Impact of Apoptosis on Sperm Morphology Indices

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Objective: Apoptosis has been implicated as a cause of male infertility. Externalization of phosphatidylserine (PS) to the sperm outer membrane leaflet is considered to mark terminal apoptosis. Magnetic cell sorting (MACS) using paramagnetic annexin V-conjugated microbeads eliminates spermatozoa with externalized PS. The procedure delivers 2 sperm fractions: annexin-negative (non-apoptotic) and annexin-positive (apoptotic). The relationship between apoptosis and the sperm morphology has not been well characterized. Sperm deformity index (SDI) is a novel quantitative expression of sperm morphological quality that has an enhanced predictive power and reproducibility. The aim of this study was to investigate the relationship between apoptosis and the sperm morphology profile including the SDI score.

Design: Prospective study. Materials and Methods: Semen specimens collected from 15 healthy donors were prepared by density gradient centrifugation followed by MACS using annexin V-conjugated microbeads. Sperm morphology was assessed using the WHO guidelines (1999) and the Tygerberg’s strict criteria. The SDI score was calculated by dividing the total number of deformities observed by the number of sperm randomly selected and evaluated, irrespective of their morphological normality. Apoptosis was evaluated in spermatozoa using flow cytometry coupled with fluorochrome stains that detect: caspase-3 activation (CP3), disruption of mitochondrial potential (MMP) and externalization of PS. Results are expressed as mean ± standard deviation. Pair-wise comparisons were made using Student’s t-test and correlation coefficients were calculated by Spearman’s rank test.

Results: The mean of percentage normal sperm morphology and the mean SDI scores in the annexin-negative and the annexin-positive fractions are shown in the table below. The annexin-negative fraction separated by MACS had significantly lower mean SDI score compared to the annexin-positive fraction (p<0.0001). On the other hand, the mean percentage of normal sperm morphology using both WHO and the strict Tygerberg’s criteria were comparable between the 2 fractions. SDI scores showed significant positive correlation with CP-3 activation (r=0.68, p<.0001), MMP integrity (r=-0.55, p=0.002) and externalization of PS (r=0.72, p<0.0001). There was no evidence of a significant
correlation between normal sperm morphology using both WHO and the strict Tygerberg’s criteria and the three apoptotic markers.  

**Table.** Mean percentage of normal sperm morphology and mean SDI scores in the annexin-negative and the annexin-positive fractions.

<table>
<thead>
<tr>
<th>Sperm fraction</th>
<th>Morphology by WHO criteria (% normal)</th>
<th>Morphology by Tygerberg's strict criteria (% normal)</th>
<th>SDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin-negative</td>
<td>21.36 ± 8.91</td>
<td>14.71 ± 5.9</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>Annexin-positive</td>
<td>20.71 ± 7.14</td>
<td>13.07 ± 6.37</td>
<td>1.65 ± 0.11</td>
</tr>
</tbody>
</table>

Conclusion: Apoptosis shows significantly positive correlation with sperm morphology using the SDI scores. Non-apoptotic spermatozoa show lower incidence of morphological anomalies. The SDI can detect subtle differences in sperm morphology that may pass undetected using routine assessment of normal sperm morphology. Support: None

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