
Assisted reproductive procedures such as intracytoplasmic sperm injection (ICSI) are possible even with a few non-motile but viable spermatozoa. Sperm viability rather than motility is critical in assisted reproduction. Motility excludes cells that are viable but nonmotile. Assessment of viability by vital stains is accurate but their effects on sperm integrity is unclear. The hypoosmotic swelling (HOS) test is advocated as an indicator of membrane integrity and normal functional ability in fresh human spermatozoa. However, reports on its use in cryopreserved samples are lacking. The purpose of this study was to determine the validity of HOS as a test of viability in fresh and frozen human spermatozoa. Fresh semen specimens from 11 normal donors were divided into two aliquots: the first aliquot received no treatment, and the other was processed by the swim-up technique. Both aliquots were then diluted with TEST-yolk buffer (1:1 v/v) and cryopreserved by the liquid nitrogen vapor method. To validate the results of HOS test each specimen was evaluated by eosin-nigrosin (EN) staining and Hoechst-33258 staining before and after freezing. Two hundred spermatozoa from each specimen were scored to assess the percentage viability. A highly positive correlation was seen in both raw and swim-up specimens ($r=0.95$, $P=0.0001$) between the viability scores of fresh sperm measured by the HOS test and by Hoechst-33258 staining. After cryopreservation, no correlation was seen between HOS test scores and Hoechst-33258 or EN scores. However, there was a high correlation between Hoechst-33258 and EN scores ($r=0.72$; $P<0.004$). HOS test is a simple and non-deleterious assay that can accurately evaluate viability in fresh human sperm. However, this test may not be clinically helpful in the selection of viable cells in cryopreserved samples for assisted reproduction.