

air in MCDM [1], supplemented with 0.5% fatty acid free BSA, 15 ng/mL NIDDK-oFSH-20, 1 µg/mL USDA-LH-β-5, 1 µg/mL E₂, 50 ng/mL EGF and 0.1 mM cysteamine. Frozen semen from one stallion was used. For each replicate, one straw (200 × 10⁶ sperm) was thawed. For the density gradient, sperm were separated through a 90/45% Percoll gradient. For the swim up, thawed semen was overlaid with 1.5 mL of FCDM [1] containing 10 µg/mL heparin. Sperm were allowed to swim into the FCDM for 4 h. Zona pellucidae were removed by vortexing with 0.5 mg/mL hyaluronidase for 60 s and incubating with 1.5 mg/mL pronase for 5 min. Oocytes were washed in FCDM and transferred in groups of 15 into 25-µL drops of FCDM supplemented with 0.5% FAF-BSA, 2 mM caffeine and 2 or 10 µg/mL heparin under mineral oil. Groups of oocytes were co-incubated with 1 × 10⁶ sperm/mL for 18 h at 38.8 °C in 5% CO₂ in air. Presumptive zygotes were cultured in well-of-well plates [2] in CDM1 [1] for 2 days and in CDM2 [1] for 4.5 days at 38.5 °C in 5% O₂, 5% CO₂ and 90% N₂. Cleavage and cell numbers were evaluated 3 days after fertilization. Uncleaved oocytes/presumptive embryos were assessed to confirm fertilization using qRT-PCR for the bovine histone H2a.o variant and the equine CGB subunit. Data were analyzed by ANOVA and Tukey's hsd. No embryos developed using sperm separated by density gradient. Numbers of oocytes that cleaved and developed to eight cells were similar ($P > 0.05$) for ZP-intact and ZP-free oocytes (cleavage, 41.9% and 54.0%; eight cells, 23.6% and 30.3%, respectively) and for fertilization medium with 2 or 10 µg/mL heparin (cleavage, 55.2% and 40.7%; eight cells, 29.8% and 24.8%, respectively). Embryos did not develop past the 8-cell stage. Quantitative RT-PCR showed gene expression of bovine H2a.o in all uncleaved ova and presumptive embryos; equine CGB in 0 of 8 uncleaved ova; and 10 of 10 eight-cell stage embryos. Separation of equine sperm by swim up incubated with heparin (10 µg/mL) for 4 h increased the fertilization capacity compared to density separation; however, none of the heparin concentrations in the fertilization medium affected fertilization rates. We concluded that ZP-intact bovine oocytes may provide a practical system to study fertilizing capacity of equine sperm.

Keywords: Sperm; In vitro fertilization; Assisted reproduction; Capacitation; Equine

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DOI: 10.1016/j.theriogenology.2007.05.036

TREATMENT EFFICACY OF TRIMETHOPRIM SULFAMETHOXAZOLE, PENTOXIFYLLINE, AND ALTRENOGEST IN EQUINE PLACENTITIS

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Introduction: Successful treatment for equine placentitis remains elusive. In recent work in our laboratory, mares with experimentally induced placentitis, using an intracervical inoculation with *Streptococcus equi subspecies. zooepidemicus*, tended to carry pregnancies longer when treated with trimethoprim sulfamethoxazole (TMS; antimicrobial) and pentoxifylline (PTX; anti-inflammatory/anti-cytokine). The objective of the present study was to determine if long-term treatment with TMS, PTX, and altrenogest (ALT), a synthetic progestin, would improve pregnancy outcome in mares with experimentally induced placentitis. Altrenogest therapy was included for inhibition of uterine contractile activity. We hypothesized that combined treatment with TMS, PTX, and ALT would delay premature parturition in mares with experimentally induced placentitis and improve neonatal viability.

Materials and methods: Seventeen normal pregnant pony mares were enrolled in the study at 280–295 days of gestation. Placentitis was induced in all mares by intracervical inoculation of *S. equi subs. zooepidemicus* (10⁷ CFU). Five mares served as infected, untreated controls (Group CON). Twelve mares (Group TXT) were infected and given trimethoprim sulfamethoxazole (30 mg/kg, PO, q 12 h), pentoxifylline (8.5 mg/kg, PO, q 12 h), and altrenogest (0.088 mg/kg, PO, q 24 h) from the onset of clinical signs to delivery of a live foal or abortion. Fetal stomach and thoracic contents were obtained for culture from dead fetuses and blood samples were cultured from live foals after parturition. Uterine swabs were obtained for culture from mares within 2 h

after foaling. Effect of treatment on foal viability was determined using Fisher's Exact Test ($P < 0.05$ was considered significant).

Results: More ($P < 0.05$) mares in Group TXT delivered live, viable foals (10/12; 83%) than mares in Group CON (0/5; 0%). Gestational length was longer ($P < 0.05$) after infection in mares from Group TXT (mean = 31 days; range: 5–55 days) than Group CON (mean = 7 days; range: 2–17 days). Ten of twelve foals (83%) in Group TXT had negative blood culture results at birth. All foals in Group CON (5/5; 100%) had positive stomach content and thoracic fluid cultures, with *S. equi subs. zooepidemicus* recovered in samples from three of five (60%) foals. Five mares (42%) from Group TXT had no growth from uterine swabs obtained after foaling, whereas uterine cultures from six mares (50%) grew predominantly *S. equi subs. zooepidemicus* and *Enterobacter* spp in one mare. Predominantly *S. equi subs. zooepidemicus* was obtained from uterine cultures of all mares in Group CON (5/5; 100%).

Discussion: Long-term treatment with oral SMZ, PTX, and ALT resulted in longer pregnancies and more viable foals in mares with placental infections than untreated mares. We inferred that this combined regimen reduced effects of infection and inflammation in initiating preterm labor, but did not reliably eliminate bacteria from the uterus.

Acknowledgements: This work was funded by the Grayson-Jockey Club Research Foundation and Intervet.

Keywords: Equine; Pregnancy; Placentitis; Preterm labor; Treatment

DOI: 10.1016/j.theriogenology.2007.05.037

PHARMACOKINETICS OF CARBETOCIN, A LONG-ACTING OXYTOCIN ANALOGUE, FOLLOWING INTRAVENOUS ADMINISTRATION IN HORSES

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Carbetocin is a long-acting, synthetic analogue of oxytocin commercially available in Europe, Canada and Mexico. The objective of the present study was to investigate the pharmacokinetics of carbetocin after intravenous administration in four healthy, adult, non-lactating anestrus mares of mixed breed and one American Quarter horse gelding. All horses were given iv 2.5 mL of Hypophysin[®] LA (0.07 mg/mL, Veyx-Pharma GmbH, Schwartzenborn, Germany) that corresponded to 8.75 units of native oxytocin. They were monitored periodically throughout the study for elevations in temperature, heart and respiratory rates, and signs of pain or discomfort. Blood samples were collected from all horses at 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, 210, 240, 270, 300, 330 and 360 min and at 7, 8, 9, 10, 11, 12, 24, 36 and 48 h after the carbetocin treatment for determination of concentrations of plasma carbetocin by radioimmunoassay. Non-compartmental pharmacokinetic analysis was performed using a commercially available software program (WinNonlin, Version 4.0, Pharsight Corp, Mountain View, CA, USA). Data were reported as mean $\pm$ S.D.

Carbetocin was very well tolerated in all horses. Minor localized sweating in the neck and inguinal areas were seen in one out of five horses. No other major adverse reactions to carbetocin were observed during the study. The half-life ($t_{1/2}$) for carbetocin was 17.22 ± 3.79 min. The volume of distribution was 6.52 ± 1.64 L/kg and the clearance was 265.7 ± 64.1 mL/kg/min.

The $t_{1/2}$ of carbetocin in other species such as the goat (22.3 min), pig (85–100 min) and humans (60 min) is higher than that documented for horses in the present study. Previous studies in cattle and pigs have shown that the administration of carbetocin can result in intense myometrial activity for up to 6 h,