A SIMPLE, RAPID, AND INEXPENSIVE TEST FOR ASSESSMENT OF SEMINAL REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN AN ANDROLOGY LABORATORY

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Objective:
Conventional methods available for ROS measurement are unable to provide information about the ROS generating activity in individual cells. Nitroblue tetrazolium (NBT) is an electron acceptor that becomes reduced in the presence of free oxygen radicals to form a blue-black compound, formazan. NBT test can be used for qualitative assessment of cellular generation of ROS. The objectives of this study were 1) to examine ROS generating activity in immature sperm and neutrophils in neat semen and 2) to determine the correlation between levels of ROS in neat semen and the results of NBT testing of sperm and neutrophils.

Design:
A prospective study in a male infertility clinic

Materials/Methods:
Semen samples were obtained from 13 infertility patients and 7 normal donors. Seminal leukocytes were quantified by Endtz test. Patients were classified into two groups: leukocytospermic (n = 5) and non-leukocytospermic (n = 8). Levels of ROS in neat semen were measured by chemiluminescence assay. Results were expressed as X 10^4 counted photons per minute (cpm)/20 million sperm/mL. One hundred microlitre of neat semen was incubated with an equal volume of 0.1% of NBT solution at 37°C for 30 minutes. Smears were air-dried and stained with Wright stain. A total of 100 immature sperm and 100 neutrophils were counted and scored using X 100 oil immersion objective. Neutrophils were scored as follows: cells filled with formazan (+++), intermediate density (++), scattered or few formazan granules (+), no formazan detectable (-). Immature sperm were scored as follows: formazan occupied ≤50% of cytoplasm (+) and >50% of cytoplasm (++)

Results:
Median (25%, 75% interquartile range) of ROS levels and results of NBT scoring for neutrophils and immature sperm are shown in the table below. In leukocytospermic samples, 54% of neutrophils stained positive for NBT. Three percent (3%) of these cells were classified as (+++), 10% (++), and 41% (+); compared to 0%(+++), 2% (++), and 5% (+) in non-leukocytospermic samples. A significant positive correlation was seen between ROS levels in neat semen and positive NBT response in neutrophils (r = 0.84, P = 0.0001) and immature sperm (r = 0.44, P = 0.04).

Conclusions:
NBT scores in both sperm and leukocytes are strongly correlated with ROS levels in neat semen as measured by chemiluminescence. In addition, NBT test is capable of identifying the cellular origin of ROS production in a given semen sample. This simple and inexpensive test can be incorporated into a routine Andrology Laboratory work-up for qualitative assessment of ROS production in semen.

Supported by:
None

<table>
<thead>
<tr>
<th>Variable</th>
<th>Donors</th>
<th>Non-leukocytospermic patients</th>
<th>Leukocytospermic patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS (X 10^4 cpm)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
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<tr>
<td></td>
<td>0.4 (0, 1)</td>
<td>5 (2, 7)</td>
<td>35 (30, 38)</td>
<td>0.016</td>
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<tr>
<td>NBT positive neutrophils (%)</td>
<td>0 (0, 2)</td>
<td>7 (6, 14)</td>
<td>54 (47, 73)</td>
<td>0.01</td>
</tr>
<tr>
<td>NBT positive immature sperm (%)</td>
<td>13 (9, 22)</td>
<td>32.5 (12, 46)</td>
<td>49 (43, 51)</td>
<td>0.39</td>
</tr>
</tbody>
</table>
a - comparison of donors vs. non-leukocytospermic samples; b - comparison of donors vs. leukocytospermic samples; c - comparison of non-leukocytospermic vs. leukocytospermic samples. Results were analyzed by Wilcoxon Rank-sum Test, P <0.05 was significant.