Effect of cryoprotective additives-reduced glutathione, acetyl-L-carnitine on sperm membrane lipid peroxidation, DNA integrity and recovery of motile human sperm

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Objective: Cryopreservation induces lethal and sub-lethal sperm damage that lead to loss of viability, acrosome loss, DNA fragmentation and poor fertilization. Reactive oxygen species, lipid peroxidation and loss of antioxidant activity such as reduced glutathione (GSH), are considered as mediators of sub-lethal cryodamage to human sperm during cryopreservation. Improvements in semen parameters have been reported by in vivo use of carnitine and acetyl-L-carnitine (ALC), possibly due to its key role in energy metabolism and antioxidant activity. The aim of this present study was to compare the effect of addition of GSH and ALC in freezing media on motility, DNA integrity and lipid peroxidation status of cryopreserved human spermatozoa. Design: Prospective in vitro study.

Materials and Methods: Normozoospermic samples (n=25) from healthy volunteers were included in the present study. Following liquefaction, each semen sample was divided into four equal parts. All aliquots were frozen, two using glycerol-based cryoprotectant with or without 5mM GSH and the other two aliquots with or without 175µM ALC as the final concentration. DNA integrity, both before and after cryopreservation, was assessed using acridine orange fluorescence. After thawing, sperm motility and lipid peroxidation (LPO) were assessed in both controls and test samples using phase contrast microscopy and malonaldehyde assay respectively.

Results: Cryoprotectant supplemented with ALC was found to improve post-thaw progressive motility from 16.4 ± 11.9 (control) to 25.4 ± 17.8% (p < 0.05) and normal DNA integrity from 59.3 ± 20.5 (control) to 72.8 ± 15.2% (p = 0.007). No difference in % motility or progressive motility was observed between control and test samples frozen with or without GSH. However, GSH group showed 67.7 ± 20.9% normal DNA integrity compared to the control (p = 0.004). GSH or ALC fractions failed to reduce post thaw LPO rate (37.00 ± 5.9 in controls; 40.2 ± 3.8 for ALC samples, 37.9 ± 4.0 for GSH samples).

Conclusion: Samples frozen as neat semen with protective environment of seminal
plasma along with supplemented antioxidants such as GSH or ALC can withstand cryo-injuries, especially during post thaw processing which involves dilution and centrifugation steps. Support: None

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