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JOURNAL ARTICLE

Osmotic tolerance of equine spermatozoa and the effects of soluble cryoprotectants on equine sperm motility, viability, and mitochondrial membrane potential

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Osmotic stress attributed to differences in the relative permeability of cryoprotectants, such as glycerol and water, appears to be an important factor in cryodamage. The objective of this study was to characterize the osmotic tolerance of equine spermatozoa, and to evaluate the effects of addition and removal of cryoprotectants from equine spermatozoa on their motility, and membrane and acrosomal integrity, as well as their mitochondrial membrane potential. Equine spermatozoa had a limited osmotic tolerance to anisotonic conditions. Although the addition of increasing concentrations of glycerol decreased the motility and viability of equine spermatozoa, the rapid removal of glycerol by dilution in isotonic media resulted in an even greater decline in motility and viability compared with spermatozoa maintained under anisotonic conditions. Likewise, the addition and rapid removal of 1.0 M glycerol, ethylene glycol, dimethylsulfoxide, or propylene glycol resulted in a significant decline in sperm motility and viability. Among these cryoprotectants, ethylene glycol had the least detrimental effect on either viability or motility of spermatozoa following the rapid addition and removal of these cryoprotectants. These data demonstrate that equine spermatozoa have a limited osmotic tolerance compared with published reports for mouse or human spermatozoa, and appear to be more similar to boar spermatozoa in their osmotic tolerance. Of the 4 cryoprotectants evaluated in equine spermatozoa, the addition and removal of glycerol resulted in a more marked osmotic stress as indicated by alterations in motility, viability, and acrosomal integrity. These data suggest that alternative cryoprotectants should be considered for cryopreservation of equine spermatozoa in order to reduce osmotic stress associated with the addition of these agents during semen freezing.

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