Title: Relationship of reactive oxygen species levels in day 3 culture media with the outcome of IVF/ICSI cycles

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Objective: Recently, it has been shown that the presence of certain levels of oxidative/antioxidant activity in day 1 culture media can impact the embryological as well as the clinical outcome of IVF cycle. The objective of this study was to examine the relationship of early human embryonic development parameters with day 3 culture media reactive oxygen species (D-3 ROS) levels.

Design: Prospective study.

Materials and Methods: Patients undergoing in vitro fertilization (IVF; n = 92; 36 with intracytoplasmic sperm injection (ICSI) and 56 without ICSI) were included. Fertilization and early culture were performed in HTF with 5% serum substitute supplement. D-3 ROS levels in the central well (sample) and the outer well (control) of each embryo culture dish were measured after being thawed using chemiluminscense method within two hours after collection using the luminol probe. The actual D-3 ROS levels were then calculated. Embryo transfer was performed at day 5 for all cases. Fertilization rate and embryo quality parameters (day 3 and day 5) were recorded for each cycle. Patients were comparable regarding age, parity, and demographic features.

Results: The overall pregnancy rate in this cohort is 50/92 (54.3%). Day-3 ROS level was significantly lower in pregnant cycles 26.8±13.9 compared to non pregnant cycles 66.4±39.4, P<0.001. Irrespective of the method of fertilization (IVF or ICSI), this relationship was maintained when the cycles were stratified to conventional IVF (27.1±5.3 vs 66.9±5.7, P<0.001) or ICSI (25.6±6.3 vs 65.5±7.0, P=0.002). After controlling for all demographic and clinical variables (Table), D-3 ROS levels were negatively correlated with all in vitro growth parameters as well as pregnancy rate.

Conclusions: Increasing levels of ROS generated in the first 3 days of in vitro culture may have a detrimental effect on the in vitro growth parameters as well as the clinical pregnancy outcome either in conventional IVF or ICSI patients.

Support: None

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<tr>
<th>Relationship of day 3 ROS with main outcomes of IVF and ICSI</th>
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<tbody>
<tr>
<td>Factor</td>
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<td>High (&gt; 7) day 3 cell number</td>
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<td>Low fragmentation rate</td>
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<td>Blastocyst rate</td>
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<td>Clinical pregnancy</td>
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