Sperm motility and fertility of cooled preserved stallion semen in either INRA96 or EquiPro CoolGuard extender
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Semen extenders are added to stallion semen when processed for preservation under cooled conditions to aid in viability and to maintain its fertility over time. Increased duration of sperm quality and fertility potential of cooled semen is desirable because of the ease of use for breeders and veterinarians. Previous studies suggest that the chemically-defined extender INRA96 (IMV Technologies USA, Maple Grove, MN) is superior to most milk-based extenders when ejaculates are incubated at 4°C for up to 48 hours. EquiPro CoolGuard (Minitube of America, Verona, WI) is a new commercial semen extender that is defined specifically to maintain quality of semen and fertility after preservation at 4°C. The objectives of this study were to: a) evaluate the motility of stallion spermatozoa after cooling for 0, 24, 48, 72, and 96 hours in both INRA96 (INRA) and EquiPro CoolGuard (CG), and b) observe the fertility rates of a small group of mares inseminated with semen extended with CG at either the day of collection or 96 hours post-collection after cooled preservation in CG. We hypothesized that CG would record similar motility data to that of INRA, but that fertility rates would decrease after cooled storage of sperm for 96 hours. For the first experiment, semen was collected daily for three days from six stallions (n=6) using a Missouri model artificial vagina, after having been collected for a week prior to the experiment. Ejaculates were centrifuged at 600g to reduce the seminal plasma to 90% and reconstituted in INRA and CG, respectively, at a concentration of 20x10⁶ spermatozoa/mL. Samples were stored in an Equitainer® (Hamilton Thorne, Beverly, MA) to be gradually cooled to a final resting temperature of 4°C. Total motility and progressive motility were recorded via a CASA system (SpermVision, Minitube of America) at 0, 24, 48, 72, and 96 hours after collection. For the second experiment, nineteen mares were bred with 1x10⁹ sperm pooled from two stallions in 10mL CG either 6 hours after collection (n=11) or at 96 hours after collection (n=8). Statistical analysis for the first experiment was done via a two-way ANOVA on repeated measures with significance set to P<0.05. While total and progressive motility decreased over time, no significant differences were found between the two extenders for either endpoint at any time. Both extenders demonstrated support of semen up to 96 hours with average progressive motility being approximately 40% at the end of the experiment. Eight of the eleven mares (72.7%) who were bred with CG on the day of collection and six of the eight mares (75%) who were bred with CG 96 hours after collection were pregnant as determined by ultrasonographic evaluation at 24 days after insemination. In conclusion, similar motility data were observed for both extenders, demonstrating the ability to maintain acceptable sperm viability for up to 96 hours after collection. Recognizing that only a small number of mares were bred in this study, we also conclude that fertility rates were comparable to the rates seen in both natural breeding as well as artificial insemination using fresh semen when semen was extended with CG for 96 hours at 4°C.

Keywords: Extender, INRA96, CoolGuard, fertility, motility

Disclosure
M.H.T. Troedsson is affiliated with Minitube of America as a consultant.