

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

## References

1. Bruemmer J, Wilson C, da Silva MC et al: Effects of hyaluronan supplementation on cryopreserved equine spermatozoa hyaluronan and cryopreserved equine spermatozoa. *J Equine Vet Sci* 2009;29:223-228.
2. Talbot P, Shur BD, Myles DG: Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion. *Biol Reprod* 2003;68:1-9.

## 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.

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**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