ALTERATIONS IN MITOCHONDRIAL MEMBRANE POTENTIAL (ΔΨ) AND OXIDATIVE STRESS IN MEN WITH MALE INFERTILITY

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Objective:
Oxidative stress due to excessive production of reactive oxygen species (ROS) is known to impair male fertility. Sperm mitochondria possess a distinct genome (mtDNA) which is more susceptible to ROS-induced damage than nuclear DNA. Fragmentation of genomic DNA is one of the hallmarks of apoptosis. Functional integrity of sperm mitochondria is indicative of apoptosis. Alterations in mitochondrial membrane potential (ΔΨ) may occur during early stages of apoptosis. The ability to discriminate between mitochondria exhibiting high membrane potential from those having low ΔΨ provides a rigorous estimate to metabolic function. The purpose of this study was to determine the effect of ROS on mitochondrial membrane potential and to evaluate its correlation with conventional semen parameter.

Design:
Controlled prospective study.

Materials/Methods:
Semen specimens were obtained from infertile men (n = 19) and normal healthy donors (n = 5) after 2 to 3 days of sexual abstinence. Each sample was divided into three aliquots after liquefaction at 37°C. Semen analysis was performed as per World Health Organization criteria. Levels of ROS were measured by chemiluminescence assay and expressed as X 10^6 counted photons per minute (cpm) using luminol as the probe. We used the carbocyanines dye DIOC₆ (3,3'-dihexyloxacarbocyanine iodide) for detecting changes in the membrane potential. As DIOC₆ has a signal wavelength emission, a high ΔΨ was attributed to cells with a high fluorescence signal. Spermatozoa (0.5 X 10^6 ml) were incubated with DIOC₆ (0.1nM) at 37°C for 5 minutes. ΔΨ was measured using flow cytometry and 10,000 cells were analyzed per sample.

Results:
ROS levels in patients with abnormal semen parameters were significantly higher than in donors (6.95 ± 19.5 vs. 0.10 ± 0.09; p< 0.05). Significantly lower levels of ΔΨ were seen in patients with abnormal semen parameter compared to donors (155.8 ± 767.7 vs. 3391.4 ± 1987.1; p< 0.02). ΔΨ was significantly correlated with sperm concentration (r = 0.59, p < 0.003) and inversely correlated with the amount of ROS produced (r = -0.42, p < 0.004).

Conclusions:
Sperm damage due to high ROS production in infertile men is associated with decreases in ΔΨ and may be one of the early events of apoptosis, if not a cause of apoptosis. ROS mediated mitochondrial damage may play a central role in spermatozoal DNA damage.

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