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JOURNAL ARTICLE

Mechanism of superoxide dismutase loss from human sperm cells during cryopreservation

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Earlier studies on human sperm cryodamage have shown that plasma membrane stress is the primary process and that phospholipid peroxidation in cryopreserved samples is not inhibited by addition of antioxidants. One consistent effect of cryopreservation is loss of enzymatic activity of the peroxidation defense enzyme, superoxide dismutase (SOD). To clarify this aspect of the freeze-thaw process and to develop a more complete resolution of the reactions leading to cryodamage, we sought to identify which of the two most probable mechanisms, loss of enzyme protein from the cells of denaturation of the protein, operates. If the first operates, cellular enzymatic activity and enzyme protein as identified by immunocytochemistry should give a linear correlation. If the second operates, there should be no correlation. In this study, five individual samples were analyzed before and after cryopreservation for immunoreactive Cu/Zn SOD and cell intactness by flow cytometry, for SOD enzymatic activity by a highly sensitive fluorimetric method, and for motility characteristics by Hamilton-Thorn motility analyzer. Fresh samples were obtained by the "swim-up" method and had > 95% intact cells with > 78% motile cells. After freeze-thaw, about half the cells were intact. SOD enzymatic activity was determined on Triton X-100 cell extracts, a method that removes all enzymatic activity from the cell structure, and compared with immunoreactive SOD in the cells as determined by indirect immunofluorescence mean intensities. Residual immunofluorescence was observed in the cells after Triton X-100 treatment; if this was taken into account, a close linear correlation between SOD enzyme activity and SOD immunoreactivity was obtained ($r = 0.90$; $P = 0.00014$). There was no correlation between SOD enzyme activity ratios for cryopreserved and fresh cells and fraction of intact cells after freeze-thaw. We conclude that loss of SOD protein from the subset of cells undergoing acute membrane damage is the most probable primary mechanism of SOD enzymatic activity loss from the sample and that resistance to cryodamage and SOD activity in any given cell are quite independent of one another.

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