

EFFECTS OF EXTENDERS AND WARMING TEMPERATURES ON COOLED STALLION SEMEN.

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Several extenders have been developed for cooling and transporting equine semen. The most commonly used are skim milk-based extenders; however, transportation of semen for assisted reproductive techniques such as GIFT or sexing of sperm may require extenders with less or no milk components. In most cases, cooled semen is inseminated into the mare's uterus with no prior warming. Samples of semen usually are rewarmed for evaluating motility after cooling; however, the warming procedure could affect final motility. Objectives of this study were to compare percentages of motile sperm (MOT), live sperm (LIVE) and sperm with high mitochondrial membrane potential (MITO) between fresh semen and semen held at 5 °C for 24 h in EZ-Mixin "CST" (CST; Animal Reproduction Systems, Chino, CA) or KMT (Padilla and Foote, 1991) and rewarmed in a water-bath at 20 or 25°C. A single ejaculate was collected from three stallions. Semen was extended with CST or KMT at 25 to 50 x 10⁶ sperm/ml. Fresh samples from each stallion and each extender (not cooled) were stained with SYBR-14/PI (LIVE) and JC-1/PI/SYBR-14 (MITO) and evaluated by flow cytometry. Semen from the same ejaculates was stored for 24 h at 5°C in an Equitainer (Hamilton Research, Inc., South Hamilton, MA). After storage, samples (10 ml) were warmed in water bath at 20 or 25 °C for 10 min and held at room temperature until evaluation by flow cytometry. Data were transformed using the arcsin and subjected to factorial ANOVA plus Tukey's h.s.d. test. Stallion main effects were significant ($P < 0.01$) for all responses (MOT, LIVE, MITO). Main effect means for LIVE and MITO were not different between fresh (81% and 61%) and cooled samples rewarmed at 20°C (80% and 59%) or 25°C (80% and 62%). MOT was higher ($P < 0.01$) for fresh sperm (82%) compared to cooled sperm rewarmed at 20°C (42%) or 25°C (50%). MOT was higher ($P < 0.05$) for samples warmed at 25°C than at 20°C, but there was no temperature effect for LIVE and MITO. When comparing CST vs KMT, main effect means for percentages of LIVE and MOT were higher for CST (83% and 61%) than KMT (78% and 56%; $P < 0.05$); however no extender effect was observed for MITO: CST (60%) and KMT (61%). In conclusion, cooling semen did not affect the percentages of live sperm and sperm with high mitochondrial membrane potential; however, cooling did have a deleterious effect on sperm motility. The percentage of motile sperm was slightly higher when semen was warmed at 25°C vs 20°C. The CST extender was more successful in maintaining sperm viability during cooling under current experimental conditions.

Research was funded through benefactors of the Preservation of Equine Genetics and the Lucy Whittier Foundation.

Keywords: Stallion, Spermatozoa, Cooled Semen, Extender