ORIGINAL ARTICLE: ASSISTED REPRODUCTION

The impact of multinucleated blastomeres on embryo developmental competence, morphokinetics, and aneuploidy

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Objective: To study the effect of human embryo multinucleation on the rate of aneuploidy, in vitro developmental morphokinetics, and pregnancy outcome.
Design: Retrospective study.
Setting: University-affiliated fertility center.
Patient(s): A total of 296 patients undergoing IVF cycles.
Intervention(s): None.
Main Outcome Measure(s): Rate of multinucleation at the 2- and 4-cell stage, time-lapse morphokinetic parameters from zygote to blastocyst stage, ploidy of embryos analyzed by means of trophectoderm biopsy and array comparative genomic hybridization (PGS), and pregnancy outcome.
Result(s): A total of 1,055 out of 2,441 (43.2%) embryos evaluated with the use of the Embryoscope time-lapse system showed blastomere multinucleation at the 2-cell stage (MN2). The frequency of this abnormality was substantially reduced in 4-cell-embryos (15.0%). Among all clinical factors analyzed, only maternal age had a positive correlation with multinucleation rate. The timing of cleavage divisions from the pronuclear fading to 5-cell embryo was significantly longer (1.0–2.5 h) in MN2 embryos than in non-MN2 control samples. Of the total embryos tested with the use of PGS (n = 607), the rates of multinucleation were similar in euploid versus aneuploid blastocysts (40.8% and 46.7%, respectively). All 24 chromosomes contributed to aneuploidy of MN2 embryos. There were 61 transfers of MN2 embryos that resulted in 45.9% clinical pregnancies and a 31.6% implantation rate.
Conclusion(s): The frequency of multinucleation is high in human embryos cultured in vitro and equally affects euploid and aneuploid human embryos. It appears that most MN embryos have the capacity for self-correction during early cleavage divisions and can develop into euploid blastocysts resulting in healthy babies. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Multinucleation, preimplantation genetic screening (PGS), aneuploidy, self-correction, time-lapse

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Blastomere multinucleation is a common nuclear abnormality observed in early human embryos (1). This phenomenon appears to be associated with diminished potential of embryo development, manifested by reduced blastocyst formation, significantly lower implantation, and decreased live birth rates (2–8). The existing reports on multinucleation are based on brief daily observations with the use of light microscopy. These studies reported a multinucleation of MN in day 2 and day 3 cleavage embryos of 11%–34% (1, 3, 5, 6, 9).

The presence of multinucleated (MN) blastomeres in human embryos, especially during the first and second mitotic divisions, is generally considered to be abnormal. Therefore, these embryos are usually not transferred. To investigate the negative impact of this nuclear abnormality on clinical outcomes, numerous preimplantation genetic screening (PGS) studies have been performed in the past with the use of fluorescence in situ hybridization (FISH; with two to nine multicolor

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specific chromosomal probes) to evaluate for the presence of aneuploidy and mosaicism (2, 4, 7, 10–15). These groups reported that 50%–100% of MN embryos examined exhibited aneuploidy or mosaicism, often complex and chaotic. Despite this high rate of aneuploidy in most studies, a proportion of MN 2-cell-stage embryos and their daughter cells were apparently not aneuploid. However, this early technique for PGS did not look at all chromosomes, so one could not be sure that the embryos are truly euploid. Interestingly, the incidence of "self-correction" to euploidy was reported by other studies using FISH when day 3 aneuploid and mosaic embryos were reanalyzed on day 5 (16–18). However, multinucleation of blastomeres was not recorded in those studies. FISH results indicate that some aneuploid embryos may be capable of "self-correction" during development to the blastocyst stage. This suggests that there are possible repair mechanisms within early preimplantation embryos that could restore them to normal ploidy. The concept of rescue mechanisms (self-corrective) in MN blastomeres could be an explanation for the sporadic cases of healthy births that have been reported after the transfer of embryos exhibiting one or two MN blastomeres (9,12,19–21).

To enhance our knowledge on multinucleation and chromosome segregation errors in human embryos, it is important to study the genetic constitution of MN embryos and the cellular mechanisms underlying this frequent phenomenon, as well as to evaluate their developmental potential and impact on IVF clinical outcomes. The introduction of time-lapse embryo morphokinetic monitoring systems offers a promising new method to evaluate embryo development. In addition, the advent of technologies to evaluate all 24 chromosomes, such as array comparative genomic hybridization (a-CGH), is a more comprehensive approach now available to investigate MN human embryos.

The aims of the present study were to: 1) use a time-lapse incubation system to compare the morphokinetics and developmental potential of human embryos with and without multinucleation; 2) analyze the impact of clinical variables such as age, number of retrieved oocytes, E_2 levels, and etiology of infertility on the frequency of embryo multinucleation; 3) reevaluate the relationship between multinucleation and embryo ploidy status with the use of contemporary PGS methodologies; and 4) examine the types of chromosomal abnormalities in embryos originating from MN blastomeres observed during early embryo cleavage stages.

MATERIALS AND METHODS

Patients

This retrospective study protocol was approved by the Health Sciences Research Ethics Board of the University of Toronto (IRB no. 31565). The study included 296 consecutive intracytoplasmic sperm injection cycles (296 patients) in which a time-lapse imaging system was used to observe embryo development (Embryoscope; Unisense Fertilitech). Among the patients, 198 (66.9%) were using their own gametes and 98 used donated ova (33.1%). In 113 (38.2%) of the cycles, patients underwent PGS. The mean ages of the oocyte providers were 37.63 ± 5.18 years for the nondonor group and 25.72 ± 3.59 years for the ovum donors. Indications for assisted reproduction included: advanced maternal age (AMA; n = 49 own/98 donor ova), polycystic ovary syndrome (PCOS; n = 18), male factor (MF) as the only cause of infertility (n = 39), other female factors, such as idiopathic infertility or endometriosis (n = 36), and combination of male factor with female factors (female factors in this group were AMA [n = 33]; and PCOS [n = 23]).

Controlled ovarian hyperstimulation was carried out with the use of a standard antagonist protocol (22). Transvaginal ultrasound–guided follicular aspiration was carried out ~36 hours after administration of a trigger injection of either hCG (Fresenius Kabi) alone, GnRH agonist alone (Abbvie), or a dual trigger of both. The embryo transfer was performed 3 or 5 days after oocyte retrieval or in a future cycle after cryopreservation by vitrification. An ongoing pregnancy was defined by the presence of fetal heart activity observed by means of ultrasound 11–12 weeks after embryo transfer.

Laboratory Procedures

Gamete preparation and embryo culture were performed in Global media (Life Global). Routine semen analysis and a two-step (40% and 80%) gradient separation technique (Sperm Filter; Gynotec, Fertilitech) were used to prepare sperm samples. Severe male factor, including samples from testicular biopsies, were excluded from the study. Embryos were placed into the Embryoscope on embryo slides containing 25 μL preequilibrated single-step medium and cultured at 6% CO_2, 5% O_2, and 37°C until embryo transfer and/or cryopreservation.

Laser-assisted trophectoderm (TE) biopsy was performed at the blastocyst stage (day 5 or 6) in PGS cases. An opening of 6–9 μm was made in the zona pellucida with the use of a Lycos laser (Hamilton-Thorn; Origio), and three to five herniated TE cells were aspirated and separated from the blastocyst by application of multiple laser pulses. Harvested TE cells were washed and placed within a polymerase chain reaction tube containing 2.5 μL phosphate-buffered saline solution. Comprehensive chromosome analysis with the use of a-CGH was carried out with the use of Bluegenome kits as described previously (23).

Time-lapse Imagining

Embryo images were recorded every 10 minutes in seven different focal planes. Embryo development was annotated by two investigators with the use of Embryoviewer image software (Unisense; Fertilitech) and verified by a third operator. The following morphokinetc parameters were recorded: time of pronuclear fading (tPNf; when both pronuclei were no longer visible) and cleavage time to the 2-, 3-, 4- and 5-cell stages (t2, t3, t4, and t5, respectively). The other kinetic parameters monitored were: abnormal zygote cleavage from 1 to 3 cells; time to complete synchronous division from 3- to 4-cell stage (t4−t3); beginning of blastulation (tSB); and formation of full blastocyst (tB). Additionally, the duration of the first cleavage, from pronuclear fading to the first cleavage furrow (t2−tPNf),