mare

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Deep horn intrauterine application of misoprostol, a synthetic prostaglandin E1 (PGE1), has been demonstrated to improve fertility in mares suspected of oviductal dysfunction.[1] Adverse reactions in horses included mild abdominal discomfort and soft feces after oral misoprostol and in women anaphylactic reactions were observed after oral and vaginal treatment. An 18-year-old Friesian mare weighing ~ 650 kg was presented for unexplained infertility of 3 years duration. Misoprostol (600 µg, Greenstone LLC, Peapack, NJ) dissolved in sterile water was deposited at the tip of each uterine horn.[1] Ten minutes after treatment the mare collapsed in the stall. Her mucous membranes appeared dark red with a prolonged capillary refill time of 4 seconds and she demonstrated tachycardia of 100 beats per minute, cool extremities, tachypnea of 60 breaths per minute, and was minimally responsive. Dexamethasone (50 mg) and flunixin meglumine (750 mg) were given intravenously in addition to 6 mg epinephrine. A 14-gauge intravenous catheter was placed and 5 liters of lactated Ringer's saline (LRS) was given. While the mare was laterally recumbent, a cuffed intrauterine catheter was inserted and the uterus lavaged with 9 liters of LRS in 3 liter aliquots. Fifteen minutes after the onset of treatment, the mare's heart rate and respiratory rate improved and she was able to achieve sternal recumbency and stand with encouragement. However, 15 minutes later she again became tachycardic and tachypneic and collapsed a second time. An additional 6 mg of epinephrine was given intravenously in conjunction with continuous bolus fluids and the uterus was again lavaged with 9 liters of LRS. Ten minutes after the second epinephrine the mare improved and regained ability to stand. Her vital signs gradually normalized, she passed normal manure and began to graze. Over the course of the 1.5-hour treatment window the mare received intravenously 50 mg dexamethasone, 750 mg flunixin meglumine, 12 liters of LRS, 2 doses (6 mg each) epinephrine, and twice 9 liters of uterine lavage with LRS. Following recovery, no further ill effects of the incident were noted. The mare was bred with fresh cooled semen 2 weeks later but failed to become pregnant. She had no previously reported medication allergies or history of drug reaction. This is the first reported adverse event of its kind associated with intrauterine PGE_1

Keywords: Misoprostol, intrauterine, adverse reaction, anaphylaxis

Reference

1. Alvarenga MA, Segabinazzi LG: Application of Misoprostol as a treatment of unexplained infertility in mares. J Equine Vet Sci 2018;71:46-50.

Next generation sequencing in deciding to discontinue antibiotic treatment in a stallion

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A 10-year-old Morgan stallion presented for test cooling of semen with a history of mares not conceiving from cooled shipped semen. Five weeks prior, the stallion was collected for semen shipment. The semen was greyish in color and had 60% progressively motile sperm (PMS), mild teratozoospermia, 58% morphologically normal sperm, and 4.48 x 10^9 total number of sperm. Penis was washed thoroughly before collection to minimize debris in the semen sample from the penis as a possible cause of semen discoloration. Semen was collected using a Missouri artificial vagina, and the semen sample was again greyish in color and had 3.4 x 10^9 total number of sperm, 60% PMS, and 66% morphologically normal sperm. Cytological examination of the semen sample with Diff-Quik stain had no leukocytes or germ cells. Large numbers of branching rods were observed on cytology and an aerobic culture was performed. A fastidious and slow-growing *Actinomyces* species was identified by culture. Antibiotic susceptibility was not possible due to the bacteria's slow-growing nature. *Actinomyces* has been reported to infect the testes and accessory sex glands in humans. Empirical antibiotic selection was based on published reports and oral doxycycline (10 mg/kg) was prescribed for 8 weeks. Stallion was presented again 43 days later. Semen appeared normal with improved number of total sperm (7.45 x 10^9), 60% PMS, and 90% normal morphology. A culture was performed, and the sample was submitted for next generation sequencing (NGS) of 16S rRNA for bacteria and ITS for fungi. The culture was negative, and NGS had 59% *Klebsiella oxytoca*, 18% *Petrimonas* sp., 7% *Streptococcus uberis*, 3% *Corynebacterium kroppenstedii*, 2% *Luteococcus* sp., and 2% *Proteiniphilum* sp. for bacteria and 91%