Time Lapse Assessment is Better Than Just Culturing to the Blastocyst Stage: Con

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Disclosures

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Choosing the Best Embryo By Time Lapse versus Standard Morphology

- TLM has several potential benefits compared with standard morphological scoring.
- Several retrospective studies are encouraging.
- Inconclusive whether improved pregnancy rates can be attributed to the incubator or the selection model.
- TLM has usefulness aside from selection power but cost-effectiveness remains to be evaluated.
Time-lapse in the IVF-lab: how should we assess potential benefit?

- Industry behind TLM has largely driven widespread adoption, touting “improved success rates”.
- Serious flaws in study design. The only identified published studies chose to randomize oocytes or embryos and not women; therefore, impossible to evaluate pregnancy outcomes per woman.
- Until phase III randomized, controlled trials have been completed, where women, not oocytes or embryos are randomized, fertility clinics should decline to include this costly intervention in assisted reproduction protocol.

Need to resolve:

1. Are the enhanced outcomes a result of the addition of time-lapse parameters or to better incubation conditions?
2. Prospective studies with randomization of patients.
4. If it is better than simply growing all embryos to the blastocyst stage, it it ethical to deny this intervention to some patients due to cost?
What indications are there that the results might be due to incubation conditions?

Can time-lapse monitoring (TMS) improve reproductive outcome over standard incubation (SI) in a multicenter trial?

Meseguer et al., Fertil Steril 98:1481-1489, 2012

- 10 centers throughout Spain
- All ICSI
- 1,390 TMS cycles vs. 5915 SI cycles
- TMS with Embryoscope, 5 images, 15’
- both systems, 5% CO2 in air
- TMS utilized hierarchical classification
Can time-lapse monitoring (TMS) improve reproductive outcome over standard incubation (SI) in a multicenter trial?

Meseguer et al., Fertil Steril 98:1481-1489, 2012

Logistic regression model, incubation method as covariate:
- type of incubation
- type of cycle (autologous, donor)
- day of transfer (d3, d5)
- oocyte source (fresh, vitrified)
- no. mature oocytes injected
- patient age, autologous cycles
- no. prior treatments
- no. embryos transferred
- stimulation protocol
- female etiology
- clinic where cycle was performed

Multicenter trial – Meseguer et al., 2012

Avg. improvement = 20% (weighted for no. of TMS cycles)
Conclusions:

- Use of TMS can improve clinical pregnancy rate by an estimated relative 20% [OR 1.201; p = 0.0043]
- Use of TMS includes less handling of embryos thus reducing the risk of loss or contamination
- TMS offers strictly controlled environment and stable incubation conditions

The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection

Basile N, Vime P, Florensa M, Aparicio-Ruiz B, Garcia Velasco JA, Remohi J, Meseguer M.

**The New Algorithm for Embryo Selection – Multicentric Study**

- **t3** = zygote to 3 cells; 34-40 h
- **cc2** = 2-3 cells; 9-12 h
- **t5** = zygote to 5 cell; 45-55 h

**Include**

- **Morph.**
  - Exclude
  - Include

- **A+**
  - **A-**
  - **B+**
  - **B-**

- **C+**
  - **C-**
  - **D+**
  - **D-**

- **E**
  - **F**

*Phase 1, development; n = 765 cycles
Phase 2, test of algorithm; n = 885 cycles

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**Culture Characteristics**

<table>
<thead>
<tr>
<th>Center</th>
<th>IVI Valencia</th>
<th>IVI Madrid</th>
<th>IVI Sevilla</th>
<th>IVI Barcelona</th>
</tr>
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<tbody>
<tr>
<td>Culture Medium</td>
<td>Cook</td>
<td>Global</td>
<td>Cook</td>
<td>Global</td>
</tr>
<tr>
<td>Mineral Oil</td>
<td>Global</td>
<td>Global</td>
<td>Global</td>
<td>Global</td>
</tr>
<tr>
<td>%CO₂</td>
<td>5.5</td>
<td>6.5</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>% O₂</td>
<td>20/74.5 N₂</td>
<td>20/73.5 N₂</td>
<td>20/74.0 N₂</td>
<td>20/74.0 N₂</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.0</td>
<td>37.0</td>
<td>37.0</td>
<td>37.0</td>
</tr>
</tbody>
</table>

*Basile et al., Hum Reprod 30:276-283, 2015*
### Implantation by Embryo Category – Basile et al., 2015

<table>
<thead>
<tr>
<th>Category</th>
<th>imp/total (%)</th>
<th>Category</th>
<th>implantation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+</td>
<td>106/333 (32)</td>
<td>A</td>
<td>32</td>
</tr>
<tr>
<td>A-</td>
<td>23/74 (31)</td>
<td>B</td>
<td>28</td>
</tr>
<tr>
<td>B+</td>
<td>40/124 (32)</td>
<td>C</td>
<td>26</td>
</tr>
<tr>
<td>B-</td>
<td>12/61 (20)</td>
<td>C-</td>
<td>20</td>
</tr>
<tr>
<td>C+</td>
<td>23/70 (33)</td>
<td>D</td>
<td>20</td>
</tr>
<tr>
<td>C-</td>
<td>14/70 (20)</td>
<td>D-</td>
<td>19</td>
</tr>
<tr>
<td>D+</td>
<td>8/38 (21)</td>
<td>E</td>
<td>17</td>
</tr>
<tr>
<td>D-</td>
<td>30/155 (19)</td>
<td>E-</td>
<td>17</td>
</tr>
</tbody>
</table>

No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: results from an RCT

Park H, Bergh C, Selleskog U, Thurin-Kjellberg A, Lundin K
Hum Reprod 30:268-275, 2015

Standard incubator: Falcon dishes, 20 µl drops

EmbryoScope: EmbryoSlides, 25 µl
Impetus for the study:

To date, it has not yet been established if adding morphokinetic data to conventional embryo selection criteria improves clinical outcomes if all embryos are cultured in a self-contained incubator allowing growth in an optimized environment without disturbance.

Endpoints:

Implantation and clinical pregnancy rates in a blinded randomized controlled trial with all embryos cultured in the Embryoscope under identical conditions.
Patients: 18-43, autologous oocytes, >3 zygotes

Randomization: patients (not embryos) with a random number generator 1:1, 116 conventional; 119 TLM

Blinding: patients, REI, staff, sonographer

Culture: 12-well EmbryoSlide, 25 µl Global with 10% SPS, 6% CO₂, 5.5% O₂ with no replenishment; all ICSI

Conventional screening:
Hours post ICSI (HPI) – 42, 66, 90, 114, 138
Parameters –
- cell #
- symmetry
- % fragmentation
- multinucleation
- progression of compaction
- blastocyst (114, 138 HPI)
  - volume % expansion
  - ICM development
  - trophectoderm organization
Conventional screening:

Optimal growth pattern

<table>
<thead>
<tr>
<th>HPI</th>
<th>day 2</th>
<th>day 3</th>
<th>day 4</th>
<th>day 5</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>42</td>
<td>66</td>
<td>90</td>
<td>114</td>
</tr>
<tr>
<td>4 cell</td>
<td>8 cell</td>
<td>morula</td>
<td>blastocyst</td>
<td></td>
</tr>
</tbody>
</table>

Time-lapse assessment: (after morph. suitable)

time to pronuclear fading (tPNf; syngamy)
time to: 2 cells (t2)
3 cells (t3)
4 cells (t4)
5 cells (t5)
8 cells (t8)
> 9 cells or partially compacting (t9)
morula (tM)
start of blastulation (tSB) [initial appearance of blastocoel]
blastocyst (tBL) [crescent-shaped fluid space]
expanded blastocyst (tEBL) [expansion visible]
Goodman LR, Goldberg J, Falcone T, Austin C, Desai N.  
Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial.  

### Time-lapse assessment:

<table>
<thead>
<tr>
<th>Calculated parameters</th>
<th>Deselection</th>
</tr>
</thead>
<tbody>
<tr>
<td>duration of second cell cycle (cc2; t3-t2)</td>
<td>reverse cleavage</td>
</tr>
<tr>
<td>third cell cycle (cc3; t4-t3)</td>
<td>direct division 1 + 3</td>
</tr>
<tr>
<td>interval between 4c &amp; 5c (t4 int.; t5-t4)</td>
<td>irregular division</td>
</tr>
<tr>
<td>2c &amp; 5c (t5-t2)</td>
<td>multinucleation</td>
</tr>
<tr>
<td>time to complete synchronous divisions</td>
<td></td>
</tr>
<tr>
<td>s1 (t2-tPNf)</td>
<td></td>
</tr>
<tr>
<td>s2 (t4-t3)</td>
<td></td>
</tr>
<tr>
<td>s3 (t8-t5)</td>
<td></td>
</tr>
</tbody>
</table>

### Embryo selection:

- **Conventional selection** – grade, growth pattern, data from once-daily assessments

- **TLM selection (morphokinetic score)** –
  - **negative points** –
    - cc2 < 5 hours (-1)
    - multinucleation (-0.5)
    - irregular division (-0.5)
  - **positive points** –
    - t5 45.8-57.0 HPI (+1)
    - s2 0 – 0.1 h (+1)
    - s3 1.4 – 7 h (+1)
    - tSB <100 HPI (+1)
Goodman LR, Goldberg J, Falcone T, Austin C, Desai N.

Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial.

**Issues:**

- Number of embryos transferred per ASRM guidelines
day 3 and day 5 transfers (IR for d5 = 3X d3)
- Morphology assessment for suitability before use of morphokinetic parameters (no TLM arm independent of morphology)

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<table>
<thead>
<tr>
<th></th>
<th>TLM</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPR (%) all</td>
<td>68</td>
<td>63</td>
</tr>
<tr>
<td>CPR (%) blast</td>
<td>74</td>
<td>67</td>
</tr>
<tr>
<td>IR all</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td>IR blast</td>
<td>56</td>
<td>51</td>
</tr>
</tbody>
</table>

Group (TLM vs CS) not a significant predictor of CPR or IR.
tSB <100 hours and morphokinetic score in TLM group were independently associated with blastocyst implantation rates.
Conclusions

Enhanced outcomes unlikely due to stable culture alone.