

THE EFFECTS OF CONTROLLED SHORT-TERM COOLING PRIOR TO CRYOPRESERVATION ON MOTILITY AND MORPHOLOGY OF EQUINE SPERMATOZOA

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Cryopreservation of equine semen requires expertise and equipment not commonly available in general veterinary practices. However, collection and preparation of semen for cooled shipment is commonly performed on farms. If semen could be collected on farms and transported under controlled conditions within hours to a laboratory, the availability of cryopreservation of semen could be improved. Previous research suggested that cooling semen to, and holding at 5 °C for 12 h prior to freezing was not more damaging for the post-thaw motility when compared to keeping semen cooled for only 2 h prior to freezing (Crockett et al., 2001). However, it was not clear if cryopreservation without prior cooling would have resulted in less damage to the spermatozoa. We hypothesized that controlled, short-term cooling in an equine semen cooling system (Equitainer, HamiltonThorne, South Hamilton, MA) for ≤ 3 h, down to a temperature of 14–15 °C would not be detrimental to semen integrity and provide a suitable product for cryopreservation. Three stallions were collected on three separate occasions. Each ejaculate was divided into four equal fractions, one for each experimental condition, and extended, with EquiPro with amikacin and penicillin (Minitube of America, Verona, WI), to an extender to semen ratio of 3:1. Semen was immediately centrifuged and frozen (Control, A), stored/cooled at room temperature for 1 h (B), stored in an Equitainer for 1 h (C) or stored in an Equitainer for 3 h (D) prior to centrifugation and cryopreservation. After centrifugation, the semen pellet was resuspended and concentration was confirmed using a hemocytometer. The semen was then extended with EquiPro CryoGuard Complete (Minitube of America, Verona, WI) without antibiotics to a concentration 400×10^6 sperm/ml. The semen was frozen in 0.5 cm³ straws using the IceCube automated freezing system (Minitube of America, Verona, WI). Semen was analyzed for progressive motility and morphological assessments prior to cryopreservation and after thawing of all samples. Data were expressed as means $\pm$ S.E.M., and analyzed for statistical differences using a Kruskal–Wallis one-way ANOVA. Significance was set at $p < 0.05$. Motility of semen after cryopreservation was generally lower than non-frozen semen ($43.5\% \pm 2.6$ and $68.2\% \pm 1.6$, respectively; $p < 0.04$). The change in motility after thawing was 22.7 ± 4.5 (A), 22.1 ± 5.3 (B), 25.5 ± 3.0 (C) and 28.7 ± 5.0 (D). None of these changes were statistically significant from each other. Changes in sperm morphology were also compared between treatments. The percentage change in morphologically normal sperm in Group A was 2.1, Group B was 3.7, Group C was 7.5 and Group D was 2.3. These changes were not significantly different from each other. We conclude that controlled, short-term cooling for up to 3 h, or storage at room temperature for 1 h prior to freezing can be performed with no deleterious effects on post-thaw semen quality. Our results suggest that semen from selected stallions, collected on farm, can be extended and shipped using current industry standards to facilities with cryopreservation capabilities for freezing within 3 h after collection.

Keywords: Equine; Cryopreservation; Semen; Cooling