Association of early apoptosis with the nuclear integrity and maturity in different seminal fractions

Author Block: T. M. Said, A. Agarwal, J. Erenpreiss, R. Mahfouz, A. Giwercman, D. P. Evenson; Cleveland Clinic, Cleveland, OH, Malmö University, Malmo, Sweden, SCSA Diagnostics, Brookings, SD

Objective: The integrity and maturity of the paternal genome is an absolute prerequisite for successful fertilization and a healthy offspring. In human spermatozoa, the relationship between apoptosis and the nuclear status remains unclear. Externalization of phosphatidylserine (PS), to the outer sperm membrane leaflet is considered to mark terminal apoptosis. The sperm chromatin structure assay (SCSA) measures the percentage of fragmented sperm DNA (DNA fragmentation index, %DFI) and also identifies the percentage of sperm with immature nuclear development (high DNA stainability index, %HDS). The objective of our study was to investigate differential expression of PS as a marker for early apoptosis with nuclear integrity and maturity of human sperm.

Design: Prospective-controlled study.

Materials and Methods: Semen samples were collected from 19 healthy donors. The neat semen samples were divided into mature and immature sperm fractions using double density gradient centrifugation (90% and 47% layers). Both fractions as well as an aliquot of the neat sample were evaluated for the incidence of early apoptosis (PS externalization) using the annexin V flow cytometric assay. In addition, the %DFI and %HDS were measured using the SCSA.

Results: The means ± standard errors of the percentages of annexin V as well as the %DFI and %HDS in neat semen, mature and immature fractions are given in the table. Externalization of PS was significantly lower in mature sperm fractions compared to the immature fractions and neat semen samples (p<0.0001 and p=0.007, respectively). Similarly, the mature sperm fractions showed lower %HDS compared to the immature fractions and neat semen samples (p<0.0001 and p=0.001, respectively). The %DFI was comparable between all fractions. Externalization of PS showed a significant positive correlation with %HDS (r=0.47, p=0.0004) but not with %DFI.

Conclusion: Mature sperm fractions show lower incidence of apoptosis and higher nuclear maturity. The association of PS externalization with %HDS in human semen samples indicates that early apoptosis is associated with immature nuclear development and subsequently abnormal nuclear chromatin (DNA and protein). Our data also supports the lack of association between early apoptosis and sperm DNA fragmentation.

Financial Support: None.

Table. The percentages of annexin V, DFI and HDS in neat semen, mature and immature fractions.

<table>
<thead>
<tr>
<th></th>
<th>Neat semen sample</th>
<th>Mature fraction</th>
<th>Immature fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin V - positive</td>
<td>17.2±4.0*</td>
<td>11.3±3.6</td>
<td>17.7±4.3*</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>16.6±3.8</td>
<td>15.5±4.4</td>
<td>17.4±3.7</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>HDS (%)</td>
<td>6.3±0.6*</td>
<td>3.2±0.5</td>
<td>7.35±1.0*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error of mean. *p<0.001 compared to the mature fraction using two tailed paired t-test.

Author Disclosure Block:  T.M. Said, None;  A. Agarwal, None;  J. Erenpreiss, None;  R. Mahfouz, None;  A. Giwercman, None;  D.P. Evenson, None.

Category (Complete):  Male Reproduction and Urology: Research (SMRU)
Topic (Complete):
  Topic : Sperm biology

Additional Information (Complete):
  Presenting Author Fellow : No
  In-Training Awards for Research : True
  ACCME Disclosure : I will not be discussing non-FDA approved products
  I agree : True

Status: Complete

If you have any questions or experience any problems with the 2006 ASRM Abstract Submitter, please contact Customer Service at asrm@dbpub.com or call (800) 375-2586 or (617) 621-1398. Customer Service hours of operation: Monday-Friday, 9:00 AM-6:00 PM EDT.

Powered by OASIS, The Online Abstract Submission and Invitation System SM © 1996 - 2006 Coe-Truman Technologies, Inc. All rights reserved.