Motility characteristics and DNA integrity of spermatozoa processed with different concentrations of reduced glutathione (GSH).

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Objective: Sperm processing methods with repeated centrifugal pelleting of unselected spermatozoa for assisted conception have been reported to result in sperm damage due to enhanced generation of reactive oxygen species (ROS) resulting in subsequent failure of pregnancy in some cases. The main objective of the present study was to assess the effect of in vitro supplementation of GSH during pellet swim-up method.

Design: Prospective study

Materials and Methods: Liquefied semen samples of normozoospermic (n=20) patients attending the andrology laboratory at University of Calcutta were mixed and centrifuged with minimal essential medium (MEM with 4mg/ml HSA) containing 2.5 mM or 5 mM GSH along with proper controls. Care was taken each time to dissolve the GSH powder to the processing media just prior to the preparation to get the maximum antioxidant capacity. The resultant pellet was layered with Quinn’s sperm preparation media for swim-up. The resultant swim-up fraction was analyzed for motility parameters according to WHO standards and DNA integrity was analyzed by acridine orange method.

Results: There was no significant improvement in % motility and DNA integrity between the control and GSH groups. However an improvement was observed in the % rapid forward motility in both the doses of GSH employed (Table1) with 5mM group showing a statistically significant value (P value = 0.036).

Conclusion: The protective effect of GSH can be attributed to its strong antioxidant activity, which might be helpful in minimizing the ROS generation during the pellet swim-up procedure. However care should be taken to the use of GSH as it may act also as a pro-oxidant. Instant addition of GSH to the processing media may be helpful rather than storage of GSH supplemented media in frozen conditions and repeated usage of frozen thawed GSH containing media.

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Table1: Effect of GSH in sperm washing media on sperm characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control swim-up</th>
<th>GSH (2.5mM)swim-up</th>
<th>P-value</th>
<th>GSH(5mM)swim-up</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>10.87 ± 10.25</td>
<td>17.70 ± 17.92</td>
<td>0.092</td>
<td>21.68 ± 18.46</td>
<td>0.056</td>
</tr>
<tr>
<td>% Motility</td>
<td>94.17 ± 6.35</td>
<td>97.22 ± 3.67</td>
<td>0.167</td>
<td>95.33 ± 4.68</td>
<td>0.633</td>
</tr>
<tr>
<td>% Rapid forward</td>
<td>16.12 ± 19.81</td>
<td>34.87 ± 17.37</td>
<td>0.070</td>
<td>32.48 ± 16.81</td>
<td>0.036</td>
</tr>
</tbody>
</table>
% Slow Forward | 62.40 ± 11.22 | 58.00 ± 20.76 | 0.575 | 55.43 ± 21.06 | 0.342 |
% Non-progressive | 15.61 ± 7.10 | 4.27 ± 4.13 | 0.037 | 6.75 ± 8.12 | 0.165 |
% Normal DNA integrity | 96.50 ± 1.22 | 97.16 ± 0.98 | 0.286 | 97.40 ± 1.76 | 0.282 |

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