Title: DOES COMPLETE HATCHING AT THE TIME OF THAW NEGATIVELY AFFECT PREGNANCY RATES?

Authors: Julian A. Gingold, MD, PhD, Jorge Rodriguez-Purata, MD, Michael C. Whitehouse BA, Joseph A. Lee BA, Benjamin Sandler, MD, Alan B. Copperman, MD

Affiliations:
1. Reproductive Medicine Associates of New York, 635 Madison Ave 10th Floor New York, New York, United States, 10022
2. Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, 1176 Fifth Avenue, 9th Floor, New York, New York, United States, 10029.

Objective: After re-warming for frozen embryo transfers (FET), embryonic expansion and hatching frequently observed in biopsied embryos, even when they were previously unhatched at time of vitrification. It is unknown whether hatching at this point is associated with poor embryo survival. This study sought to determine whether single embryo transfers (SETs) of euploid embryos that hatched during the warming process were associated with lower pregnancy rates (PRs).

Design: Retrospective study

Materials and Methods: Patients underwent trophectoderm biopsy with qPCR-based comprehensive chromosome screening (CCS) from June 2011 to January 2015. Only patients who underwent SET of a frozen euploid embryo were included. All embryos were graded according to the Gardner scale both prior to freeze by vitrification and after thaw prior to transfer. Patients were segregated into two groups: A) FETs of embryos that hatched from expansion 4 or 5 to expansion 6 during the warming process; and B) FETs of embryos expansion 4 or 5 that remained the same after re-warming. Contingency tables were generated for PR and clinical PR based on embryo hatching stage and the PR and clinical PR were computed. Statistical analysis was calculated by fisher exact test with significance at p< 0.05.

Results: Couples underwent 262 euploid frozen SETs of oocytes aged 23.5-44.3 yo. Embryos that had hatched prior to biopsy to grade 6 at transfer (n=86) experienced PRs (0.76, 0.65-0.84 95%
CI) that were not significantly different from those (n=176) that remained grade 4 or 5 (0.78, 0.71-0.84 95% CI, p=0.75). Clinical PRs from euploid SET in embryos that had hatched from grade 4 or 5 prior to biopsy to grade 6 (0.65, 0.54-0.75 95% CI) were not significantly different from those that remained grade 4 or 5 (0.66, 0.59-0.73 95% CI, p=0.89).

**Table:**

<table>
<thead>
<tr>
<th>Blast 4/5 Embryos at Freeze</th>
<th>At Transfer</th>
<th>Blast 6 vs 4/5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blast 4/5</td>
<td>Blast 6</td>
</tr>
<tr>
<td>ET (n)</td>
<td>176</td>
<td>86</td>
</tr>
<tr>
<td>BP (n)</td>
<td>137</td>
<td>65</td>
</tr>
<tr>
<td>BP Rate</td>
<td>0.78</td>
<td>0.76</td>
</tr>
<tr>
<td>OP (n)</td>
<td>117</td>
<td>56</td>
</tr>
<tr>
<td>OP Rate</td>
<td>0.66</td>
<td>0.65</td>
</tr>
</tbody>
</table>

ET: embryos transferred  
BP: biochemical pregnancy  
OP: ongoing clinical pregnancy  
OR: odds ratio

**Conclusions:**  
Extended culture has been improved and is more commonly applied in practice, consequently increasing the prevalence of blastocyst cryopreservation. Embryos hatching from their zona pellucida during the re-warming process experienced comparable PR and clinical PRs to those hatching prior to cryopreservation. There is no cause for concern when upon rewarming an unhatched embryo, complete escape from the zona pellucida occurs. Further studies utilizing larger subsets of patients are needed to confirm this study’s findings.

**Support:**  
None