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JOURNAL ARTICLE

Cryopreservation-Thawing of fractionated human spermatozoa is associated with membrane phosphatidylserine externalization and not DNA fragmentation

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The objective of these studies was to evaluate the effect of cryopreservation-thawing of human spermatozoa on DNA fragmentation and membrane integrity. This was a prospective, controlled cohort study, performed at a university-based infertility center. Ejaculates were examined from 5 donors and 16 men undergoing infertility evaluation. Purified sperm populations were prepared by gradient centrifugation, cryopreserved using a manual method and TEST-yolk buffer and glycerol (TYB-G), followed by quick-thaw. Annexin V binding was used for assessing membrane translocation of phosphatidylserine, and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was utilized for the evaluation of DNA fragmentation. The results were as follows: the percentage of live cells with intact membranes (annexin V-, live) was significantly reduced after cryopreservation-thawing. On the other hand, the percentages of live cells with phosphatidylserine translocation (annexin V+, live) and of necrotic (dead) cells increased significantly after thawing. TUNEL revealed percentages of cells with DNA fragmentation in the prefreeze and postthaw samples that were not significantly different. In a further attempt to examine differences in response to various cryoprotection protocols, experiments were carried out using no cryoprotection, glycerol alone, or TYB-G. Samples frozen with TYB-G demonstrated significantly higher percentages of live cells without phosphatidylserine translocation than the other conditions. We concluded that cryopreservation-thawing of human sperm from patients and donors was associated with membrane change, as revealed by membrane translocation of phosphatidylserine, while having no major impact on DNA fragmentation.

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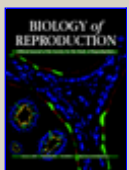
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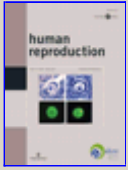
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