Objective: Semen processing is used during assisted reproductive techniques to eliminate unwanted, damaging cells and debris from semen samples. To date little is known about the sperm DNA content and its relation to apoptosis or DNA fragmentation in neat semen and following density gradient centrifugation. Sperm DNA content can be evaluated by flow cytometry using propidium iodide (PI) stain. Flow cytometric analysis could quantitatively differentiate between intensities of haploid spermatid, spermatozoa with condensed chromatin and sub-haploid with low DNA content. Our objective was to investigate the DNA content of the late apoptotic and necrotic sperm cells in neat and processed semen following density gradient centrifugation.

Design: Prospective-controlled study.

Materials and Methods: Semen samples were collected from 22 donors. The neat samples were divided into mature and sperm immature 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 and necrosis using the annexin V/PI assay using flow cytometry. To evaluate the DNA content, PI-positive cells were gated and subjected to analysis. In addition, the DNA fragmentation index (%DFI) and high DNA stainability index (%HDS) were measured using the sperm chromatin structure assay.

Results: Mature sperm fractions showed significantly lower abnormal DNA content parameters compared to neat samples: sub-haploid (p=0.003) and spermatid (p=0.03), while the number of sperm with condensed chromatin was comparable (Table). Neat semen and mature fractions showed significantly higher proportion of spermatozoa with condensed chromatin compared with immature fraction (p=0.02 and p=0.01, respectively). There was no significant difference between mature and immature fractions regarding the number of sub-haploid cells. In neat semen, there was a significant negative correlation between haploid apoptotic cells and %HDS (r=-0.48, p=0.04), while positive correlation was found between immature and sub-haploid apoptotic cells with %DFI (r=0.6, p<0.01). In mature and immature fractions there was strong positive correlation between each apoptotic haploid and spermatid with DFI (r=0.5, p<0.01 and r= 0.6, p=0.005, respectively).

Conclusion: Mature sperm fractions in processed semen contain more normal DNA content than neat and mature semen. The %DFI in neat semen could be related to immature and low DNA content cells, while %HDS could be related to diploid immature cells.

Financial Support: None.

Table: DNA content of PI positive cells in neat, mature and immature fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Sub-Haploid</th>
<th>Spermatid</th>
<th>Condensed Chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>0.003</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Mature</td>
<td>0.003</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>
DNA content of PI positive cells | Neat semen sample | Mature fraction | Immature fraction |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-haploid</td>
<td>15.5±1.4a</td>
<td>10.4±1.1</td>
<td>12.6±1.6</td>
</tr>
<tr>
<td>Haploid spermatid</td>
<td>3.6±0.5a</td>
<td>3.5±1.4</td>
<td>5.1±1.8</td>
</tr>
<tr>
<td>Sperm with condensed chromatin</td>
<td>17.1±2b</td>
<td>18.3±2.7c</td>
<td>11.4±1.7</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error of mean. a: significant difference between neat semen and mature fraction; b: significant difference between neat semen and immature fraction; c: significant difference between mature and immature fractions.

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