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Contact/Presenting Author: Reda Mahfouz, M.D.

Department/Institution: Center for Reproductive Medicine, Cleveland Clinic

Address: E 9500, Euclid Avenue, Desk A 19.1

City/State/Zip/Country: Cleveland, United States

Phone: 1-216-444-9485 Cell Phone: Fax: 1-216-445-6049 E-mail: agarwaa@ccf.org

Abstract Category: 10. Male Factor: ART (SART)

Abstract Topic: 42. Sperm Biology

Poster presentation only? No

Are you a fellow in training in an American Board of Obstetrics and Gynecology approved program leading to certification in Reproductive Endocrinology and Infertility? No

Data is submitted for a separate scientific abstract and video presentation: No

Awards: IRB Approval: The study material in this abstract has been approved by the local Institutional Review Board (IRB) if any human subjects or any human material was utilized.

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Six Author Rule: All authors named in this abstract have agreed to its submission for presentation at the 63rd Annual Meeting of the American Society for Reproductive Medicine and are familiar with the 6-author rule.

Accept Complete Responsibility: I accept complete responsibility for the data at the time of submission.

Title: ASSESSMENT OF INTRACELULAR HUMAN SPERM REACTIVE OXYGEN SPECIES AFTER HYDROGEN PEROXIDE EXPOSURE USING FOUR DIFFERENT PROBES
**Objective:** Oxidative stress occurs when intracellular ROS level exceeds the antioxidant capacity of the cell. Luminol is commonly used to measure ROS by chemiluminescence. Recently flowcytometry using dichlorofluorescein diacetate (DCFH-DA) and the dihydroethidium (DHE) dyes has been reoported. Our objective was to investigate intracellular ROS changes after exposure to H$_2$O$_2$ using these different probes.

**Design:** Prospective study.

**Materials and Methods:** 12 donor semen samples were collected and a portion of neat samples was subjected to density gradient centrifugation for separating mature and immature sperm. Each fraction was resuspended in PBS buffer and divided into two aliquots, one aliquot incubated with 50µL of freshly prepared H$_2$O$_2$ added to 1 mL sperm suspension with a final concentration 20µM for 15 min at 37°C and the other aliquot served as control. Subsequently aliquots from all fractions were evaluated for sperm count and motility. ROS levels in all samples were assessed using chemiluminescence and by flowcytometry.

**Results:** Exposure of mature and immature sperm fractions to H$_2$O$_2$ was associated with statistically significant increase in %DHE +ve sperm. The significant increase in %DCF +ve sperm was noted only in the exposed mature fractions but not in the immature fractions.

<table>
<thead>
<tr>
<th></th>
<th>Mature (n=12)</th>
<th>Immature (n=12)</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% DCF +ve sperm</td>
<td>25.3 ± 14.2</td>
<td>50 ± 24</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>% DHE +ve sperm</td>
<td>1.5 ± 1.2</td>
<td>16.7 ± 13</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Luminol (cpm)</td>
<td>0.26 ± 0.54</td>
<td>61 ± 43</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Lucigenin (cpm)</td>
<td>0.04 ± 0.14</td>
<td>3.9 ± 3.3</td>
<td>0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; Paired Student t-test was utilized to compare ROS levels in different sperm fractions. P < 0.05 was considered significant.

Traditional assessment of ROS production using luminol and lucigenin detected significant increase in ROS after the exposure of mature and immature sperm fractions to H$_2$O$_2$.

**Conclusions:** Exposure of human spermatozoa to exogenous hydrogen peroxide is associated with increased intracellular ROS which can be detected by either flowcytometry or chemiluminescence probes. Mature spermatozoa may be more susceptible to exogenous H$_2$O$_2$ exposure as it showed increase in both intracellular O$_2$•- (DHE%) and H$_2$O$_2$ (DCFH%).

**Support:** none.

**Author:** Reda Mahfouz, M.D.
**Department/Institution:** Center for Reproductive Medicine, Cleveland Clinic
**Address:** E 9500, Euclid Avenue, Desk A 19.1
**City/State/Zip/Country:** Cleveland, United States
**Phone:** 1-216-444-9485 **Cell Phone:** 1-216-445-6049 **E-mail:** agarwaa@ccf.org

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Author: Nabil Aziz, M.D.
Department/Institution: Liverpool Women's Hospital
Address: Liverpool, United Kingdom
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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Author: Rakesh Sharma, Ph.D.
Department/Institution: Cleveland Clinic
Address:
City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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Author: Maria Bykova
Department/Institution: Cleveland Clinic
Address:
City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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Author: Edmund Sabanegh, M.D.
Department/Institution: Cleveland
Address: Cleveland, United States
Phone: Cell Phone:  Fax: E-mail: agarwaa@ccf.org

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