ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Type of chromosome abnormality affects embryo morphology dynamics

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Objective: To study the differences in the cleavage time between types of embryo chromosomal abnormalities and elaborate algorithm to exclude aneuploid embryos according to the likelihood to be euploid.
Design: Retrospective cohort study.
Setting: University affiliated private center.
Patient(s): Preimplantational genetic screening patients (n = 112) including cases of advanced maternal age, repeated implantation failure, and recurrent miscarriage. A total of 485 embryos were analyzed.
Intervention(s): None.
Main Outcome Measure(s): All biopsied embryos were cultured in an incubator with time-lapse technology, cleavage timing from insemination to day 3 and all kinetic parameters that have been described in previous studies by our group.
Result(s): Logistic regression analysis were used to identify morphokinetic parameters and some were strongly associated with complex aneuploid embryos; t3 (odds ratio = 0.590, 95% confidence interval 0.359–0.971) and t5–t2 (odds ratio = 0.151, 95% confidence interval 0.082–0.278).
Conclusion(s): Embryo morphokinetics are affected by chromosome aneuploidy and further analysis of the chromosome content reveals higher differences when the complexity in the chromosome disorders is increased. The use of time-lapse monitoring, although not able to detect an abnormal embryo, may be potentially useful to discard those embryos with high risk of complex chromosomal abnormalities. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Embryo kinetics, complex abnormalities, array CGH, time lapse

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Selecting an embryo according to its morphology has the disadvantage of the moment we observed it and the subjectivity of the observer (1–4). Another concern that has been historically used in assisted reproductive technique (ART) is multiple pregnancies. We must avoid those at-risk pregnancies, and to achieve that, the best solution is transferring only one embryo (5–7).

One of the great inconveniences about selecting an embryo using morphological criteria is that we are not considering the chromosomal content of the embryo (8). The correlation between embryo morphology and implantation potential is relatively weak (9, 10). Even embryos considered to be morphologically good do not always succeed in implanting in the uterus. Before implantation, chromosomal abnormality is extremely common, affecting more than half of whole cohort of embryos produced by women >35 years of age (11, 12). We cannot obviate that parameter. In addition, in patients with repeated implantation failure or recurrent miscarriage, the only way to determine an euploid embryo is by using preimplantation genetic screening (PGS). In many of these patients, the use of PGS implies a huge effort (economically and psychologically). In addition, this genetic diagnosis cannot always be done, for many reasons, like clinical infrastructure, moral reasons, legal reasons, or for any other reason.

Time lapse is a noninvasive method that allows us to observe embryos for 24 hours. Therefore, we can increase the amount and quality of information about the embryo without affecting negatively the culture conditions (13–16). This type of image analysis is not a new technology; it has been commercialized and has become accessible to many clinics around the world (17–20).

Observing the embryo kinetics has helped us determine the behavior of

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those embryos and know that we can propose another tool for those patients in whom PGS cannot be performed. Recent publications show that chromosomally normal and abnormal embryos have different kinetic behaviors. Davis (21), Chavez (22), and Basile (23) and their colleagues reported differences between euploid and aneuploid embryos during the early stages of development. Yet Campbell et al. (24) observed no difference between euploid and aneuploid embryos during those early stages of development. However, in the periblastulation phase, aneuploid embryos had a significant delay in development compared with euploid embryos.

Nevertheless, all chromosomal abnormalities are not equal; depending on each specific abnormality, the development of the embryo will be different (25). Our goal was to separate the embryos according to each chromosomal abnormality and to find a correlation between the type of aneuploidy and embryo morphokinetic. The objective of the present study was to compare embryo kinetics depending on the type of chromosome abnormality (monosomy, trisomy, and complex) in euploid embryos and try to build a model based on the kinetic variables that can predict the chromosomal status of an embryo.

MATERIALS AND METHODS

This research project was conducted at the Instituto Valencianode Infertilidad (IVI Valencia and Madrid) and was performed from March 2013 until August 2014. The procedure and protocol for analysis of embryos were approved by an Institutional Review Board (1407-MAD-053-NB), which regulates and approves database analysis and clinical IVF procedures for research at IVI. In addition, the project complies with the Spanish law governing ART (14/2006). The present retrospective cohort study was drawn from a total of 112 cycles from patients undergoing PGS due to advanced maternal age (>39 years old), recurrent pregnancy loss, or repeated implantation failure. Recurrent miscarriage was defined as two or more miscarriages before 20 weeks of pregnancy and repeated implantation failure was defined as the absence of a gestational sac on ultrasound at $\geq$5 after ET and after transferring three high quality embryos (26). Embryo development was retrospectively analyzed using a time-lapse imaging system (Embryoscope, UnisenseFertilitech). A total of 485 embryos were successfully biopsied on day 3 of development and analyzed with array comparative genome hybridization (CGH) for all chromosomes. The IVF centre (IVI Madrid and Valencia) and the PGD laboratory (IGENOMIX) complies with all quality controls, external and internal. They are accreditation ISO 15189 and report dates about cycles of PGD to the European Society for Human Reproduction and Embryology (ESRHE) and the Spanish health authorities. Embryo biopsies and genetic analyzes were performed by a highly qualified staff.

Ovarian Stimulation and Oocyte Retrieval

For ovarian stimulation and oocyte retrieval, the patients were treated as described previously (27). Briefly, the women received a starting dose of recombinant FSH (Puregon, Organon; Gonal F, Serono) ranging from 150–225 IU (maximum) and 0.25 mg of the GnRH antagonist ganirelix (Orgalutran, Organon) daily starting on day 5 or 6 after FSH administration. The patient's cycle was monitored according to the individual policy of the clinic. Recombinant hCG (Ovitrelle, Serono) was administered as soon as two leading follicles reached a mean diameter of $\geq$17 mm, and oocyte retrieval was performed 36 hours later.

Embryo Culture Evaluation and Embryo Biopsy

Fertilization was confirmed 16–20 hours after insemination by the presence of two pronuclei (2PN) and extrusion of the second polar body. Normal fertilized oocytes were cultured in a microdroplet of culture media (Vitrolife, Scandinavian IVF) until the day of blastomere biopsy. Embryos were evaluated on days 2 and 3. Cell number, fragmentation pattern (defined as the embryonic volume occupied by the enucleated cytoplasm and expressed as a percentage), symmetry, and multinucleation were recorded.

Embryo biopsy was performed on day 3 in embryos with more than five cells and <25% fragmentation (28). The zona pellucida (ZP) was perforated using laser technology (OCTAX). Biopsied embryos were cultured up to day 5. Not biopsied embryos were discarded on day 3. Embryo transfer was performed on day 5 when a chromosomal normal embryo was available. The maximum number of embryos transferred was two.

CGH Analysis

Array CGH was performed as described elsewhere (28). 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 date (BlueGnome). The entire protocol for array CGH analysis was completed in 24 hours.

For internal quality control, measures were as specified by the array manufacturer for labeling, hybridization, and scanning. The quality and accuracy of the profile and results from reference male and female DNA samples also were checked as an internal quality control. All parts of the work-up and diagnostic procedure from the initial referral to the delivery of the final report were monitored with suitable controls and calibrators. Regular audit of these parameters enables the laboratory to assess the performance of their service and objectively measure improvements. Two main groups of embryos were studied: chromosomally normal embryos and chromosomally abnormal embryos. In the latter group we performed a further separation according to the chromosomal abnormality, as follows: embryos with monosomies (those embryos that lose a chromosome); trisomies (those embryos that have an extra copy of a chromosome); chaotic embryos (have all of chromosomes altered); complex