ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy?

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Objective: To determine whether cleavage anomalies, multinucleation, and specific cellular kinetic parameters available from time-lapse imaging are predictive of developmental capacity or blastocyst chromosomal status.
Design: Retrospective analysis of prospectively collected data.
Setting: Single academic center.
Patient(s): A total of 1,478 zygotes from patients with blastocysts biopsied for preimplantation genetic screening were cultured in the EmbryoScope.
Intervention(s): Trophectoderm biopsy.
Main Outcome Measure(s): Embryo dysmorphisms, developmental kinetics, and euploidy.
Result(s): Of the 767 biopsied blastocysts, 41.6% (95% confidence interval [CI], 38%–45%) were diagnosed as euploid. Individual dysmorphisms such as multinucleation, reverse cleavage, irregular chaotic division, or direct uneven cleavage were not associated with aneuploidy. Direct uneven cleavage and irregular chaotic division embryos did, however, exhibit lower developmental potential. The presence of two or more dysmorphisms was associated with an overall lower euploidy rate, 27.6% (95% CI 19%–39%). Early embryo kinetics were predictive of blastocyst development but not ploidy status. In contrast, chromosomal status correlated significantly with start time of blastulation (tSB), expansion (tEB), and the tEB-tSB interval. A lower euploidy rate, 36.6% (95% CI 33%–42%) was observed with tSB $\geq$ 96.2 hours, compared with 48.2% with tSB $<$ 96.2 (95% CI 42%–54%). A drop in euploidy rate to 30% (95% CI 25%–37%) was observed in blastocysts with delayed expansion (tEB $>$ 116). The proportion of euploid blastocysts was increased with tEB-tSB intervals of $\leq$13 hours. A logistic regression model to enhance the probability of selecting a euploid blastocyst was constructed.
Conclusion(s): Morphokinetics may aid in selection of euploid embryos from a cohort of day 5/6 blastocysts. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Blastocyst development, preimplantation genetic screening, morphokinetics, multinucleation, aneuploidy

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Recognition of the health risks and financial costs associated with multiple pregnancies has been a driving force for IVF laboratories to move toward elective single ET (eSET). The adoption of an eSET policy demands not only optimization of culture technology but also embryo selection/deselection techniques that enhance the possibility of the selection of embryos with the greatest implantation potential. Embryonic aneuploidy contributes significantly to failed implantation. An interesting study by Yang et al. reported a euploidy rate of only 44% in good-prognosis patients undergoing IVF (1). Clearly, blastocyst selection with the use of conventional morphology alone has its limitations. The last several years have seen a steep rise in the use of preimplantation genetic screening (PGS) as a tool to encourage eSET through selection of a single euploid blastocyst for transfer (2–4). Trophectoderm biopsy for PGS is, however, an invasive, labor-intensive technique, presenting some risk to the embryo and requiring considerable laboratory expertise. Moreover, genetic screening adds significant cost to the IVF cycle and may therefore not be affordable to all patients. Also, in many countries, PGS screening of embryos is not as widely practiced as in the United States or as accessible to all patients.

The association between embryonic growth rate and embryo quality is well recognized. Implantation and live-birth rates are lower with slow growing embryos not blastulating until the sixth day of culture (5–7). PGS data indicate lower euploidy rates with day 6 versus day 5 biopsied blastocysts (8, 9). Sophisticated incubation chambers with continuous live imaging of embryos in culture have produced a wealth of new information and raised the intriguing possibility of using such data to refine blastocyst selection and/or deselection criteria. Time-lapse (TL) imaging of developing embryos offers more accurate quantification of cellular kinetics and cell cycle events than was possible with conventional static morphology assessment. A wide array of kinetic markers have been proposed to be predictive for continued embryonic development and blastocyst formation (8, 10–16). TL has also revealed the prevalence of specific dysmorphisms such as multinucleation (MU), reverse cleavage (RC), direct uneven cleavage (DUC), and irregular chaotic division (ICD) in human preimplantation embryos (10, 17–20). Determining the ploidy status of such embryos may help to better assess their potential. Studies have suggested that atypical growth patterns and MU may be associated with lower embryonic growth potential (5, 17, 20–23). However, limited data are available on the implantation potential of embryos with such dysmorphisms as there is a general tendency to deselect these embryos for possible transfer.

The combining of TL culture with PGS screening of embryos for euploidy has opened a new avenue of research. The probability of selecting chromosomally normal cleavage-stage embryos was shown by Basile et al. (24) to increase with the use of morphokinetic criteria. A correlation between delayed blastulation and blastocyst aneuploidy has also been reported (1, 5, 25, 26). Campbell and colleagues have proposed an aneuploidy risk classification model for blastocysts based on morphokinetics (26, 27). However, use of TL imaging as a technique to enhance the probability of selecting euploid embryos remains controversial (28–31). Rienzi et al. did not find an association between the morphokinetic characteristics of the growing embryos and ploidy status (30). Kramer and colleagues attempted to use Campbell's risk classification model to discriminate between euploid and aneuploid embryos but concluded that morphokinetics did not give sufficient accuracy to replace PGS screening (28). Clearly more work needs to be done by laboratories around the world to determine whether TL morphokinetics (TLM) can enhance selection of euploid blastocysts. To accomplish this, we need to monitor embryo kinetic behavior and observed dysmorphisms and determine whether they correlate with chromosomal status.

The primary objective of this study was to combine morphokinetic data from TL imaging of embryos with chromosome data from PGS screening to determine whether cleavage anomalies, MU, and specific cell developmental kinetics are predictive of not only blastocyst formation but also of embryo chromosomal status. Such information could be invaluable in supporting the use of TL as a noninvasive technique to enhance selection of competent embryos likely to have the greatest implantation potential. This may be of particular benefit to patients desiring elective single blastocyst transfer without PGS screening.

MATERIALS AND METHODS

Study Design

This study involves the retrospective analysis of prospectively collected morphokinetic and PGS results for 130 consecutive patients undergoing an intracytoplasmic sperm injection cycle (ICSI) at the Cleveland Clinic between April 2012 and June 2016. PGS was elective and offered to all patients but more strongly encouraged for our patients age 37 and older. A minimum number of embryos to proceed with PGS was not set. Data collection for this study was approved by our Institutional Review Board (IVF data registry IRB no. 5251 and EmbryoScope data registry IRB no. 14-566). All embryos were individually cultured in the EmbryoScope TL imaging chamber (VitroLife). This study was performed within the guidelines established by the Cleveland Clinic Institutional Review Board.

Ovarian Stimulation

Ovarian stimulation protocol selection was based on patient age, serum antimüllerian hormone levels, antral follicle counts, and prior response to gonadotropins. Women were treated with either a GnRH agonist or antagonist to suppress ovulation until follicle maturity was attained. In antagonist cycles, the GnRH antagonist was administered when the lead follicle reached 12 mm in size. Recombinant FSH, with or without urinary menotropins, was used for ovarian stimulation. Final follicular maturation was triggered with hCG and/or a GnRH agonist when at least two lead follicles measured 18 mm in mean diameter. Oocytes were collected 36 hours later by transvaginal ultrasound-guided needle aspiration of follicles.