Can embryo morphokinetic parameters predict recurrent implantation failure?

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• The process of implantation involves two main components:

A healthy embryo that should have the potential to implant.
A receptive endometrium that should enable implantation.

• The “cross-talk” between the embryo and the endometrium that finally leads to apposition, attachment and invasion of embryos is mandatory for successful implantation and subsequent normal placentation.
• Abnormal karyotype of the embryo is one of the major reasons for failure of implantation and miscarriage.
• Increased incidence of chromosomal translocations, mosaics, inversions and deletions of genetic material were reported in patients with RIF.
• Almost twice as many chromosomal abnormalities were detected in embryos from RIF patients as in embryos of controls (67.4 % versus 36.3 %) (Simon, 2015).
• PGT for aneuploidy (PGT-A), PGT for monogenic/single gene defects (PGTM) and PGT for chromosomal structural rearrangements (PGT-SR).

• Indications for PGT-A include advanced maternal age (AMA), recurrent *implantation failure (RIF)*, and couples with normal karyotypes who have experienced recurrent pregnancy loss (RPL).
• Genetic composition of the inner cell mass (ICM) and trophectoderm (TE) can differ, and mosaicism could occur in the trophectoderm.
• PGS requires careful conduct which may lead to false results if not properly executed.
• The invasive and complicated nature of PGS operation
• Legal or social reasons or simply because the clinic cannot perform the technique.
TIME LAPSE EMBRYOSCOPE
Time-lapse embryo monitoring system
Time-lapse monitoring system
• During a conventional IVF cycle the dishes should be removed from the incubator to evaluate the embryos at least once a day.
• Using time-lapse system, the prevention of the stress factors due to movement and the unfavorable changes in the environment (e.g., humidity, gas composition and light conditions).
• To avoid missing and also follow the important events during the cycle which confirmed that appear and disappear in a period of time.
Time-lapse monitoring system
<table>
<thead>
<tr>
<th>Time-lapse kinetics</th>
<th>Definition</th>
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<tbody>
<tr>
<td>tPNf</td>
<td>The time when both (or the last) PN disappear</td>
</tr>
<tr>
<td>t2</td>
<td>The time of the first cell cleavage, or mitosis. t2 is the first frame at which the two blastomeres are completely separated by individual cell membranes.</td>
</tr>
<tr>
<td>t3</td>
<td>The first observation of three discrete cells; the three cells stage marks initiation of the second round of cleavage.</td>
</tr>
<tr>
<td>t4</td>
<td>The first time the four discrete cells are observed.</td>
</tr>
<tr>
<td>t5</td>
<td>The first time the five discrete cells are observed.</td>
</tr>
<tr>
<td>t6</td>
<td>The first time the six discrete cells are observed.</td>
</tr>
<tr>
<td>t7</td>
<td>The first time the seven discrete cells are observed.</td>
</tr>
<tr>
<td>t8</td>
<td>The first time the eight discrete cells are observed.</td>
</tr>
<tr>
<td>cc2</td>
<td>Duration of the second cell cycle; the time from the division to a two blastomeres embryo until the time to the division to a three blastomeres; (cc2=t3-t2).</td>
</tr>
<tr>
<td>cc3</td>
<td>Duration of the third cell cycle; the time from the division to a three blastomeres embryo until the time to the division to a five blastomeres; (cc3=t5-t3).</td>
</tr>
<tr>
<td>s1</td>
<td>The synchronicity of the two blastomere divisions within the second cell cycle, calculated as t2-tPNf.</td>
</tr>
<tr>
<td>s2</td>
<td>The synchronicity of the two blastomere divisions within the second cell cycle, calculated as t4 -t3.</td>
</tr>
<tr>
<td>s3</td>
<td>The synchronicity of the four blastomere divisions within the third cell cycle, calculated as t8 - t5.</td>
</tr>
</tbody>
</table>
Time-lapse imaging and ART outcomes

• A 19% increase in the incidence of live birth using morphokinetic data to select embryos (Fishel et al, 2017).

• The timing of morula compaction and regular blastocyst formation is important as an indicator of high-quality blastocysts to increase odds for pregnancy after embryo transfer (Harada et al 2019).

• Time of morulation (tM) and trophectoderm quality were outlined as putative predictors of live birth (Rienzi et al, 2019).

• There is insufficient good-quality evidence of differences in live birth or ongoing pregnancy, miscarriage and stillbirth, or clinical pregnancy to choose between TLS, with or without embryo selection software, and conventional incubation (Armstrong et al, 2019).
Time-lapse imaging and RPL/RIF
Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis

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• Retrospective analysis
• A time-lapse imaging system in a total of 125 RPL/RIF patients undergoing PGS
• Embryo (n=504) biopsy was done on day 3 and comprehensive chromosome screening performed through arrayCGH.
• Kinetic variables included the time to 2 (t2), 3 (t3), 4 (t4), and 5 (t5) cells, the length of the second (cc2) and third (cc3) cell cycle, the synchrony in the division from 2 to 4 cells (s2), and the interval t5- t2.
• A logistic regression analysis identified t5-t2 and cc3 as the most relevant variables related to normal chromosomal content.
• An algorithm for embryo selection is proposed to classify embryos from A to D.
Biopsied

ok

≥20.5 h

11-18h

yes

cc3

no

11-18h

yes

Grade A

no

Grade B

yes

Grade C

no

Grade D

Discarded

nonviable
Summary:

• Chromosomally normal and abnormal embryos have different kinetic behavior.
• On the basis of these differences, the proposed algorithm serves as a tool to classify embryos and to increase the probability of noninvasively selecting normal embryos.
Type of chromosome abnormality affects embryo morphology dynamics

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• Retrospective cohort study.
• PGS patients (n= 112) including cases of advanced maternal age, repeated implantation failure, and recurrent miscarriage.
• All biopsied embryos (n=485) were cultured in an incubator with time-lapse technology.
• Chromosomal abnormality, as follows: embryos with monosomies (those embryos that lose a chromosome); trisomies (those embryos that have an extra copy of a chromosome) complex (embryos that have more than one chromosome altered)
• The embryos with monosomies showed an intermediate kinetics between complex and normal
• Embryos with trisomies showed very similar kinetics to normal embryos for all the variables studied; there was no statistically significant difference
• Complexes embryos showed faster divisions than normal embryos
• The difference was statistically significant for t3, t5, cc2, cc3, s2 and t5-t2
• Logistic regression analysis showed that t3 and t5–t2 were strongly associated with complex aneuploid embryos
• A hierarchical model was developed which subdivided embryos into four categories (A–D)
Biopsed

ok

t5-t2

yes

t3

35-40h

>21 h

no

35-40h

Grade A

Grade B

Grade C

Grade D
The bar chart shows the percentage of normal and complex embryos across different categories labeled A to D.

- Category A: 70.6% normal, 29.4% complex (n=84, n=35)
- Category B: 62.6% normal, 37.4% complex (n=67, n=40)
- Category C: 35.7% normal, 64.3% complex (n=10, n=18)
- Category D: 14.6% normal, 85.4% complex (n=7, n=41)
Summary:

• Embryo morphokinettics are affected by chromosome aneuploidy
• The use of time-lapse monitoring, although not able to detect an abnormal embryo, may be potentially useful to discard those embryos with high risk of complex chromosomal abnormalities
Can time-lapse parameters predict embryo ploidy? A systematic review

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KEY MESSAGE
Studies reporting an association between morphokinetic parameters and embryo ploidy status are controversial and do not support the predictive value of time-lapse analysis for embryo aneuploidy screening.
• 13 studies were selected for data collection on the predictive value of morphokinetic analysis for human embryo ploidy in IVF–PGS cycles

• The number of cycles included varied significantly, ranging from 25 to 444.

• The number of embryos included was heterogeneous in these studies, ranging from 53 to 928.
Table 1 – Principal characteristics of the studies reporting on the value of morphokinetic parameters as predictors of embryo ploidy. Studies are listed in chronological order.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Number of couple/ cycles</th>
<th>Clinical indication for PGS</th>
<th>Number of embryos</th>
<th>Embryo stage for biopsy</th>
<th>PGS technique</th>
<th>Time- lapse device</th>
<th>Atmosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chavez et al. (2012)</td>
<td>45/NA</td>
<td>NA</td>
<td>53</td>
<td>Day 2</td>
<td>aCGH</td>
<td>custom-built miniature microscope system</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Campbell et al. (2013a)</td>
<td>25/25</td>
<td>AMA, RIF, recurrent miscarriage, severe male factor</td>
<td>98</td>
<td>Blastocyst</td>
<td>aCGH or SNP array</td>
<td>Embryoscope®</td>
<td>5.5% CO2, 5% O2</td>
</tr>
<tr>
<td>Campbell et al. (2013b)</td>
<td>69/69</td>
<td>Unknown</td>
<td>88</td>
<td>Blastocyst</td>
<td>aCGH or SNP array</td>
<td>Embryoscope®</td>
<td>5.5% CO2, 5% O2</td>
</tr>
<tr>
<td>Basile et al. (2014)</td>
<td>87/125</td>
<td>RIF and recurrent miscarriage</td>
<td>504</td>
<td>Day 3</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>Not described</td>
</tr>
<tr>
<td>Kramer et al. (2016)</td>
<td>25/25</td>
<td>Recurrent miscarriage, AMA, others</td>
<td>169</td>
<td>Blastocyst</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Yang et al. (2014)</td>
<td>NA</td>
<td>RPL, RIF, PCA</td>
<td>285</td>
<td>Blastocyst</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Chawla et al. (2015)</td>
<td>132/132</td>
<td>Sex selection</td>
<td>460</td>
<td>Day 3</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>Not described</td>
</tr>
<tr>
<td>Rienzi et al. (2015)</td>
<td>138/138</td>
<td>AMA, RIF, recurrent miscarriage</td>
<td>455</td>
<td>Blastocyst</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Minasi et al. (2016)</td>
<td>444/530</td>
<td>Unknown</td>
<td>1730/928 cultured in time-lapse</td>
<td>Blastocyst</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Balakier et al. (2016)</td>
<td>296 (113 with PGS) / 296 (113)</td>
<td>AMA, PCOS, male factor and others</td>
<td>2441/607 with PGS</td>
<td>Blastocyst</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6% CO2, 5% O2</td>
</tr>
<tr>
<td>Patel et al. (2016)</td>
<td>26/29</td>
<td>AMA, RIF, recurrent miscarriage</td>
<td>167</td>
<td>Day 3</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>Not described</td>
</tr>
<tr>
<td>Mumusoglu et al. (2017)</td>
<td>103/103</td>
<td>AMA, PGD</td>
<td>415</td>
<td>Day 3</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>6.8% CO2, 5% O2</td>
</tr>
<tr>
<td>Del Carmen Nogales et al. (2017)</td>
<td>112/112</td>
<td>AMA, RIF, and recurrent miscarriage</td>
<td>485</td>
<td>Day 3</td>
<td>aCGH</td>
<td>Embryoscope®</td>
<td>Not described</td>
</tr>
</tbody>
</table>
• Finally, most, but not all, investigators reported significant differences in morphokinetic pattern between euploid and aneuploid embryos.
• The clinical significance of these results was not clear.
• All investigators concluded that time-lapse should not be still considered as an accurate non-invasive method for embryo ploidy assessment.
Overall, review of the literature on the effectiveness of time-lapse as a predictor of embryo ploidy revealed:

- Morphokinetic parameters can aid in differentiating between euploid and aneuploid embryos, although they are not sufficiently accurate to replace preimplantation genetic testing for aneuploidy.
• Most of the current studies are retrospective
• Large heterogeneity of the studies published to date
• Patients basal characteristics
• The stage at which embryo biopsy was carried out (cleavage/blastocyst)
• Various technical approaches can be used for PGS (aCGH)/new technologies for embryo aneuploidy screening such as next-generation sequencing
Conclusions…:

• Neither a unique morphokinetic nor combined parameters could predict embryo ploidy with enough sensitivity, specificity, or both, to be used clinically for embryo selection.

• Morphokinetic assessment together with chromosomal screening may ultimately help identify euploid embryos with the highest developmental potential
...Conclusions:

- An excellent selection tool for patients who are indicated for PGS (history of implantation failure or early pregnancy loss) but who for any legal, social, or economic reasons do not wish or cannot have PGS performed.
- More large-scale prospective studies, conducted in homogeneous populations with standard culture and biopsy protocol, adjusted to patients’ characteristics, are needed.
Thank you for your attention