ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system

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Objective: To describe the events associated with the blastocyst formation and implantation that occur in embryos during preimplantation development based on the largest sample size ever described with time-lapse monitoring.
Design: Observational, retrospective, single-center clinical study.
Setting: University-affiliated private IVF center.
Patient(s): A total of 7,483 zygotes from 990 first treatments of intracytoplasmic sperm injection (ICSI; 627 of oocyte donor vs. 363 autologous oocyte cycles), of which 832 blastocysts were transferred.
Intervention(s): No patient intervention. Embryos were cultured in a time-lapse monitoring system, and the embryos were transferred on day 5 after ICSI. Embryo selection was based on the multivariable model previously developed and on blastocyst morphology.
Main Outcome Measure(s): Using a time-lapse system, embryo images were acquired every 15 minutes for 120 hours. Embryos cleavage time points up to the 9-cell stage (t2–t9) as well as to the morula stage (tM) and blastocyst formation (tB) were registered in hours after ICSI. Additionally, duration of the cell cycle and synchrony of the second and third cell cycles were defined. As a result, we have monitored the embryonic development of a total of 3,215 blastocysts, of which 832 were transferred. Finally, we analyzed the characteristics of embryonic development of blastocyst (phase 1) and of implanted and not implanted (phase 2) embryos as finally validated in an independent data set (phase 3).
Result(s): A detailed retrospective analysis of cleavage times was made for 7,483 zygotes. We analyzed 17 parameters and found several significantly correlated with subsequent blastocyst formation and implantation. The most predictive parameters for blastocyst formation were time of morula formation, tM (81.28–96.0 hours after ICSI), and t8–t5 ($\leq$8.78 hours) or time of transition of 5-blastomere embryos to 8-blastomere embryos with a receiver operating characteristic curve (ROC) value = 0.849 (95% confidence interval [CI], 0.835–0.854; phase 1). These parameters were less predictive of implantation, with a ROC value = 0.546 (95% CI, 0.507–0.585). We also observed that time for expansion blastocyst, tEB (107.9–112.9 hours after ICSI), and t8–t5 ($\leq$5.67 hours after ICSI) predict blastocyst implantation, with a ROC value = 0.591 (95% CI, 0.552–0.630; phase 2). The model was validated on an independent data set and gave a ROC of 0.596 (0.526–0.666; phase 3).
Conclusion(s): The inclusion of kinetic parameters into score evaluation may improve blastocyst selection criteria and can predict blastocyst formation with high accuracy. We propose two multivariable models based on our findings to classify embryos according to their probabilities of blastocyst stage and implantation in the largest data set ever reported of human blastocysts. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Embryo quality, implantation, time lapse, assisted reproductive technologies, morphokinetic parameters

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The morphological classification of gametes and embryos has been used since the beginning of IVF until today as a tool to assess embryo development (1) and to select the best embryo for transfer (2). Embryos are routinely evaluated at a single or few time points on day 2 or 3 (D2 or D3). This handling is likely to cause fluctuations in temperature and pH during routine microscopic evaluation, which can harm embryos. Although used in most laboratories as a general indicator of embryo quality, this methodology may not select the most competent embryo, as a consequence of the nonspecific and subjective characteristics of this method. In addition, the conventional embryo selection method is associated with a relatively low IVF success rate (3). Therefore, the final decision on which embryo to transfer is traditionally based predominantly on the number and symmetry of blastomeres at the day of transfer, taking into account morphology evaluation at earlier observations. As a compensative approach, extended embryo culture and transfer at the blastocyst stage (D5 or D6) becomes an alternative that permits the selection of embryos at more advanced stages of development and minimizes the risk of multiple pregnancies, a figure that in 2010 reached an alarming threshold of 20.6% (4). Although multiple observations give a better understanding of embryo development, it is well known that each observation also involves exposure to suboptimal conditions outside the controlled environment of an incubator, potentially affecting the success of the treatment.

In contrast, automatic time-lapse systems offer the possibility to monitor embryo development continuously throughout the culture period (5). This is obtained through the digital image capture defined by programmed time intervals and increases the quality and quantity of the information. In addition, time-lapse technology allows maintaining more stable culture conditions and is considered a safe tool in IVF laboratories, mainly because no adverse effects on the embryo can be detected (6–9). Since the first analysis of human embryonic development with time-lapse imaging that included the process of fertilization and assessment of early events (10), numerous studies have used this technology (11, 12), including the search for noninvasive prognostic markers that predict embryo development and the outcome of IVF treatments. Meseguer et al. (13) introduced specific temporal development markers that were related to subsequent implantation. They evaluated the chronological pattern of cell divisions as well as other morphological features to identify connections between the time taken to reach each embryonic event and the implantation potential of the specific embryo and proposed a hierarchical classification based on the parameters t5 (time division 5 cells), t4–t3, and t3–t2 to select the most viable embryos for transfer in each treatment cycle. However, this model only assesses embryos until D3 of development and is not associated with the formation and quality of blastocysts or the outcomes of success in IVF treatments that focus on blastocyst culture.

Conversely, other studies using this tool have focused on identifying morphokinetic parameters that predict blastocyst formation and blastocyst morphology (14–18), which could potentially improve the reproductive outcome compared with the methodology of conventional incubation (8).

Wong et al. (19) found that development of human embryos to the blastocyst stage was linked to key timings in early embryos development like the duration of the first cytoplasm cleavage from 1 to 2 cells (cytokinesis) and the length of the interval between divisions in the first stages of embryonic development. Later Campbell et al. described the risk to embryos of having single or multiple aneuploid chromosome constitution, and their results showed that multiple aneuploid embryos were delayed at the initiation of compaction, initiation of blastulation, and the time to reach full blastocyst stage. So they developed a predictive algorithm based on the dynamic events (20). Although in the retrospective analysis to evaluate the effectiveness and potential impact of this model (21) their results showed that this model offers a good prediction of pregnancy rates and outcomes and although other investigators (20, 22–24) defined optimal ranges of embryonic development associated with chromosomal normality, no prospective study or test comparing these algorithms with the conventional embryo selection procedure and conversely has been published.

Thus, time-lapse technology becomes an important tool in IVF that allows identification of a number of critical events during embryonic development. Therefore, the present study offers a unique opportunity for a comprehensive retrospective analysis and evaluates blastocyst development through time-lapse monitoring (TLM) and the effect on treatment outcome.

The purpose of this study was to assess the time of embryonic events and determine which have the ability to predict blastocyst formation and implantation potential using a TLM and a multivariable morphokinetic model until D5.

MATERIALS AND METHODS

This research project was conducted at the Instituto Valenciano de Infertilidad (IVI) Valencia and was performed from May 2010 until May 2014. The procedure and protocol for analysis of embryos were approved by an Institutional Review Board (IRB reference 1404-VLC-014-YM), which regulates and approves database analysis and clinical IVF procedures for research at IVI. Additionally, the project complies with the Spanish law governing assisted reproductive technologies (14/2006). The present retrospective cohort study was drawn from a total of 990 first treatments of intracytoplasmic sperm injection (ICSI). Among the embryos included in the study, 3,215 (42.96%) developed to the blastocyst stage, 832 (11.11%) blastocysts were transferred in fresh treatment, and the remaining 2,383 (31.84%) were vitrified. Finally, 4,268 (57.03%) embryos did not reach blastocyst stage (nonviable and discarded; Supplemental Fig. 1).

Phase 1 of the study included the generation of the algorithm for blastocyst formation. The data to develop the new algorithm were obtained from a total of 990 cycles giving rise to 7,483 zygotes and 3,215 blastocysts. Phase 2 included the generation of the algorithm for implantation blastocysts. The data to develop the new algorithm were obtained from 832 blastocyst with known implantation (KID) transferred blastocysts, from which 383 implanted. Phase 3 included