Cloprostenol sodium and dinoprost tromethamine are 2 most widely used and available $PGF_{2\alpha}$, with the former being a natural and the latter a synthetic prostaglandin. Label dose treatment of $PGF_{2\alpha}$ results in colic-like signs in mares, but the impact is unknown in donkeys. The latter species are thought to be more pain-tolerant than horses. This study aimed to objectively assess luteolysis and side effects of jennies receiving standard horse doses of cloprostenol and dinoprost. We hypothesized that the luteolytic doses widely recommended for horses have no side effects in donkeys and both types of $PGF_{2\alpha}$ have equivalent luteolytic properties. Eight jennies (144 ± 22.5 kg; height 95.5 ± 113 cm) were used. Five days after ovulation, jennies were randomly assigned in a cross-over design and received either intramuscular dinoprost (5 mg) or cloprostenol (250 µg). B-Mode and Doppler ultrasonography were performed starting 15 minutes before $PGF_{2\alpha}$, and then repeated at 15 minute intervals until 1 hour after $PGF_{2\alpha}$ and then at 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours. At these times, serum samples were collected for progesterone concentrations by RIA (Beckman Coulter, US). Animals were observed from a distance for side effects (sweating, abdominal discomfort, and diarrhea) at 15 minute intervals starting before and for 1 hour after $PGF_{2\alpha}$. Data normality was assessed with the Shapiro-Wilk's test and comparisons of the CL area and luteal blood flow were performed using PROC MIXED of SAS 9.4. The study was approved by the Ethics Committee on the use of animals – CEUA (UNESP, Brazil) under protocol 0028/2019. Jennies were accounted as random effect whereas time and luteolytic agent were fixed effects. Interactions of fixed effects were also assessed. Significance was considered as $p \leq 0.05$. An increase ($p < 0.05$) in CL blood flow was observed 60 minutes and 45 minutes after treatment with dinoprost and cloprostenol, respectively. There was an increase ($p < 0.05$) in CL blood flow at 4 hours after dinoprost compared to cloprostenol treatment. However, at hours 5, 6, and 7, jennies that received cloprostenol had higher CL vascularity than dinoprost-treated cycles. Blood flow and CL area decreased gradually during the first 24 hours in both groups. Both prostaglandins reduced ($p < 0.005$) serum progesterone concentrations within 30 minutes after treatment with no differences ($p > 0.05$) between groups. Dinoprost resulted in major score of sweating ($p \leq 0.05$) whereas higher ($p \leq 0.05$) abdominal discomfort and diarrhea were detected in cloprostenol. In conclusion, both prostaglandins and doses used were equivalent in inducing luteolysis in donkeys. However, both prostaglandins caused adverse reactions, leading us to believe that horse doses used are inappropriate for small-frame donkeys.

Keywords: Jennies, corpus luteum, Doppler ultrasonography, progesterone, side effects

Sperm-filter enhanced semen parameters and fertility of stallion poor cooled semen

Lorenzo Segabinazzi,[a,b] Igor Canisso,[b] Marcio Carmo,[a] Camila Dell'Aqua,[a] Jose Dell'Aqua Jr.,[a] Fredrico Papa,[a] Marco Alvarenga[a]

[a]Department of Animal Reproduction, São Paulo State University Botucatu, Brazil; [b]Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL

Cooled-shipped semen is the horse industry's primary approach to breed mares. Whereas most stallion ejaculates tolerate cooling some have inadequate responses to cooling. Despite the development of new extenders in the past 2 decades, some stallions still have poor semen cooling quality. Therefore, there is a critical need to develop tools to process semen for stallions with a poor response to cooling. Sperm-Filter® (SF, Botupharma) is a porous membrane used as an alternative to centrifugation. This technology has yet to be tested in stallions with poor semen cooling. Therefore, this study's objective was to assess semen parameters and fertility of cooled-stored stallion semen processed with SF or conventional centrifugation ([C] 600 x g for 10 minutes) and reextended in 3 commercial extenders. We hypothesized that SF enhances semen parameters and improves the fertility of stallions with poor semen cooling ability. The ejaculates were obtained from 7 stallions known to have poor semen cooling ability (i.e. < 25% total motility (TM) 24 hours postcooling at 5°C). After collection, semen was extended to 50 x 106 sperm/ml with a skim milk-based extender ([SM] BotuSemen, Botupharma) and stored at 5°C for 24 hours. At 24 hours postcooling, samples were split into 7 groups. Control (CT) consisted of cooled semen with no further processing and the remaining 6 groups were submitted to SF or C, then resuspended in either SM, SM containing pentoxifylline ([P] BotuTurbo, Botupharma), or an egg yolk-based extender ([EY] BotuCrio, Botupharma). Total and progressive motility (PM) and percentage of sperm with rapid motility (RAP) were assessed with CASA (IVOS 12, Hamilton Thorne, Beverly, MA). Plasma membrane integrity (PMI), and mitochondrial membrane potential (MMP) were assessed with the combination of Yo-Pro® and MitoStatusRed with flow cytometry (LSR-Fortessa, Becton Dickinson, Mountain View, CA). Five stallions (4 - 8 ejaculates) were used for breeding mares (CT, n = 19; SF-SM-P, n = 9; SF-EY, n = 18 estrous cycles). Data were analyzed with GraphPad Pris 8.0.1. (GraphPad, San Diego, CA). Parametric data were analyzed with ANOVA-RM with Tukey's as post-hoc. Nonparametric data were analyzed by Kruskal-Wallis followed by Wilcoxon–Mann–Whitney. Pregnancy rates were compared by multivariate regression. Significance was set at $p \leq 0.05$. Sperm kinetics (TM, PM, and RAP) increased ($p < 0.05$) in all samples resuspended EY compared to CT, SM, and semen centrifuged and resuspended in SM-P. Semen processed by SF and resuspended in SM-P was similar ($p > 0.05$) to EY groups. SM-P had superior ($p < 0.05$) results in all processed semen by SF compared to CT, whereas centrifuged semen had intermediate values ($p > 0.05$). There were no differences ($p > 0.05$) in PMI between CT and semen processed by SF. However, centrifuged semen had less ($p > 0.05$) PMI than SF processed semen. Additionally, mares inseminated with SF-SM-P (66%) or SF-EY (67%) had higher ($p < 0.05$) pregnancy rates than mares inseminated with CT (13%). In conclusion, sperm parameters of stallions with poor semen cooling ability were enhanced by

the removal of the supernatant and sperm resuspension with YG or SM-P. Additionally, processing semen by SpermFilter enhanced PMI compared to centrifuged semen. Fertility rates of poor cooled semen improved by semen processing by SF and resuspended in SM-P or EY.

Keywords: Stallion, extender, sperm kinetics, bad cooler

Pregnancy rates and subsequent pregnancy losses of in vitro produced embryos from oocytes

Etta Bradecamp,[a] Carly Garcia,[b] Charlie Scoggin,[a] Maria Schnobrich,[a] Crystal Howard,[a] Jamie Kaczor,[a] Erin Lohbeck,[a] Holly Hersey[a]

[a]Rood and Riddle Equine Hospital, Lexington, KY; [b]Lazy E Ranch, Guthrie, OK

Over the past 5 years there has been an increased utilization of transvaginal aspiration of oocytes and intracytoplasmic sperm injection (ICSI) to produce equine embryos. With this increase in demand has come the increase in the number of commercial ICSI labs and in the number of ICSI-produced embryos being shipped to commercial recipient herds for transfer. There are limited data in the literature describing the pregnancy rates and any subsequent pregnancy losses associated with these shipped ICSI-produced embryos. Data were collected from 2 facilities that performed a total of 572 aspirations over 3 breeding seasons and shipped the oocytes to 5 commercial ICSI labs. Embryos produced were shipped to 2 commercial recipient herds. Pregnancy rates and subsequent losses were calculated for 3 of the ICSI labs; 2 of the labs were not included due to a very small number of embryos transferred from these facilities. In total, 208 fresh embryos were shipped for transfer. Fourteen-day pregnancy rates ranged from 41 to 75%; pregnancy loss rates varied from 12.8 to 60% depending on the TVA Facility/ICSI Lab combination and year. Due to the variability in transfer results both between and within the same facilities, more in-depth research needs to be performed to identify the ideal shipping conditions (media, time in transport, etc.) to maximize pregnancy rates and minimize subsequent pregnancy losses.

TVA Facility/ICSI Lab/Year	Embryos transferred	14-day pregnancies	Pregnancies lost
TVA Facility 1/ICSI Lab A 2018	21	10 (47%)	6 (60%)
TVA Facility 1/ICSI Lab A 2019	12	9 (75%)	2 (22%) - 1 due to twins
TVA Facility 1/ICSI Lab A 2020	53	29 (55%)	8 (27.5%) - 3 due to twins
TVA Facility 1/ICSI Lab B 2019	31	15 (48%)	8 (53%)
TVA Facility 1/ICSI Lab B 2020	24	10 (41%)	5 (50%)
TVA Facility 2/ICSI Lab A 2020	21	14 (66%)	8 (57%) plus 2 late term
TVA Facility 2/ICSI Lab B 2019	13	8 (61%)	2 (25%)
TVA Facility 2/ICSI Lab B 2020	14	8 (57%)	1 (12.8%)
TVA Facility 2/ICSI Lab C 2020	19	13 (68%)	2 (15%)

Keywords: Mare, embryo, ICSI, trans-vaginal aspiration, pregnancy

Incidence rate of reproductive problems in nonpregnant mares

Patrick McCue, Melissa Prell, Christian Bisiau, Christina Divine, Jennifer Hatzel

[a]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Broodmares may be affected by a variety of reproductive issues. The goal of this retrospective study was to document reproductive abnormalities encountered in broodmare veterinary practice. Reproductive records of mares managed at Colorado State University were evaluated retrospectively. Reproductive issues were broadly categorized into abnormalities of the ovary, oviduct, uterus, cervix, vagina, perineum, mammary gland, and behavioral concerns. The abnormalities were then assigned to specific subcategories within each broad category. Reproductive records were evaluated for 636 individual mares over a 3-year period (2018 - 2020). A mare was considered positive for an abnormality if the issue was noted at least once during a breeding season. The incidence rate (IR) was calculated as the percentage of mares with a specific abnormality compared to the overall population of mares. Data are presented as the mean ± standard deviation. A total of 862 mare-years were evaluated, as some mares were evaluated over multiple breeding seasons. The average age of the mare population was 11.9 ± 4.8 years and ranged from 3 to 26 years. The most common breeds were American Quarter Horse (383 mares, 60.2% of total), Warmbloods (all breeds combined) (55 mares, 8.6%) and Arabians (26 mares, 4.1%). Most common ovarian issues noted were after 250 µg of cloprostenol treatment were, persistence of luteal tissue (62 cases; 7.2% IR) and hemorrhagic anovulatory follicles