Response of immature and mature mouse oocyte spindle structure to oxidative stress

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Objective: The present study was undertaken to evaluate the effect of oxidative stress (OS) on the spindle structure of immature and mature oocytes induced by exogenous exposure to hydrogen peroxide during in vitro culture. Design: Prospective, animal study Materials and Methods: Based on the presence or absence of first polar body, a total of 52 oocytes were grouped as mature (n = 32) and immature (n = 20) and exposed to 25µM of hydrogen peroxide (H₂O₂) for 30 min. Mature oocytes (n = 50) incubated with human tubal fluid (HTF) media alone served as control. After fixation, immunohistochemical staining was done to evaluate the effects of H₂O₂ on the oocyte microtubule morphology (MT) and staining with propidium iodide staining to evaluate alteration of chromosomal alignment (CH). Stained oocytes were scored for alterations in MT and CH under a Fluorescent and scanning confocal microscope. Spindle structure was scored for microtubule and chromosome alterations (modified from Saunders and Parks, 1999). Microtubules were scored as: 1) normal = score 1-2; 2) abnormal = score 3; and 3) missing = score 4. For chromosomes: 1) normal = score 1-2; 2) slightly abnormal = score 3; and 3) abnormal = score 4. Results: Results of the alterations in MT are in Table 1 and CH analyses in Table 2. Compared with control, statistically significant differences were seen in both abnormal and missing microtubules category from both mature and immature oocytes following H₂O₂ exposure (P <0.05). An inverse trend was seen in the proportion of abnormal and missing microtubules in the mature and immature oocytes (P <0.05) following H₂O₂ exposure. Statistically significant differences were observed in both slightly abnormal and abnormal CH from mature and immature oocytes compared with control (P <0.05). However there were no differences in slightly abnormal and abnormal CH seen in both mature and immature oocytes.

Table 1: Percentage of mature and immature oocytes showing alterations in the MT following H₂O₂ exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal (%)</th>
<th>Abnormal (%)</th>
<th>Missing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mature (n = 50)</td>
<td>78.00a</td>
<td>16.00a</td>
<td>6.00a</td>
</tr>
</tbody>
</table>
Treated Mature (n = 32) 0.00\textsuperscript{b} 65.63\textsuperscript{b} 34.38\textsuperscript{b}  
Treated Immature (n = 20) 0.00\textsuperscript{b} 35.00\textsuperscript{c} 65.00\textsuperscript{c}  
\textsuperscript{a,b,c}Values with different superscripts within each column are significantly different, p<0.05 by Chi square test.

Table 2. Analysis of CH between mature and immature groups following H\textsubscript{2}O\textsubscript{2} exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal (%)</th>
<th>Slightly abnormal (%)</th>
<th>Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mature (n = 50)</td>
<td>80.00\textsuperscript{a}</td>
<td>12.00\textsuperscript{a}</td>
<td>8.00\textsuperscript{a}</td>
</tr>
<tr>
<td>Treated Mature (n = 32)</td>
<td>0.00\textsuperscript{b}</td>
<td>68.75\textsuperscript{b}</td>
<td>31.35\textsuperscript{b}</td>
</tr>
<tr>
<td>Treated Immature (n = 20)</td>
<td>5.00\textsuperscript{b}</td>
<td>65.00\textsuperscript{b}</td>
<td>30.00\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b}Values with different superscripts within each column are significantly different, p<0.05 by Chi square test.

Conclusion: Microtubules in immature oocytes are more sensitive to OS compared to mature oocyte. Alterations in CH are comparable between mature and immature oocytes. Mature oocytes show more resistance to oxidative stress than immature oocytes. It is critical to have minimal exposure to OS conditions as the immature oocytes retrieved from the patient are subjected to subsequent in vitro maturation, in vitro fertilization, or oocyte freezing. Support: None

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