Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis

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Objective: To study the differences in the cleavage time between chromosomally normal and abnormal embryos and to elaborate an algorithm to increase the probability of noninvasively selecting chromosomally normal embryos.
Design: Retrospective cohort study.
Setting: University-affiliated infertility center.
Patient(s): Preimplantation genetic screening patients (n = 125; n = 77 with ET), including cases of repeated implantation failure or recurrent miscarriage. A total of 504 embryos were analyzed.
Intervention(s): Embryo culture within a time-lapse system.
Main Outcome Measure(s): Kinetic variables included the time to 2 (t2), 3 (t3), 4 (t4), and 5 (t5) cells as well as the length of the second (cc2 = t3 − t2) and third (cc3 = t5 − t3) cell cycle, the synchrony in the division from 2 to 4 cells (s2 = t4 − t3), and the interval t5 − t2. Implantation and clinical pregnancy rates were also analyzed.
Result(s): A logistic regression analysis identified t5 − t2 (odds ratio [OR] = 2.853; 95% confidence interval [CI], 1.763–4.616), followed by cc3 (OR = 2.095; 95% CI, 1.356–3.238) as the most relevant variables related to normal chromosomal content. On the basis of these results, an algorithm for embryo selection is proposed to classify embryos from A to D. Each category exhibited significant differences in the percentage of normal embryos (A, 35.9%; B, 26.4%; C, 12.1%; D, 9.8%).
Conclusion(s): 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. (Fertil Steril® 2014;101: 699–704. ©2014 by American Society for Reproductive Medicine.)
Key Words: Embryo kinetics, chromosome, arrayCGH, time lapse

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Tremendous advances have occurred in the field of assisted reproduction technology (ART) over the past 30 years as the result of a combination of different factors in the IVF laboratory, including the introduction of groundbreaking techniques, such as intracytoplasmic sperm injection (ICSI) (1, 2), great improvements in culture media, and the introduction of preimplantation genetic diagnosis (PGD) (3). From a clinical point of view, physicians have learned to handle more pure and more powerful stimulation drugs that, along with increasing knowledge about the pathophysiology of ovarian hyperstimulation syndrome, have made the frequency of this syndrome almost anecdotic and the process of preparing a patient for IVF a controlled situation.

However, IVF success rates remain relatively low, with clinical pregnancy rates (PRs) of ~30% per transfer as reported by the Society for Assisted Reproductive Technology and by the European registers of the European Society of Human Reproduction and Embryology (https://www.sartcorsonline.com, 4). In addition, the number

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of multiple births still remains a concern, and the pressing need to reduce the number of transferred embryos demands better selection methods. Thus, a big question remains: how can we move on to single ET (SET) programs while maintaining, or even improving, our clinical outcomes?

One approach could be based on the method used to evaluate and select embryos in the laboratory. Before the introduction of time-lapse technology, embryo grading and, most importantly, embryo selection were based only on static observations. These observations are inevitably linked to specific time points during the day and do not describe a dynamic process, such as embryo development, well enough. This subjective procedure has large flaws, such as inter- and intraobserver variation (5, 6), and controversial benefits for almost every scoring system.

The problem with applying a static scoring system to a dynamic process was solved in our laboratories by the introduction of time-lapse technology. Since that step, new kinetic markers associated with higher implantation potential have been proposed (7), the safety of these systems validated (8–11), and the effects of different intrinsic and extrinsic factors on the morphokinetic behavior of the human embryo analyzed (12–15). A morphokinetic era has evidently started in IVF.

Another approach could be based on the genetic screening of embryos. Several studies have demonstrated that chromosomal abnormalities are one of the most common causes of abnormal embryos in IVF (16–20), which translates into poor clinical outcomes. A recent publication highlighted the inherent imprecision of SET when conventional morphology is used alone, observing a 44.9% aneuploidy rate for blastocysts from patients with good prognosis (21).

Thus, embryo selection using morphokinetic markers combined with preimplantation genetic screening (PGS) could be the solution. Even though time-lapse technology is increasingly used in IVF laboratories, PGS is not always possible owing to legal or social reasons or simply because the clinic cannot perform the technique. The objective of the present study was to analyze the morphokinetic behavior of chromosomally normal and abnormal embryos to develop a new selection tool that increases the probability of noninvasively selecting chromosomally normal embryos. We retrospectively analyzed embryos using array-comparative genome hybridization (arrayCGH).

MATERIALS AND METHODS

Embryo development was retrospectively analyzed using a time-lapse imaging system (Embryoscope, Unisense Fertilitech) in a total of 125 consecutive patients undergoing PGS (n = 504 embryos); of those, 77 received an ET between March 2011 and August 2012. All procedures and protocols were approved by the Institutional Review Board, which regulates and approves database analysis and clinical IVF procedures for research at IVI.

Embryo biopsy was done on day 3 and comprehensive chromosome screening performed through arrayCGH. This study included patients undergoing PGS for recurrent miscarriage (RM) and repeated implantation failure (RIF) only. RM was defined as two or more miscarriages before 20 weeks of pregnancy, and RIF was defined as the failure of a couple to conceive after 10 or more good-quality embryos transferred or after three IVF cycles (22).

Ovarian Stimulation

Ovarian stimulation was carried out as described elsewhere (23). Briefly, patients received a starting dose of recombinant FSH (Puregon, MSD; Gonal F, Merck-Serono) ranging from 150 to a maximum of 225 IU. GnRH antagonist (0.25 mg Ganirelix, Orgalutran) was administered daily starting on day 5 or 6 after FSH administration. Recombinant hCG (Ovitrelle, Merck-Serono) was administered as soon as two leading follicles reached a mean diameter $\geq$17 mm.

Oocyte Retrieval, Embryo Culture, and Embryo Biopsy

Oocyte retrieval was performed 36 hours after hCG under ultrasound guidance. After retrieval, the oocytes were kept in culture for 4 hours at 37.3°C and 5.8% CO_2 until oocyte denudation. Oocyte denudation was performed by mechanically pipetting with 40 IU/mL of hyaluronidase (Vitrolife). ICSI was subsequently performed on all metaphase II oocytes. Fertilized oocytes were cultured individually in microdroplets of culture media (Vitrolife) until the day of blastomere biopsy. Biopsy was performed on day 3 for all embryos that were made up of 6 or more cells with less than 25% fragmentation as described elsewhere (24). Briefly, embryos were placed individually on a droplet containing Ca^{+2}/Mg^{+2}-free medium (G-PGD, Vitrolife), and the zona pellucida was perforated using laser technology (OCTAX). Patients with normal embryos were scheduled for blastocyst transfer on day 5 (approximately 120 hours) of development. Treatment cycles were selected at random for investigation by time-lapse image acquisition and subsequent retrospective analysis of the morphokinetic parameters of embryo development.

ArrayCGH

ArrayCGH was performed as described elsewhere (24). Briefly, a single cell from embryos was amplified using the Sureplex DNA amplification system (BlueGnome). Amplification quality was ensured by gel electrophoresis (Lonza). Cy3 and Cy5 fluorophores were used to label the sample and control DNA, respectively. Labeling mixes were combined and hybridized on a 24sure array (BlueGnome) for 6–12 hours. Final results were obtained on day 5 using a laser scanner (710 Innoscan, Innopsys; and Powerscanner, TECAN). BlueFuse software was used to analyze the data (BlueGnome). The entire protocol for arrayCGH analysis was completed in 24 hours.

Time Lapse Analysis and Recording of Kinetic Parameters

The exact times for each embryo division and developmental parameters were calculated in hours postmicroinjection (hpi). Time-lapse images of each embryo were retrospectively analyzed using an EmbryoViewer software workstation (Unisense FertiliTech). Images were acquired every 15 minutes in five different focal planes over 120 hours of culture.