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Chimpanzee (*Pan troglodytes*) Spermatozoa Osmotic Tolerance and Cryoprotectant Permeability Characteristics

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Osmotic properties of chimpanzee spermatozoa were studied at 22°C. An electronic particle counter was used to determine the isosmotic cell volume, and the volume response after exposure to four commonly used cryoprotectants: dimethyl sulfoxide, glycerol, propylene glycol, and ethylene glycol. The data were analyzed to determine the hydraulic conductivity and the permeability coefficients for the four cryoprotectants. The osmotically inactive volume fraction was determined using a Boyle van't Hoff plot of cells exposed to sodium chloride solutions. A computer-assisted semen analysis system was used to determine the osmotic tolerance of chimp spermatozoa, as well as the effects of a one-step addition and removal of 1 M permeating cryoprotectant on sperm motility. The isosmotic volume of chimpanzee sperm is 27.7 μm^3. The osmotically inactive cell fraction is 69%. Hydraulic conductivity was higher in the presence of ethylene glycol: 4.09 ± 0.76 (mean ± SEM) and propylene glycol: 3.91 ± 0.71 as compared to dimethyl sulfoxide: 3.49 ± 0.79 and glycerol: 2.83 ± 0.40 µm/min per atmosphere. The permeability of chimpanzee sperm in ethylene glycol (2.18 ± 0.40 x 10^{-3} cm/min) and propylene glycol (1.75 ± 0.17 x 10^{-3} cm/min) was higher than in glycerol (1.42 ± 0.12 x 10^{-3} cm/min) and dimethyl sulfoxide (0.82 ± 0.015 x 10^{-3} cm/min). Although chimpanzee sperm tolerated osmotic stress in the range of 169–400 mOsm very well, loss of motility was observed as the solution concentrations diverged from isosmotic condition. Exposure to the four cryoprotectants at 1 M did not cause a significant reduction in sperm motility. This information on membrane permeability characteristics and cryoprotectant tolerance will aid in designing more reliable cryopreservation protocols for chimpanzee sperm.

Key words: Cryopreservation, membrane permeability