Effect Of Exogenous $\beta$-Nicotinamide Adenine Dinucleotide Phosphate Reduced (NADPH) On Sperm DNA Integrity

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**Objective:** Human spermatozoa have been shown to generate reactive oxygen species (ROS) via oxidation of endogenous NADPH that is involved in sperm capacitation. However, excessive ROS generation is also known to cause sperm DNA damage. The objective of our study was to detect the ability of mature and immature spermatozoa to generate ROS in response to exogenous NADPH and to measure the extent of the subsequent DNA damage.

**Design:** Prospective-controlled study.

**Materials and Methods:** Semen samples were collected from five donors with normal standard semen parameters. Samples were divided into mature and immature fractions using Isolate double density gradient (90%, 47%). Each fraction was further subdivided into 2 subsets; the first was divided into 3 aliquots and incubated with 5 mM NADPH for 0, 3 and 24 hours. The second subset was similarly divided and incubated without NADPH to serve as control. Sperm DNA damage and ROS levels were assessed using terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labeling (TUNEL)-coupled flow cytometry and chemiluminescence assay ($x 10^6$ counted photons per minute) using lucigenin as a probe.

**Results:** The median and interquartile values (25th and 75th percentiles) of DNA damage are shown in the table. Significantly higher DNA damage ($P < 0.05$) was seen in both NADPH treated sample fractions compared to controls only after 24 hours incubation. Levels of ROS in the mature fraction (90%) were significantly higher than controls (16.4 ± 9.6 vs. 2.36 ± 3.8, $P = 0.032$) only after 24 hours of incubation, while in the immature fraction (47%) ROS levels were significantly higher at both 3 (1.45 ± 1.15 vs. 0.4 ± 0.3, $P = 0.032$) and 24 hours (18.38 ± 13.46 vs. 3.2 ± 6.03, $P = 0.032$). DNA fragmented sperm showed a positive correlation with ROS levels ($r = 0.54$, $P = 0.002$) in samples incubated with NADPH for 24 hours.

**Conclusions:** Our results indicate that NADPH may play a role in sperm ROS production and DNA fragmentation. ROS generation is triggered earlier in the immature fraction but DNA damage takes longer time to develop.

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<table>
<thead>
<tr>
<th>Fraction</th>
<th>Incubation</th>
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<tr>
<td></td>
<td>0 hour</td>
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<tr>
<td>Mature spermatozoa (NADPH)</td>
<td>3.3 (2.4, 3.2)</td>
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<tr>
<td>Mature spermatozoa (Control)</td>
<td>6.8 (4.2, 6.6)</td>
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<tr>
<td>Immature spermatozoa (NADPH)</td>
<td>7.5 (7.1, 10.8)</td>
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</table>
Immature spermatozoa (Control) | 6.4 (5.6, 7.1) | 12.3 (8.8, 13.3) | 10.5 (7.6, 13.2)

P value <0.05 considered significant in samples compared to controls.

P value <0.05 considered significant after incubation for 24 hours compared to 0 and 3 hours.

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- I Agree : True

**Status:** Complete