SUPEROXIDE GENERATION IN SPERMATOZOA OF INFERTILE PATIENTS: RELATIONSHIPS WITH SEMEN QUALITY
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Reactive oxygen species (ROS) generation by abnormal spermatozoa is considered to be mediated by the excess generation of NADPH leading to production of the superoxide (O$_2^-$) radical. However, in most studies, luminol, a probe specific for hydrogen peroxide (H$_2$O$_2$), has been used. Our objective was to examine the levels of O$_2$ and H$_2$O$_2$ generation in a group of infertile men and estimate the correlation between semen quality and the levels of ROS generation triggered by exogenous NADPH. Semen samples were obtained from 11 infertile men and 6 healthy donors. The basal levels of H$_2$O$_2$ and O$_2$ generation were estimated in washed spermatozoa by chemiluminescence assay using luminol and lucigenin as probes, respectively. We further evaluated their generation after incubation with 2 concentrations of NADPH (5mM and 10mM). Results are expressed in X 10$^6$ counted photons/20 X 10$^6$ sperm. A significantly higher level of O$_2^-$ generation was observed in spermatozoa of infertile patients (.73 (.5, 5.5)) as compared with healthy donors (.20 (0.0, 0.5) p <0.02). H$_2$O$_2$ followed the same trend but was not significant. NADPH at both concentrations triggered high levels of O$_2$ generation (p< .001 for each). O$_2^-$ generation triggered by NADPH was negatively correlated with sperm concentration (r = -0.75; p <0.001), motility (r = -0.69; p <0.01), and % normal morphology (r = -0.78; p <0.01) based on WHO criteria. We conclude that spermatozoa from infertile men produce significantly high levels of O$_2$ compared with normal donors. The ability of spermatozoa to generate O$_2$ increase as the semen quality declines. We speculate that the superoxide radical plays an important, independent role in the pathogenesis of male infertility. It is, therefore, prudent to measure both O$_2$ and H$_2$O$_2$ radicals while reporting the levels of oxidative stress in a semen sample.