

Comparison of Dimethyl Formamide and Glycerol for Cryopreservation of Equine Spermatozoa

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Glycerol is the primary cryoprotectant used for freezing equine spermatozoa. However, not all stallions have sperm that survive the freeze/thaw process. Thus, alternative cryoprotectants have been suggested as a means of improving the survivability of sperm during freezing and thawing. Several studies have shown that progressive motility post-thaw is higher for sperm frozen in extenders containing dimethyl formamide (DMF) versus glycerol but limited fertility trials are available. The objective of this study was to evaluate post-thaw motility of equine sperm frozen in various extenders with glycerol or DMF and to determine the fertility of mares inseminated with semen extended in lactose-EDTA extender with either glycerol or DMF.

In the first experiment, 20 ejaculates were collected from 14 stallions. Each ejaculate was diluted to 50×10^6 /ml with skim milk and centrifuged at $400 \times g$ for 12 min. Sperm were then resuspended in either a modified French extender (FR5), lactose-EDTA extender or E9-Papa extender (Papa et al., SFT, 1993). Within extender, one-half of the tubes contained 4% DMF and the other contained 4% glycerol. Sperm was frozen at a concentration of 400×10^6 /ml in 0.5-ml straws using a programmable cell freezer. Semen was thawed at 37°C for 30 sec. The thawed semen was diluted to 50×10^6 /ml in skim milk and evaluated for motility at 0, 10 and 30 min post-thaw. There was no effect of extender treatment on total or progressive motility.

Additional stallion semen was frozen in lactose-EDTA with either 4% DMF or 4% glycerol. This semen was evaluated in a fertility trial in Canada as well as in Colorado. At CSU, mares were inseminated 24 and 40 hr after hCG and 30 and 48 hr after administration of deslorelin acetate. Pregnancy rates were higher for mares inseminated with semen containing DMF (6/8) than glycerol (2/7). In Canada, mares were assigned to be inseminated with two treatments: lactose-EDTA with DMF or lactose-EDTA with glycerol. Mares were inseminated at 1 of 3 frequencies after hCG. There was no difference in fertility due to frequency of AI. Pregnancy rates were higher for those mares inseminated with lactose-EDTA containing DMF (8 of 22) than glycerol (3 of 27). When data for both fertility trials were combined, pregnancy rates were higher ($P < 0.05$) for mares inseminated with lactose-EDTA containing DMF (14 of 30) versus glycerol (5 of 34).

In summary, motility was not altered by the addition of DMF to any of the extenders, but pregnancy rates were higher for mares inseminated with extenders containing DMF versus those containing glycerol.

Key words: Cryoprotectant, Frozen semen, Motility, Fertility