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Title: COMPARING FLOWCYTOMETRY AND CHEMOLUMINESCENSE IN ASSESSING HUMAN SPERM PRODUCTION OF SUPEROXIDE AND HYDROGEN PEROXIDE IN DIFFERENT SPERM FRACTIONS
Objective: Chemiluminescence is a routine method for measuring reactive oxygen species (ROS) in a given sample using luminol and Lucigenin probes. However, the assay is not specific for any individual oxidant, does not specifically target intracellular ROS, and requires relatively large number of cells. Recently, intracellular ROS has been measured specifically using flowcytometry. Our objective was to compare the intracellular levels of the spermatozoal $O_2^-$ and $H_2O_2$ using flowcytometry (FACS) with chemiluminescence using luminol and lucigenin probes.

Design: Prospective study.

Materials and Methods: Semen samples from 17 men were collected. After liquefaction, neat semen was subjected to double density gradient centrifugation to separate the mature and immature sperm fractions. The mature and immature fractions were evaluated within 2 hour by: a) chemiluminescence assay using both luminol and lucigenin probes, and also for b) flowcytometry intracellular ROS assay using two probes: DHE for $O_2^-$ and DCFH-DA for $H_2O_2$.

Results: The mean (±SD) values of ROS assessment using luminometer and flowcytometry is given in the Table. The proportions of sperm cells stained for intracellular $O_2^-$ (HE%) and for $H_2O_2$ (DCFH%) were significantly higher in the immature fractions compared to the mature fractions.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mature (n=17)</th>
<th>Immature (n=17)</th>
<th>Mature vs. Immature P#</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHE %</td>
<td>1.4 ± 1.1</td>
<td>7.7 ± 2.8</td>
<td>P = 0.0038</td>
</tr>
<tr>
<td>DCFH-DA %</td>
<td>20 ± 15</td>
<td>29 ± 16.5</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Luminol (cpm/20 x 10^6 sperm)</td>
<td>0.5 ± 0.7</td>
<td>2 ± 5.7</td>
<td>P = 0.46</td>
</tr>
<tr>
<td>Lucigenin (cpm/20 x 10^6 sperm)</td>
<td>0.04 ± 0.16</td>
<td>0.15 ± 0.6</td>
<td>P &gt; 0.9999</td>
</tr>
</tbody>
</table>

# Wilcoxon’s signed ranks test

On the other hand routine chemiluminescence assay using both luminol and lucigenin probes did not show any significant difference in ROS production between sperm fractions. There was no evidence of interdependence between sperm intracellular $O_2^-$ and lucigenin ROS assessment, and between intracellular $H_2O_2$ level and luminol ROS assessment.

Conclusions: Our results indicate that there is no interdependence between sperm intracellular $O_2^-$ and lucigenin ROS assessment, or between intracellular $H_2O_2$ level and luminol ROS assessment. This confirms differential affinities of luminol and lucigenin and their reaction to global different radicals.
Support: none

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