Objective: To determine the prognostic impact of the nuclear status at the two-cell stage on intracytoplasmic sperm injection (ICSI) outcomes.

Design: Retrospective study.

Setting: Hospital.

Patient(s): Only ICSI cycles with time-lapse monitoring of transferred embryos with known implantation/delivery data from November 2012 to December 2014 were included. A total of 2,449 embryos were assessed for multinucleation rates at the two- and four-cell stages, and 608 transferred embryos were studied for ICSI outcomes.

Intervention(s): None.

Main Outcome Measure(s): Implantation rate (IR) and live birth rate (LBR) according to the number of multinucleated blastomeres at the two-cell stage: none (Without-MNB2cell), one (MNB1/2cell), and two (MNB2/2cell); morphokinetics of MNB2cell embryos.

Result(s): Embryos with MNB1/2cell led to lower IR (27.7%) and LBR (22.7%) than embryos Without-MNB2cell (33.4% and 29.8%, respectively). The MNB2/2cell embryos led to significantly lower IR (18.3%) and LBR (13.4%) than embryos Without-MNB2cell. This difference remained significant in multivariate analysis for implantation (odds ratio 0.57; 95% confidence interval 0.34–0.94) and birth (odds ratio 0.46; 95% confidence interval 0.26–0.80), independently of the other significant parameters (women’s age, time of two-cell formation, and multinucleation at the four-cell stage). Among implanted MNB2cell, if cleavage into four cells occurred later than 37 hours after insemination, embryos were significantly more likely to lead to birth.

Conclusion(s): The presence of multinucleation at the two-cell stage and more specifically in both blastomeres had a significant negative impact on birth potential. Thus, embryo multinucleation at the two-cell stage should be used as an additional noninvasive criterion for embryo selection. (Fertil Steril® 2017;107:97–103. ©2016 by American Society for Reproductive Medicine.)

Key Words: Birth rate, embryo multinucleation, ICSI outcomes, implantation, time-lapse system

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Because single embryo transfers are now applied to reduce multiple pregnancies after IVF (1), the selection of embryos with higher developmental potential is now crucial to ensure high implantation and birth rates (2–4). Traditionally, and according to the European Society of Human Reproduction and Embryology (ESHRE)/ALPHA consensus, the embryo morphologic grade, like the time of the first cleavage, the number of blastomeres, and the degree of fragmentation, are useful criteria for embryo selection (5). The recent development of time-lapse systems offers new insights into embryo development that allow embryologists to use additional morphologic criteria (6–8) and to develop decisional algorithms for embryo transfer (9).

Moreover, by allowing a wide and precise observation of the nuclear status, time-lapse imaging leads to a better...
assessed implantation potential (10, 23) reported a negative effect of potential increased risk of miscarriage. Available data on multinucleation at the two-cell stage (MNB2cell) may not affect blastocyst development (11), it is now well known to be unfavorable for embryo implantation and birth potential (12–18), hence the importance of detecting multinucleation at day 2.

Concerning the nuclear status at the two-cell stage, the time-lapse system reveals a high proportion of multinucleated embryos. Almost half of the embryos (42.5%) are multinucleated at this stage (19), thus raising questions about their potential development. In particular, correlations have been demonstrated between multinucleation and increased rates of aneuploidy and chromosomal abnormalities (20–22), suggesting a potential increased risk of miscarriage. Available data on implantation potential (10, 23) reported a negative effect of multinucleation at the two-cell stage (MNB2cell) on implantation rates. However, the influence of multinucleation at the two-cell stage per se on the probability of birth is still not known.

To address this issue, the present study retrospectively examined implantation rates and live birth rates in univariate and multivariate analysis according to the number of multinucleated blastomeres at the two-cell stage assessed by time-lapse monitoring. The number of multinucleated blastomeres, the kinetics, and the type of multinucleation at the two-cell stage were assessed to determine whether they could predict implantation and birth. These data emphasized that careful observation of nuclei at day 1 is also crucial to optimize noninvasive strategies used for embryo selection to improve births of healthy babies after intracytoplasmic sperm injection (ICSI).

MATERIALS AND METHODS

In this retrospective study, only ICSI cycles with time-lapse monitoring of transferred embryos with known implantation/delivery data from November 2012 to December 2014 at the University Hospital of Dijon were included. Indeed, the analyses exclusively concerned transfers of day-2 or day-3 embryos with exact traceability (i.e., implantation and developmental potential to delivery could be traced). All single embryo transfers were included. Among two-embryo transfers, only transfers resulting in no pregnancy or a clinical pregnancy with two gestational sacs followed by no or two births were included. Transfers of three or more embryos and ICSI with oocyte donors were excluded from the study. In our study, the multinucleation rates at the two- and four-cell stage were assessed among the 420 ICSI cycles included from 335 ICSI patients (generating 2,449 embryos). For the ICSI outcomes analysis, a total of 608 transferred embryos (fresh and frozen) were studied. For all of them, embryos reached the four-cell stage of development before 45 hours after microinjection (time proposed by ESHRE/ALPHA consensus) (5). Therefore, no transfers of embryos with major delayed cleavage were included in this study. Institutional review board approval was obtained for the study protocol and the collection of data of couples who had undergone ICSI cycles (no. 1941917v0).

ICSI Protocol and Embryo Culture

The controlled ovarian hyperstimulation protocols consisted of GnRH agonist down-regulation, followed by recombinant FSH/hMG and hCG, or of antagonist protocols. Oocyte retrieval was performed by transvaginal ultrasound-guided follicle aspiration 36 hours after hCG injection. Sperm preparation for ICSI was performed as previously described (24–26). After micro-injection, inseminated oocytes were immediately transferred into Embryoslide (Unisense Fertilitech) with 25 μL of culture medium (Global, LifeGlobal) under oil (Nidoil, Nidalan). Then, Embryoslides were incubated in the time-lapse system (EmbryoScope, Unisense Fertilitech) at 37.0°C, 6% CO2, 5% O2. Embryo development was recorded every 20 minutes in seven different focal planes. Images and related data were stored in the EmbryoViewer (Unisense Fertilitech) and subsequently analyzed.

Embryo Morphologic Records and Transfer

Fertilization was assessed 17 hours ± 30 minutes after ICSI by checking the number of pronuclei. Nucleation features were checked from the first cleavage (two-cell stage) to the second cleavage (three-cell and four-cell stages). At the two-cell stage, embryos in which multinucleation was observed were recorded as MNB2cell. If only one blastomere was multinucleated the embryo was annotated MNB1/2cell, and if multinucleation was present in both blastomeres the embryo was identified as MNB2/2cell. MNB4cell was recorded if multinucleation was observed at the four-cell stage. Nucleation features at the two-cell stage were annotated as proposed by Ciray et al. (27): mononucleated (only one nucleus was seen), binucleated (nBI, number of blastomeres in which two nuclei per cell are visible), and multinucleated (nMN, number of blastomeres in which more than two nuclei are visible, definition including micronuclei). The morphologic appearance of day-2 embryos was monitored according to the number and the size of the blastomeres (regular or irregular cleavage), as well as the percentage of aneuploidy fragments (18). Embryos fertilized at day 1 with regular four- to five-cell embryos at day 2 with less than 20% fragmentation were regarded as “TOP” grade (3, 18). Only embryos from oocytes exhibiting two pronuclei were transferred. Depending on the age of the women, the number of previous cycles, and the number and quality of embryos available, 1 or 2 embryos were transferred at either day 2 or day 3 after oocyte retrieval. Embryo cryopreservation by slow-cooling and embryo thawing were performed at day 2 or day 3, as previously described (28). The thawed-embryo transfers were accomplished without additional embryo culture.

Morphokinetic Events and Nuclear Status Monitoring of Implanted MNB2cell Embryos

Nuclear events were annotated as suggested by Ciray et al. (27). Fade out of the two pronuclei (tPNf), exact time of