Assessment of embryo morphology and developmental dynamics by time-lapse microscopy: is there a relation to implantation and ploidy?

Nikica Zaninovic, Ph.D.,[a] Mohamad Irani, M.D.,[b] and Marcos Meseguer, Ph.D.[b]

[a] The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, New York; and [b] Instituto Valenciano de Infertilidad, Universidad de Valencia, Valencia, Spain

Time-lapse microscopy (TLM) is an exciting novel technology with great potential for enhancing embryo selection in the embryology laboratory. This non-invasive objective assessment of embryos has provided a new tool for predicting embryo development and implantation potential. TLM detects several morphological phenomena that are often missed with static observations using conventional incubators, such as irregular divisions, blastocyst collapse and re-expansion, timing of blastocoel appearance, and timing of formation and internalization of fragments. Nevertheless, it should be recognized that conventional morphological assessment has been widely accepted as the gold standard by most embryologists. TLM can enhance conventional morphological assessments to improve embryo selection and subsequent reproductive outcomes. Furthermore, morphokinetic parameters can aid in differentiating between euploid and aneuploid embryos, although they are not sufficiently accurate to replace preimplantation genetic testing for aneuploidy. Morphokinetic assessment together with chromosomal screening may ultimately help identify euploid embryos with the highest developmental potential. (Fertil Steril® 2017;108:722–9. ©2017 by American Society for Reproductive Medicine.)

Key Words: Time-lapse microscopy, euploid embryos, morphokinetic assessment, chromosomal screening, implantation

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Despite major advances in assisted reproductive technologies over the last three decades, a key challenge facing in vitro fertilization (IVF) remains the selection of the best single embryo for transfer (1). This advancement will maximize IVF success rates while minimizing the risks of multiple gestation pregnancies along with the associated maternal and fetal complications (2). An essential step to achieve a singleton live birth after each elective single embryo transfer (eSET) is to enhance the embryo selection process. There are currently invasive and non-invasive technologies to select embryos. Non-invasive strategies include embryo morphology, time-lapse microscopy (TLM), metabolomics and proteomic profiles, while invasive techniques involve embryo biopsy for genetic and/or chromosomal testing.

Preimplantation genetic testing for aneuploidy (PGT-A) has been shown to be an important tool to determine embryo ploidy and to improve transfer success rates (3–5). However, its invasive nature is associated with a decrease in cumulative pregnancy rates when biopsies are performed at the cleavage stage (6, 7). In addition, PGT-A may only improve the pregnancy rate per transfer in women older than 37 years (8). Furthermore, the recent development of genetic technologies, particularly high-resolution next generation sequencing, has increased the sensitivity of detecting mosaicism, a finding of yet unknown significance (9–11). Indeed, many mosaic embryos are discarded despite their potential of becoming healthy babies. Moreover, with the implementation of blastocyst biopsy, patients undergoing PGT-A cycles using next generation sequencing or array comparative genomic hybridization require frozen-thawed embryo transfer to avoid the apparent lower success rates associated with day 6 fresh embryo transfers (12). Therefore, despite its clinical advantages, PGT-A still requires further optimization and validation of its role in assisted reproductive technology.

In the context of PGT-A limitations, it is paramount to optimize non-invasive technologies with the goal of selecting embryos with the highest developmental potential. Since

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Correspondence: Nikica Zaninovic, Ph.D., The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, 1305 York Avenue, New York, New York 10021 (E-mail: nizanin@med.cornell.edu).

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the establishment of IVF, embryo morphology has been the most common method utilized by embryologists for monitoring embryo growth and selection of optimal embryo(s) for transfer (13, 14). Standard morphological evaluation includes the assessment of several parameters, including cell number, rate of division, degree of fragmentation, presence of multinucleation, blastomere size and symmetry, and thickness of zona pellucida. At the blastocyst stage, assessment of blastocoel expansion, and number, shape, and cohesion of cells within the trophectoderm and inner cell mass, is used (15, 16). These parameters are good clinical predictors of live birth rates following fresh or frozen cycles especially at the blastocyst stage (13, 17, 18). Furthermore, blastocyst grading has been successfully used to predict, although imperfectly, embryo ploidy status and implantation and live birth rates of euploid blastocysts (19, 20).

However, given the dynamic nature of embryo development during the preimplantation period, assessing morphology through static observations using conventional incubators is misleading (21). This inaccuracy is even more relevant to the current trend toward limiting the frequency of microscopic observations to minimize the potential negative effect of handling the embryos outside the incubator (22). Additionally, the inconsistency of time-points assessment may contribute to equivocal findings. For instance, day-2 embryos may be at the two-cell stage in the morning of day 2, but found to be at the four-cell stage if assessed a few hours later. Indeed, standard morphological assessment is susceptible to the timing of observations and inherent variability in embryo scoring among embryologists.

The introduction of TLM has offered solutions to overcome some of the pitfalls of standard morphological assessment. First, TLM has safely allowed the incubation of embryos in stable culture conditions by minimizing the potential impact of changes in temperature or gas composition (23–26). Secondly, TLM allows for continuous observation of embryo development, thereby both enhancing our knowledge of embryokinetics (exact timing of embryo cleavages) and preventing possible inaccurate findings of static morphological assessments (27). Lastly, TLM allows for the evaluation of quantitative and qualitative objective parameters, thus reducing inter- and intra-observer variations.

Here, we aim to describe morphokinetic markers and to review the most pertinent studies pertaining to the role of TLM in embryo selection and potential association with the embryo ploidy.

TIME-LAPSE MICROSCOPY PREDICTS EMBRYO DEVELOPMENT AND IMPLANTATION

The multiple benefits of TLM include allowing users to observe the precise occurrence and duration of cell divisions (cytokinesis), duration of cell cycles (time interval between cleavages) and to precisely monitor morphological parameters. These TLM advantages have been correlated with embryo development and implantation rates when compared with the use of conventional incubators (28–30). TLM also allows the detection of several phenomena such as irregular divisions, timing and formation of fragments, and the appearance of the blastocoel (31–33). In 1997, Payne et al. (34) were the first to use TLM in a clinical setting. They used it in the initial 17-20 hours after intracytoplasmic sperm injection (ICSI), wherein they described several key events such as second polar body extrusion and the formation of the male and female pronuclei. Soon thereafter, Shoukir et al. reported that embryos that cleaved to the two-cell stage 25 hours post-insemination with conventional IVF yielded higher clinical pregnancy rates compared to those that did not complete the first division by that time; this was confirmed by the same authors for embryos fertilized with ICSI (35, 36). In 2010, Wong et al. correlated TLM analysis with embryo gene expression, suggesting that TLM can predict embryo progression to blastocyst with >93% sensitivity and specificity (28). The authors used frozen-thawed embryos and proposed the mean ± standard deviation durations of first cytokinesis (14.3 ± 6 min), the time interval between the first and second mitosis -from the 2- to 3-cell stage (11.1 ± 2.2 h), and the time interval between the second and third mitosis from the 3- to 4-cell stage (1 ± 1.6 h) were good predictors of blastocyst formation (28). Based on this technology, the Eeva automated cleavage annotation system was developed under dark field microscopy. The Eeva system showed limited clinical use and thus was discontinued.

Early TLM findings encouraged many other groups to investigate whether kinetic markers can assist in embryo selection and, thus, predict implantation potential (29, 37). In 2011, a landmark paper by Meseguer et al. (29) described the development of 522 embryos monitored by TLM for at least 64 hours. They analyzed kinetic parameters of 247 transferred embryos with known implantation data and found that the following criteria correlate with implantation: t5, representing the time between ICSI and the 5-cell stage (48.8–56.6 h); s2, reflecting the time between the division to 3 cells and the division to 4 cells (≤0.76 h); and cc2, illustrating the time between the division to 2 cells and the division to 3 cells (≤11.9 h). The authors also described some aberrant morphological events that are associated with poor implantation potential, which could otherwise not be detected by conventional incubators: direct cleavage from the 2- to 3-cell stage (cc2 = t3-t2 < 5 hours); 2-cell stage with uneven blastomere size during the interphase where the nuclei are visible; and 4-cell stage with multinucleation during the interphase where the nuclei are visible (29). Meseguer et al. (29) proposed an algorithm for embryo selection based on morphological and morphokinetic characteristics classifying embryos into 10 categories (A+ to F) that are associated with decreasing implantation rates.

Time-lapse Microscopy and Abnormal Cytokinesis

Rubio et al. (37) conducted a multicenter retrospective study consisting of 5,225 embryos to evaluate the effect of direct cleavage from the 1 to 3-cell stage on pregnancy outcomes. They confirmed that embryos with direct cleavage (t3-t2 < 5 h) exhibited very low implantation rates (1.2%), suggesting that excluding them from transfer would improve