among treatments at any time point. In conclusion, addition of hyaluronic acid to 2 milk-based extenders did not affect motility parameters of fresh-cooled equine semen. Additional work is necessary to determine whether there is any benefit to stallion fertility with HA in semen extenders.

Keywords: Stallions, sperm, motility, fertility, hyaluronic acid

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Methods to prepare platelet-rich plasma

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Platelet-rich plasma (PRP) is a biological by-product commonly used in clinical practice to treat orthopedic and dermatologic conditions. Recently, use of PRP has become popular in management of a mare's reproduction to mitigate postbreeding induced endometritis and improve fertility. Currently, there are no standardized methods to prepare PRP for intrauterine use in mares. The aim of this study was to compare 3 methods of PRP preparation. Eighteen clinically healthy mares had blood collected via venipuncture in a blood transfusion bag (method 1), blood tubes (method 2), and a syringe (method 3). In method 1, blood was collected in a 150 ml blood transfusion bag containing 21 ml of citrate-phosphate-dextrose solution with adenine as an anticoagulant (CPD-A). After collection, blood was divided into 50 ml conical tubes and centrifuged at 400 x g for 10 minutes. Resulting plasma was split into 15 ml conical tubes and subjected to centrifugation at 1000 x g for 20 minutes. After second centrifugation, the 2.5 ml in the bottom of each tube was considered PRP, and the remaining plasma as platelet-poor-plasma (PPP). Method 2 involved centrifugation of blood collected in 4.5 ml vacutainer tubes containing 3.2% sodium citrate at 120 x g for 10 minutes. The top third layer of the plasma was deemed as PPP, while the remaining portion was considered PRP. In method 3, blood was collected in a 60 ml syringe containing 7 ml of CPD-A; after collection, each syringe was wrapped in aluminum foil and placed in an upright position for 4 hours. The top 10 ml of plasma was considered PPP, and the remaining plasma (including sedimented blood cells) was deemed PRP. After processing by 3 methods, PRP and PPP were extracted and assessed for red and white blood cell counts, platelet counts, and viability. In a subset of mares (n = 6), samples of PRP were also evaluated at 6 and 24 hours postcooling at 5°C. Method 1 resulted in the highest, and method 3 in the lowest, platelet concentrations; the latter had higher ($p < 0.05$) WBC than others. Platelet viability was similar among treatments. The recovery factor (i.e. the ratio of the PRP volume to the whole blood volume) of plasma recovered as PRP was different ($p < 0.0001$) among methods; method 1, 10.5%; method 2, 33.1%; method 3, 27.2%). Cooling for 24 hours did not affect ($p > 0.05$). platelet counts. However, platelet viability was reduced ($p < 0.05$) after cooling in PRP produced by method 3, and agglutination increased over time among methods. In conclusion, the 3 methods resulted in satisfactory PRP yield without compromising platelet viability. Method 1 (i.e. involving double centrifugation) resulted in the greatest platelet concentrations whereas method 3 (sedimentation) resulted in the lowest platelet concentration and tended to be more contaminated with leukocytes. Cooling affected platelet viability in PRP obtained by method 3 and increased platelet agglutination over time among methods. Clinical efficacy of PRP with these methods of cooling remains to be determined.
Funding: Cesarean section, hypercoagulable, pulmonary embolism, venous thromboembolism

Keywords: Platelet concentrates, horse, endometritis, tissue regeneration

Fetal bones in the uterus of a Thoroughbred mare

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Fetal mummification has been reported occasionally in domestic species. It is more common in polytocous species, but has been observed in the horse, most commonly in twin pregnancies. Mummification is typically a sterile process in which fetal death occurs, the conceptus dehydrates, and is retained within the uterus. This describes a case in a 7-year-old Thoroughbred mare diagnosed pregnant with 1 conceptus (evaluations normal with transrectal ultrasonography at 15, 17, 29, and 42 days postovulation). Transrectal palpation confirmed pregnancy at 5 months of pregnancy. At 7 months of pregnancy, ultrasonography revealed that the mare was not pregnant. Two uterine lavages were performed and 6 grams Timentin was infused into the uterus after lavage. Two months later, uterine cytology and aerobic culture performed prior to breeding season revealed severe inflammation and moderate growth of *Escherichia coli* and *Enterobacter aerogenes*. Mare's uterus was lavaged for 4 days and infused with 2 grams Amikacin, and a Caslick's was placed. Transrectal ultrasonography of the uterus performed 1 month later revealed multiple small (2 cm) hyperechoic linear structures in the uterine lumen at the base of the uterine horns extending

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.