A comparative study on nuclear DNA integrity and morphology of human spermatozoa processed by three different methods

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Objective: Various sperm preparation methods are in use to isolate best quality sperm for use in assisted reproductive technology programs. The swim up procedure either from neat semen or from a washed pellet and density gradient centrifugation (DGC) is the most commonly used techniques to enrich spermatozoa. Our objective was to study the effects of DGC and swim up techniques on nuclear DNA integrity and morphology of human spermatozoa. We also evaluated the efficacy of swim up method after DGC in isolating spermatozoa with chromatin integrity and normal morphology.

Design: Prospective study.

Materials and Methods: Twenty men attending an assisted reproduction laboratory for female factor infertility were included in this study. Ejaculates were divided into three aliquots. Sperm DNA normality was assessed by acridine orange (AO) fluorescence and morphology by Pap staining method, both before and after 1) double-layered DGC (PureSperm-40% and 80%), 2) direct swim up from neat semen, 3) swim up from washed pellet and 4) swim up using pellet from DGC. The AO status of spermatozoa in 40% fraction was also assessed.

Results: Following DGC and swim up procedures, the percentage of nuclear maturity increased significantly compared to the neat semen (89.91±6.58) (Table). The nuclear maturity values (mean±SD) were highest after swim up from 80% fraction of DGC (97.08±3.93). A nuclear maturity value of 87.35±6.89 in 40% fraction of density gradient was significantly lower compared to neat semen (P = 0.0118). In addition, there was a significant improvement in morphologically normal forms after the different processing techniques (Table). Best enrichment of normal morphology spermatozoa noted after swim-up procedure from 80% fraction of DGC.

Conclusion: The results indicate that a swim up step, following density gradient centrifugation, can significantly improve the percentage of spermatozoa with normal nuclear maturity and morphology compared to neat semen sample and other sperm
processing techniques. Although, a high yield of morphologically normal forms can be separated after a swim up from washed pellet, the integrity of sperm DNA may be compromised due to the high levels of reactive oxygen species generated during washing procedure. This is especially relevant if the sample contain high levels of leukocytes and immature spermatozoa.

Support: None

<table>
<thead>
<tr>
<th>Variable</th>
<th>% Spermatozoa with normal DNA</th>
<th>P value compared with neat semen</th>
<th>% Spermatozoa with normal morphology</th>
<th>P value compared with neat semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat semen</td>
<td>89.91 ± 6.58</td>
<td></td>
<td>68.44 ± 12.91</td>
<td></td>
</tr>
<tr>
<td>Swim-up from neat semen</td>
<td>93.22 ± 4.17</td>
<td>0.0074</td>
<td>87.35 ± 4.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Swim-up from washed pellet</td>
<td>90.76 ± 5.02</td>
<td>0.47</td>
<td>92.48 ± 2.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Swim-up from 80% fraction of DGC</td>
<td>97.08 ± 3.93</td>
<td>&lt;0.0001</td>
<td>93.67 ± 2.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>80% fraction after DGC</td>
<td>94.4 ± 5.74</td>
<td>0.0002</td>
<td>88.07 ± 3.71</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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- **I Agree:** True

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