The Significance of DNA oxidation in sperm quality, semen freezing and capacitation process: A new marker of sperm quality

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Objective: Free radical damage is an important factor in sperm function. Despite extensive research efforts in biomarkers in recent years, there is a great need for additional research on the applicability of free radical damage in humans. One product resulting from free radical damage is the DNA-hydroxylation products known as DNA oxidation. Our aim with this study was to understand oxidative stress effects on DNA damage and determine its role in sperm function by correlating DNA oxidation levels with classic seminal parameters.

Materials and Methods: Semen samples were obtained from infertile males (n = 38) of couples undergoing oocyte donation cycles after 3-5 days of sexual abstinence. All aliquots were examined for ejaculate volume, concentration and motility as per WHO guidelines. Nineteen samples were previously frozen and thawed with controlled glycerol based method by doing sperm tablets on dry ice surface. The other 19 samples were freshly processed. Thawed and fresh samples were capacitated by swim-up method by using Human Tubal Fluid (HTF) media. OXIDNA assay kit was used based on the direct binding of a fluorescent probe to the DNA adduct 8-oxoguanine. In this assay, FITC-conjugate binds to 8-oxoguanine in damaged cells. Fluorescence from ejaculated and capacitated sperm was quantified using flow cytometry (excitation 495 nm, emission 515 nm). Percentage of stained cells and the average of staining intensity were obtained in Fluorescence Arbitrary Units (FAU).

Results: In fresh samples, no significant differences were seen in the percentage of stained cells before after swim-up (40.64% SD=12.6 vs. 43.90% SD=12.5).. After thawing, a significantly higher increase in percentage of cells with oxidized DNA was seen in fresh samples (28.53% SD=11.71 vs 43.98% SD=10.8) meanwhile capacitated samples did not differ from sperm previously thawed or not.

Table: Correlation between percentage of cells with DNA damage and sperm parameters before and after swim-up (independently of being fresh or frozen samples).

Parameter	Volume	Concentration	Progressive Motility	Total progressive sperm	Morphology (%Normal Forms)
Fresh	-0.536*	0.124	-0.395*	-0.505*	0.052
Swim-up	-0.168	-0.271	0.018	-0.241	0.109

* Denote a significant linear relationship.

Conclusion: Oxidative damage in the DNA is clearly increased in frozen-thawed samples, this damage is reflected by a decrease in sperm motility. Nevertheless the DNA damage disappears when the sperm cells are capacitated. Cryopreservation is a stressful process that produces various structural and functional sperm modifications producing an increased oxidative damage in the DNA. Oxidative damage in the DNA measured by the hydroxylation of 8-oxoguanine is a good biomarker of sperm quality reflecting the free radical damage in human sperm.