ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Time-lapse deselection model for human day 3 in vitro fertilization embryos: the combination of qualitative and quantitative measures of embryo growth

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Objective: To present a time-lapse deselection model involving both qualitative and quantitative parameters for assessing embryos on day 3.
Design: Retrospective cohort study and prospective validation.
Setting: Private IVF center.
Patient(s): A total of 270 embryos with known implantation data (KID) after day 3 transfer from 212 IVF/intracytoplasmic sperm injection (ICSI) cycles were retrospectively analyzed for building the proposed deselection model, followed by prospective validation using an additional 66 KID embryos.
Intervention(s): None.
Main Outcome Measure(s): Morphological score on day 3, embryo morphokinetic parameters, abnormal cleavage patterns, and known implantation results.
Result(s): All included embryos were categorized either retrospectively or prospectively into 7 grades (A+, A, B, C, D, E, F). Qualitative deselection parameters included poor conventional day 3 morphology, abnormal cleavage patterns identified via time-lapse monitoring, and <8 cells at 68 hours postinsemination. Quantitative parameters included time from pronuclear fading (PNF) to 5-cell stage and duration of 3-cell stage. KID implantation rates of embryos graded from A+ to F were 52.9%, 36.1%, 25.0%, 13.8%, 15.6%, 3.1%, and 0 respectively (area under the curve [AUC] = 0.762; 95% confidence interval [CI], 0.701–0.824), and a similar pattern was seen in either IVF (AUC = 0.721; 95% CI, 0.622–0.821) or ICSI embryos (AUC = 0.790; 95% CI, 0.711–0.868). Preliminary prospective validation using 66 KID embryos also showed statistically significant prediction in Medicult (AUC = 0.750; 95% CI, 0.588–0.912) and Vitrolife G-Series (AUC = 0.820; 95% CI, 0.671–0.969) suites of culture media.
Conclusion(s): The proposed model involving both qualitative and quantitative deselection effectively predicts day 3 embryo implantation potential and is applicable to all IVF embryos regardless of insemination method by using PNF as the reference starting time point. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Embryo, time-lapse, implantation, pronuclear fading, deselection

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After the initial attempts at applying time-lapse embryo selection to clinical IVF practice (1, 2), a number of publications have shown promising results using morphokinetic data to predict embryo implantation (1,3–6). One of the most influential morphokinetic grading algorithms was published by Meseguer et al. (1) in 2011. Regarded as one of the foundation clinical studies of human embryo morphokinetics, this algorithm has, however, been questioned more recently regarding its transferability between different laboratories (7–10). Embryo morphokinetic results are thought to be the subject of a number of factors, such as culture media (11), oxygen concentration in culture (12), patient population (13, 14),

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ovarian stimulating protocols (15), hormone levels in the female partner (16), or even sperm DNA fragmentation (17). As the transferability of previously published morphokinetic algorithms is likely to be influenced by these factors, one should be cautious when implementing an embryo morphokinetic algorithm that was constructed in a different laboratory. In addition, some qualitative deselection parameters identified via time-lapse imaging of embryos have been reported, showing an encouraging potential to identify embryos with a low probability to implant (18–21). The advantage of using such parameters for embryo deselection is their qualitative nature, with the measurement being either positive or negative and also independent of absolute cell cleavage timings. As a result, interlaboratory transferability may possibly be improved using algorithms encompassing such parameters.

The majority of published time-lapse studies to date are based on embryos fertilized via intracytoplasmic sperm injection (ICSI) (1, 4) rather than conventional IVF, largely due to the difficulty in defining sperm entry time in the latter. However, even in ICSI cases, the sperm entry time point for each individual embryo may also be imprecise as seen, for example, in particular time-lapse equipment such as the Embryoscope (Vitrolife), where all embryos cultured on the same slide share one single starting time point (22). Furthermore, nuclear mature oocytes were shown to extrude the second polar body at various timings (ranging from 0.70 to 10.15 hours post-ICSI) (23), indicating that metaphase II oocytes may have different degrees of cytoplasmic maturity at the point of sperm injection. Recently, pronuclear fading (PNF), which is a biological time point, has been proposed as an alternative reference starting time point, rather than insemination, which is a procedural time point (24). Using PNF enables IVF and ICSI embryos to be integrated into the same algorithm and minimizes the variations in early stage timings owing to the procedural issues (24). Based on the above findings, the current study aims to present a time-lapse deselection model for predicting the implantation potential of embryos regardless of insemination method, including both qualitative and quantitative measures of the growth of early cleavage human embryos.

MATERIALS AND METHODS

Patient Management and Embryo Culture

The retrospective part of this study included a total of 212 treatment cycles (84 IVF and 128 ICSI cycles; females age 34.63 ± 4.41 vs. 34.45 ± 4.51 years, not significant) performed at Fertility North between February 2013 and December 2014, with all transferred embryos having known implantation data (KID) (21). In total, 270 (105 IVF and 165 ICSI embryos) fully annotated KID embryos that had reached at least the 5-cell stage were analyzed after culture in the Medicult media suite (Origio). The prospective part of this study included [1] 36 KID embryos cultured in the Medicult media suite from 30 IVF/ICSI cycles (females ages 35.11 ± 4.03 years) performed between May and July 2015 and [2] 30 KID embryos cultured in the G-Series media suite (Vitrolife) from 23 IVF/ICSI cycles (females ages 35.97 ± 5.31 years, not significant) performed between July and September 2015. The use of the Embryoscope was registered as an innovative procedure with the Reproductive Technology Council (Department of Health, East Perth, Australia), and accordingly all participating couples gave consent to use the Embryoscope as an incubator for embryo culture. Retrospective data analysis was approved by the Human Research Ethics Committees at both Joondalup Health Campus and Edith Cowan University.

Ovarian stimulation, gamete collection, and insemination using either conventional IVF or ICSI were performed as described elsewhere (19), with oocyte collection being day 0. Gametes were prepared in the Universal IVF (Origio) or G-IVF Plus medium (Vitrolife). Fertilized oocytes were placed in the Embryoscope for 3 days of culture in either ISM1 (Origio) or G-1 Plus medium (Vitrolife) before uterine transfer. Culture conditions were set at 37°C with 6% CO_2, 5% O_2, and balanced N_2, with images taken every 10 minutes across seven focal planes of the embryos.

Morphokinetic and Conventional Assessment of Embryo Development

All the embryos cultured in the Embryoscope were retrospectively annotated on day 3 by one embryologist (Y.L.), using the Embryoviewer (Vitrolife) software. The timing parameters considered in the present study included time from pronuclear fading to 5-cell stage (T5_PNF, hour) and duration of 3-cell stage (S2, hour), which had been regarded as two major implantation predictors (t5 relative to sperm injection instead of PNF and s2) in previous publications (1, 5, 8). Qualitative parameters used for deselecting embryos were also recorded, including [1] direct cleavage (DC) where either the 2- or 4-cell stage was less than 5 hours (20, 21), [2] reverse cleavage (RC) where either daughter cells fused after cleavage division or the blastomere failed to divide after karyokinesis (19), and [3] <6 intercellular contact points (ICCP) at the end of the 4-cell stage (21). Conventional morphological assessment was also performed based on the embryo image captured at 68 hours postinsemination (hpi) according to criteria previously published (25), analyzing cell count, symmetry, and degree of fragmentation.

Grading of Day 3 Embryos using the Proposed Model

Day 3 embryos were graded either retrospectively or prospectively using a series of questions as illustrated in Figure 1. Briefly, [1] if one was determined to be a poor-quality embryo (PQE) according to conventional morphology assessment at 68 hpi, it is categorized a grade F, otherwise subject to further criteria; [2] if one had displayed abnormal cleavage pattern(s) such as DC, RC, or <6 ICCP at the end of the 4-cell stage, it is categorized a grade E, otherwise subject to further criteria; [3] if one had <8 cells at 68 hpi, it is categorized a grade D, otherwise subject to further criteria; [4] if one had S2 > 0.84 hour, it is categorized a grade C, otherwise subject to further criteria; [5] if one had T5_PNF > 28.01 hours, it is categorized a grade B, otherwise subject to further criteria; [6] if one had T5_PNF