OXIDATIVE STRESS MEDIATED SPERM DNA DAMAGE IN NORMOZOOSPERMIC INFERTILE PATIENTS – ROLE OF NADPH
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Human spermatozoa has been shown to generate reactive oxygen species (ROS) via oxidation of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH). When in excess, ROS affect the sperm genomic integrity. It is hypothesized that the source of these oxygen radicals, especially in poor quality semen samples, is NADPH present in the sperm cytoplasmic residues. In order to validate this hypothesis and evaluate the impact of NADPH we compared oxidative stress-induced DNA damage in sperm of normal donors and normozoospermic infertile patients. Semen samples were collected from 16 healthy donors and 12 normozoospermic infertile male patients. Specimens were separated into mature and immature fractions using double density gradient (90%, 47%). Each fraction was subdivided into 3 aliquots and incubated in the absence or presence of 5 mM NADPH for 0, 3 and 24 hours. ROS levels were assessed by chemiluminescence assay and sperm DNA damage by flow cytometric TUNEL assay. Results are expressed as median (25%, 75% percentiles). All immature sperm fractions of donor and infertile patients revealed higher percentage of spermatozoa with cytoplasmic droplets compared to the mature fractions. Patients had higher percentage of spermatozoa with cytoplasmic droplets than donors in both mature [1 (3, 4.5) vs. 0 (0, 1.5), P = 0.002] and immature [10 (10.5, 19) vs. 3.75 (1, 6), P = 0.01] fractions. Samples treated with exogenous NADPH had significantly higher levels of DNA damaged sperm mainly after 24 hours of incubation and this correlated positively with ROS levels. Degree of sperm DNA damage was higher in patients compared to donors [11.3 (7.18, 54.4 vs. 4.6 (2.6, 8.7), P < 0.0001] with greater damage observed in immature fraction compared to the mature fraction [9.4 (4.7, 22.7) vs. 6.6 (3.9, 21.1), P = 0.02] regardless of NADPH exposure. In conclusion, male infertility patients with immature spermatozoa exhibit high levels of sperm DNA damage that appears to be a time-dependent consequence of increased oxidative stress.