into the right uterine horn. Uterine lavage was performed to aid in the removal of these structures with no success. A repeat uterine culture and cytology revealed moderate inflammation and a light growth of *Escherichia coli*. Hysteroscopy revealed several bony fragments within the right uterine horn and were extracted. There were in total 7 fetal bones, ranging from 1.5 to 2 cm consistent with 2 scapula, 2 pelvic bones, and 3 long bones. Mare was given a dose of broad-spectrum systemic antibiotics, the uterus was lavaged, and acetylcysteine was infused. Uterine lavage was continued for 3 more days. Two weeks later, a culture and cytology were performed and were negative. Mare was bred over 2 estrous cycles (~ 30 and 55 days after the procedure) and became pregnant with twins after the second estrus. One embryonic vesicle was successfully reduced and the mare was confirmed in foal with 1 fetus at 49 days of pregnancy. Due to the low prevalence of fetal mummification in the horse, the underlying cause of this phenomenon has been difficult to discern. When twins are present, placental insufficiency is typically the cause of fetal demise of 1 fetus followed by fetal fluid resorption. In singleton pregnancies, there has been no established cause for fetal mummification and why they are retained within the uterus. This case demonstrated the future fertility of mares after fetal mummification treatment.

Keywords: Mare, fetal mummy, uterine foreign body, endometritis

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Intra-uterine injection of amnion-derived acellular bioscaffold product in mares: systemic and intra-uterine effects over 21 days

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Amnion-derived acellular bioscaffold product (ADABP) has been used as an antiinflammatory agent to promote healing in human and veterinary medicine. Proteins and cytokines present in ADABP are reported to decrease fibroblast formation and fibrosis.[1] Thus, ADABP may be beneficial in the treatment of uterine adhesions, uterine cyst ablations and remodeling of scar tissue. The safety of uterine injection of ADABP is unknown. We studied the systemic and uterine effects after uterine injection. Twelve clinically healthy light-breed mares (mean age 11.5 years; range 5 - 22) were the subjects. Rectal temperature and behavior were recorded for the duration of the study. On day 0, all mares underwent a hysteroscopic examination, control mares (n = 3) received 3 ml injection of sterile saline in the base of 1 uterine horn, and AniCell mares (n = 9) received 3 ml of ADABP (EquusCell StemWrap D™, AniCell Biotech, Arizona) in the base of 1 uterine horn. Blood (for serum amyloid A [SAA], fibrinogen [FIB], and white blood cell count [WBC]), endometrial cytology and aerobic cultures were obtained prior to hysteroscopy. Four days (day 4) after injection, mares were evaluated via transrectal ultrasonography and blood was obtained. Twenty-one days (day 21) after injection, endometrial cytology, aerobic culture, and hysteroscopy were performed. Continuous data were analyzed to determine the main effects of group, day and their interaction using the SAS MIXED procedure with a repeated statement. Categorical data were analyzed using the SAS LOGISTIC procedure. No mares experienced an elevation in rectal temperature during the 21 days after injection. There were no differences in bloodwork for markers of inflammation (SAA, FIB, WBC) from day 0 to day 4 either in the control or AniCell group. Similarly, there were no differences in uterine cytology and culture results between groups or among days within groups. Hysteroscopy following injection demonstrated no gross evidence of detrimental effects in any mare examined. In 1 mare that received a saline injection, a small 1 cm bleb of fibrous tissue was noticed and that remained for 21 days after injection. This study demonstrated that ADABP had no detrimental effect on the systemic health of the mare and it is as safe as hysteroscopy and saline intrauterine injection up to 21 days after injection. Further work is continuing, evaluating histological changes in the mares' endometrium after injection and in clinical cases where injection is performed into uterine tissue, as ADABP may be a useful tool to promote endometrial healing in the mare.

Keywords: Amnion-derived cell product, endometritis, hysteroscopy, uterine injection

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Luteal blood flow and side effects of luteolytic doses of dinoprost tromethamine and cloprostenol sodium in jennies

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Exogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment revolutionized the breeding management of livestock and horses. However, despite 4 decades of its continued use in theriogenology, the optimal luteolytic dose for donkeys has not been determined.

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

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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