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JOURNAL ARTICLE

Cryopreservation in different concentrations of glycerol alters boar sperm and their membranes

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To test the hypothesis that glycerol would concomitantly affect sperm membrane structure and the function of the intact cells, boar semen (4 ejaculates from 4 boars) was cryopreserved in an egg yolk extender with 0%, 2%, 4%, or 8% glycerol in 0.5-mL straws using previously derived optimal cooling and thawing rates. Increasing glycerol concentrations increased spermatozoal progressive motility immediately after thawing and after 2 hours at 43 degrees C, but decreased the percentage of sperm with normal acrosomal morphology. The mathematical products of the motility and acrosomal integrity scores (MOT x NAR index) were low in 0% and 8% glycerol, and significantly higher in 2% and 4% glycerol. The fluidity of sperm-head plasma membranes, a measure of molecular interaction, was assessed with the lipid probes trans-parinaric acid and cisparinaric acid (tPNA, cPNA), during a 2.5-hour incubation with or without 1 mM Ca2+. Membrane fluidity detected by each probe differed significantly, indicating the presence of at least 2 domains whose constituent molecules had unique dynamics. Behavior of each domain was radically altered by cryopreservation. Increasing glycerol concentration caused a variably faster loss of fluidity in the cPNA domain, and had highly variable effects on fluidity change over time in the tPNA domain. Normal acrosomal ridge (NAR) and the MOT x NAR index correlated significantly with the fluidity of the more mobile cPNA domain (+/- 1 mM Ca2+), supporting the hypothesis of an interrelationship of glycerol concentration during cryopreservation with sperm membrane structure and cell function. The MOT x NAR index may be a useful guide in choosing optimal cryoprotectant concentrations.