Embryo multinucleation at the two-cell stage is an independent predictor of intracytoplasmic sperm injection outcomes

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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 stage, 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-MNB^{2cell}), one ($MNB^{1/2cell}$), and two ($MNB^{2/2cell}$); morphokinetics of MNB^{2cell} embryos.
Result(s): Embryos with $MNB^{1/2cell}$ led to lower IR (27.7%) and LBR (22.7%) than embryos Without-MNB^{2cell} (33.4% and 29.8%, respectively). The $MNB^{2/2cell}$ embryos led to significantly lower IR (18.3%) and LBR (13.4%) than embryos Without-MNB^{2cell}. 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 MNB^{2cell}, 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

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assessment of multinucleation. Indeed, up to 72.3% of transferred embryos with multinucleated blastomeres detected by a time-lapse system cannot be identified with conventional incubator systems and the embryo control timing guidelines proposed by the ESHRE/ALPHA consensus (27 hours ± 1 hour at the two-cell stage or 44 hours ± 1 hour at day 2) (5, 10). However, even though blastomere multinucleation at day 2 (MNB^{4cell}) 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 (MNB^{2cell}) 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, Nidacon). Then, Embryoslides were incubated in the time-lapse system (EmbryoScope, Unisense Fertilitech) at 37.0°C, 6% CO_2, 5% O_2. 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 MNB^{2cell}. If only one blastomere was multinucleated the embryo was annotated $MNB^{1/2cell}$, and if multinucleation was present in both blastomeres the embryo was identified as $MNB^{2/2cell}$. MNB^{4cell} 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 anucleate 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 MNB^{2cell} Embryos

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

each embryo division (T2: time of appearance of two cells; T3: time of appearance of three cells; and T4: time of appearance of four cells), nucleation features at the two-cell stage, and the appearance and disappearance of each nucleus at the two-cell stage was annotated.

ICSI Outcomes

The implantation rate (IR) was the ratio between the number of gestational sacs and the number of transferred embryos. The embryo developmental capacity after implantation was assessed as previously described (18, 29), as the loss rate of gestational sacs calculated as the ratio between the number of intrauterine sacs that were not followed by the birth of a child and the total number of gestational sacs observed. The live birth rate (LBR) was the ratio between the number of live births and the number of embryos transferred.

Statistical Analysis

Univariate analysis was first conducted to compare patients' characteristics (two groups of patients, depending on the embryo nuclear status: transfers with embryos without multinucleation, and transfers with MNB^{2cell} embryos) and then to compare implantation and birth rates according to nuclei morphology among embryos with one or two multinucleated blastomeres.

Quantitative variables were described as means with their standard deviations and compared by a Student test or Mann-Whitney test after assessment of their distribution with the Kolmogorov-Smirnoff test. Qualitative variables were expressed as frequencies and compared by a χ^2 or Fischer's exact test as appropriate. Independent predictors of implantation or birth were investigated by multivariate logistic regression models. To take into account the correlation between embryos, we used a generalized estimating equations approach to logistic regression, assuming an exchangeable correlation structure. Predictors to be entered in the model were as follows: the woman's age (treated as a continuous variable), the woman's body mass index (BMI; continuous variable), quality at the four-cell stage (TOP [reference]/non-TOP), time of two-cell formation (continuous variable), MNB at the two-cell stage (0 MNB [reference]; $MNB^{1/2cell}$; $MNB^{2/2cell}$), nuclear status at the four-cell stage (multinucleated embryo described as MNB^{4cell} or not [reference]), day of embryo transfer (day 2 [reference] or day 3), and embryo state (fresh [reference], cryopreserved). All analyses were performed using STATA 12.0 (StataCorp). A P value of $< .05$ was considered significant for all tests. Odd ratios (ORs) are given with their 95% confidence intervals (CIs).

RESULTS

Analyses were based on 420 embryo transfers: 235 transfers without MNB^{2cell} and 185 with at least one MNB^{2cell}. Patients' characteristics were similar between transfers with or without MNB^{2cell} (Supplemental Table 1, available online).

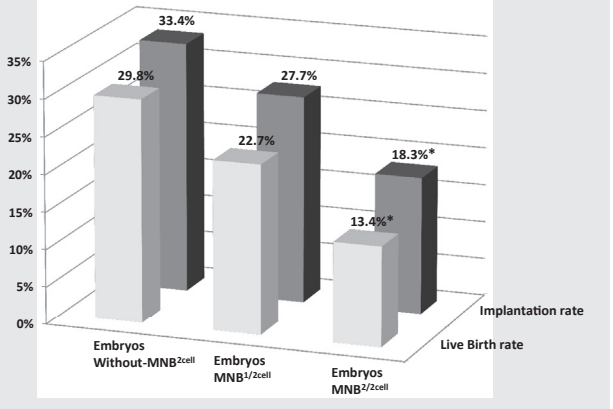
Among the embryos generated after ICSI included in the study (n = 2,449), 43.7% were multinucleated at the two-cell stage. Among the 608 transferred embryos, 36.7% were MNB^{2cell}, and 16.1% of them exhibited multinucleation at the four-cell stage (MNB^{4cell}). The proportion of embryos "TOP" cryopreserved or transferred at day 3 were identical between the transfers of embryos observed with and without multinucleation at the two-cell stage (Supplemental Table 1).

Of the 608 embryos with known implantation/delivery data, embryos with $MNB^{1/2cell}$ led to lower IR (27.7%, $P=.212$) and LBR (22.7%, $P=.109$) than did embryos Without-MNB^{2cell} (33.4% and 29.8%, respectively). In the same way, embryos with $MNB^{2/2cell}$ led to significantly lower IR (18.3%, $P=.007$) and LBR (13.4%, $P=.002$) (Fig. 1). The loss rate of gestational sacs was higher for $MNB^{1/2cell}$ (17.9%, $P=.188$) and $MNB^{2/2cell}$ (26.7%, $P=.042$) embryos than for those Without-MNB^{2cell} (10.7%) (Fig. 2A).

In a multivariate analysis, woman's age, time of two-cell formation, and multinucleation at the four-cell stage (MNB^{4cell}) significantly impacted implantation and the birth of a child (Table 1). In addition, a significant negative effect of multinucleation at the two-cell stage per se on implantation ($MNB^{2/2cell}$: OR 0.57, 95% CI 0.34–0.94; $P=.030$) and on birth ($MNB^{2/2cell}$: OR 0.46, 95% CI 0.26–0.80; $P=.007$) was also found in the multivariate analysis. The day of the ET and the embryo state were found not statistically significant (Table 1).

To evaluate the impact of MNB^{2cell} embryo morphokinetics on outcome, we compared the kinetics and nuclei features of MNB^{2cell} embryos, first between implanted and non-implanted embryos and second between those leading to birth and those arrested in their further development. Concerning the type of multinucleation at the two-cell stage, no difference was observed (Supplemental Table 2). Multinucleated or binucleated had no impact on implantation or further embryo development. The times of two-cell and four-cell formation were also not modified by the features of multinucleation at the two-cell stage (Supplemental Table 3). The loss of

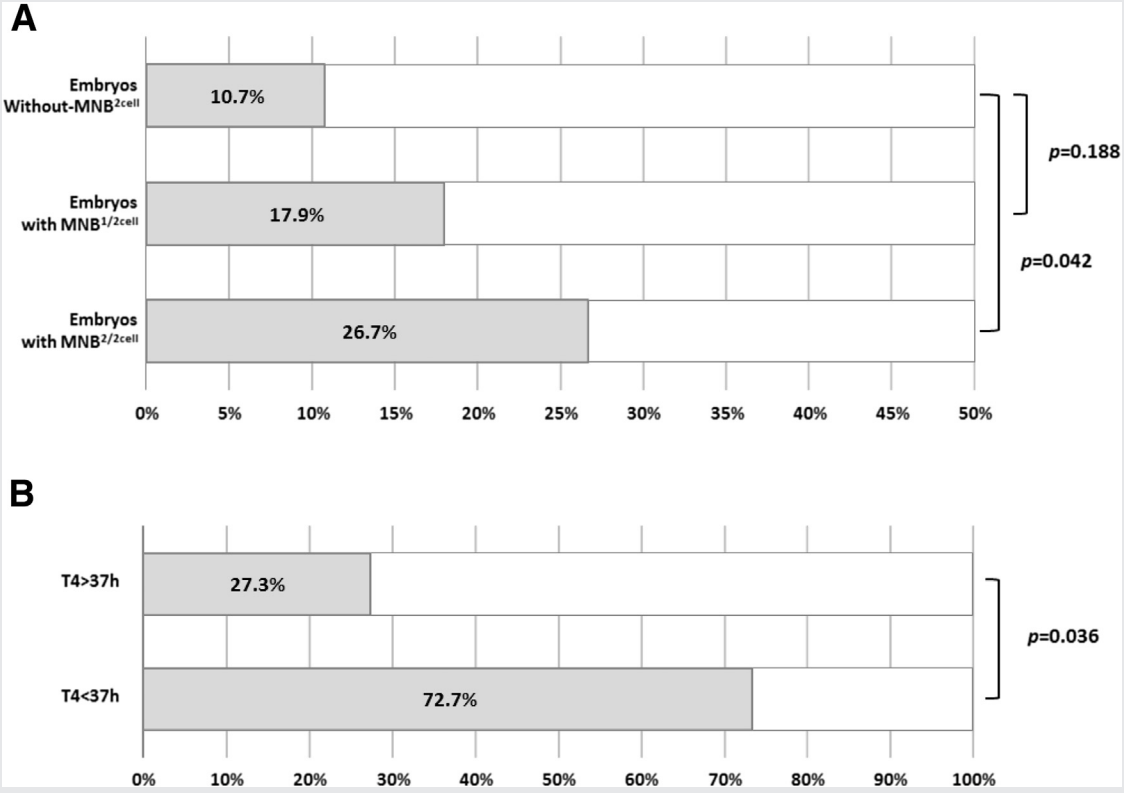
FIGURE 1



Implantation and birth rates according to the nuclear status at the two-cell stage. MNB^{2cell} = multinucleated embryo at the two-cell stage; $MNB^{1/2cell}$ = only one multinucleated blastomere at the two-cell stage; $MNB^{2/2cell}$ = both blastomeres multinucleated at the two-cell stage. *$P<.05$, χ^2 test.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

FIGURE 2



Loss rates of gestational sacs were compared (**A**) according to nuclear status at the two-cell stage among all transferred embryos (n = 608); and (**B**) according to the time to cleavage into four cells (T4, before or after 37 hours) among implanted MNB^{2cell} embryos (χ^2 test, $P<.05$).

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

gestational sac rate tended to be associated with faster embryo kinetics, mostly highlighted at T4 timing (Supplemental Fig. 1), and it was significantly higher when the four-cell stage occurred before 37 hours ($P=.036$) (Fig. 2B). No difference depending on the embryo quality (87.5% vs. 90.9% of TOP embryos), women's age (30.8 ± 3.3 years vs. 30.8 ± 4.1 years) and the number of multinucleated blastomeres at the two-cell stage (Supplemental Fig. 1) was found between embryos reaching the four-cell stage before and after 37 hours.

DISCUSSION

To our knowledge, no prior studies have explored the impact of multinucleation observed at the two-cell stage along with other embryo morphologic parameters (high grade and multinucleation at the four-cell stage) or clinical parameters (woman's age and BMI) on the birth rate in a multivariate analysis. Our data revealed that the presence of multinucleation at the two-cell stage had a significant negative impact on implantation and birth rates even if the embryos are at the four-cell stage within the reference range of timing (proposed by ESHRE/ALPHA consensus [5]). These findings are crucial to enhance our IVF successes, which are today defined as the ability to generate the birth of a healthy baby. Our results also reconfirmed that age, time of appearance of two cells, and multinucleation status at the four-cell stage are key factors for determining the embryo's fate (11, 18, 30).

Recently two teams assessed the impact of two-cell stage multinucleation on implantation for embryos undelayed in their development at the day-2 embryo assessment (10, 19). Both studies were retrospective and performed in univariate analyses to evaluate implantation potential. However, they reported contradictory results. Indeed, Aguilar et al. (19) found that the presence of multinucleation at the two-cell stage did not affect implantation, whereas Ergin et al. (10) concluded the opposite. However, if we look closely at the data provided in the Aguilar et al. article (19), it is true that the difference was not significant but the implantation rate was lower with embryos presenting at least one multinucleated blastomere at the two-cell stage (94 of 409, 23.0%) than with embryos without multinucleation (203 of 717, 28.3%). Moreover, the results of the Aguilar et al. study (19) in embryos with known implantation data depending on the nuclear status were in close agreement with ours. Indeed, the implantation rates in the Aguilar et al. study (19) were 28.3%, 26.3% (45 of 171), and 20.6% (49 of 238) for embryos without multinucleation, in embryos with one and in those with two multinucleated blastomeres at the two-cell stage, and 33.4%, 27.7%, and 18.3% in our study, respectively. In addition, the results were statistically different between embryos without multinucleation and embryos in which

TABLE 1

Multivariate analysis for implantation and live birth.

	Implantation				Live birth			
Variable	OR	95%	CI	*P* value	OR	95%	CI	*P* value
Woman's age[a]	0.88	0.85	0.93	<.001	0.88	0.84	0.92	<.001
Woman's BMI[a]	0.96	0.92	1.01	.193	0.98	0.94	1.03	.636
Embryo of TOP grade	1.30	0.85	1.99	.215	1.34	0.85	2.12	.203
$MNB^{1/2cell}$	0.83	0.58	1.19	.319	0.73	0.49	1.09	.129
$MNB^{2/2cell}$	0.57	0.34	0.94	.030	0.46	0.26	0.80	.007
MNB^{4cell}	0.51	0.28	0.94	.032	0.52	0.28	0.96	.038
T2[a]	0.91	0.85	0.97	.009	0.92	0.86	0.99	.038
Embryo state[b]	1.58	0.94	2.66	.082	1.22	0.73	2.04	.434
Day of transfer[c]	0.58	0.23	1.44	.248	0.51	0.21	1.26	.149

[a] Treated as a continuous variable.
[b] Fresh or cryopreserved embryo.
[c] Day 2 or day 3.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

both blastomeres were multinucleated. It is worth noting that even though the included population (only embryos from oocyte donors were included in the Aguilar et al. study) and the laboratory environment (e.g., culture media, ICSI procedures) were completely different, the implantation rates depending on nuclear features at the two-cell stage were extremely similar, suggesting that this parameter could be considered an important factor for implantation potential.

To test the impact of nuclear status at the two-cell stage per se, independently of other factors known to influence implantation (embryo morphologic grade, time of two-cell formation, multinucleation at the four-cell stage, woman's age and BMI), we performed a multivariate analysis. In this assessment, for the first time, we confirmed the negative effect of MNB^{2cell} on implantation and more specifically when embryos had multinucleation in both blastomeres. In their logistic regression analysis, Ergin et al. (10) reported a similar influence of the presence of multinucleation within at least one of the blastomeres of two-cell embryos on the ability to achieve clinical pregnancy (OR 0.37, CI 0.24–0.56; $P<.001$). In line with the loss of gestational sac rates, which were higher for embryos with than without multinucleation, our multivariate analysis highlighted that multinucleation in both blastomeres at the two-cell stage per se was associated with a poor prognosis after implantation. As previously reported by our team and others, we also found that women's age, time of two-cell appearance, and the presence of multinucleation at the four-cell stage were significant independent predictors of birth rate. However, the present results suggest that the presence of earlier multinucleation in both blastomeres at the two-cell stage could also independently compromise further development of the embryo even after implantation. Given this, multinucleation at the two-cell stage, like that at the four-cell stage, should be considered an embryo deselection criterion (14, 18).

Nonetheless, as previously reported by several authors, the presence of multinucleation at the two-cell stage even for embryos reaching the four-cell stage in the reference timing range is relatively frequent and more frequent than multinucleation in four cells (19, 31, 32). In the present study they accounted for 36.7% of the transferred embryos included (and 36.8% of these were multinucleated in both blastomeres). In addition, some of these retained the ability to develop to term (10, 19, 32, 33). Consequently, it would be difficult in practice to reject all of these for ET. In this regard, we assessed among transferred embryos with multinucleation at the two-cell stage, whether the kinetics or the type of multinucleation could be predictive of their evolution to term. In accordance with previous published data on implantation (10, 19), we found no particular multinucleation profile specific to embryos that implanted or developed to term as compared with those that aborted. Nevertheless, we observed that a significant proportion of MNB^{2cell} embryos leading to a birth reached the four-cell stage after a longer time (more than 37 hours after fertilization) than those with a shorter cleavage time. Our findings suggest that two-cell-stage embryos that accomplished the second mitosis before 37 hours carry an almost threefold greater risk of a loss of gestation than is the case in embryos that take more than 37 hours. This could be related to the same phenomenon that Aguilar and colleagues found, namely a longer first embryo cell cycle S-phase in implantable embryos (19). Moreover, this long time could be needed to allow functional repair mechanisms. Previously it has been proposed that there could be a rearrangement phenomenon of chromosomal anomalies (32, 34, 35), thus explaining how MNB^{2cell} embryos can lead to mononucleated blastomeres at the four-cell stage (33, 36) and successful deliveries (33–35). This phenomenon probably needs time to take place. The repair procedures could be less efficient in older women, but in our series of aborted MNB^{2cell} embryos, almost all of the women were aged <37 years. Nevertheless, because the number of implanted $MNB^{2/2cell}$ was low in our study, further research is needed to confirm whether this time parameter could be an independent predictor of the potential of embryos to develop.

Thus, the present study demonstrated that the presence of multinucleation at the two-cell stage per se and more specifically in both blastomeres has a detrimental impact not only on implantation rates but also on birth rates. We can hypothesize that these worse outcomes may be due to high rates of chromosomal aneuploidy in such embryos (23, 31). Notably, Staessen and Van Steirteghem (31), using three fluorescence in situ hybridization probes, found that only 30.4% of day-2/3 embryos developing from two-cell embryos with multinuclear blastomeres contained mononuclear diploid blastomeres; the remaining embryos displayed abnormal genetic content. In addition, Meriano et al. (23) reported that the multinucleation phenotype at the two-cell stage was related to different chromosomal contents. Indeed, at the two-cell stage observed at day 2, multinucleated embryos (defined in this study as embryos with at least two nuclei of different size in one or both blastomeres) had higher chromosomal abnormalities than binucleated embryos (defined as embryos with at least two nuclei of the same size in one or both blastomeres) (23). However, in both studies these chromosomal analyses were performed from MNB^{2cell} embryos but delayed in their cleavage because they were at the two-cell stage at

day 2. Several questions remain: first, whether multinucleated embryos at the two-cell stage observed at day 1 or day 2 (thus with delayed cleavage) have identical chromosomal aneuploidy rates; second, in line with our clinical consequences, whether $MNB^{2/2cell}$ embryos at day 1 have higher abnormal chromosomal contents than those found multinucleated in only one blastomere ($MNB^{1/2cell}$ embryos). Some responses concerning the impact of the presence of multinucleation observed at day 1 can be found in a recent study. Indeed, Balakier et al. (32) showed that among euploid and aneuploid blastocysts, the proportion of $MNB^{two\text{-}cell}$ embryos were similar. Consequently, the extended culture may not be useful to select embryos without MNB at the two-cell stage at day 1. Unfortunately, in this study the impact of nuclear features and the number of multinucleated blastomeres were not assessed (32). In addition, it will be interesting to investigate the products of conception from embryos depending on the nuclear status.

Several explanations were proposed: karyokinesis without cytokinesis (37), fragmentation of nuclei (38), changes in temperature (39) and suboptimal culture conditions (40), in vitro–maturated oocytes (41), and cytoskeletal and spindle malfunction leading to defects in migration of chromosomes at the mitotic anaphase (20, 31, 42). In the present study the embryo culture was limited to 2 or 3 days and under conditions that also allowed low temperature and gas exchanges (because embryo observations were done using a time-lapse system). In our multivariate analysis, embryo quality had no statistically significant effect, probably because in the present study almost all of the transferred embryos were of high grade.

In conclusion, multinucleation at the two-cell stage in two blastomeres should be considered an additional and independent criterion of development potential even for high-grade embryos reaching the four-cell stage within the reference timing range. Thus, careful observations of the nuclei at day 1 should be included in strategies used to select embryos for transfer or cryopreservation. Indeed, this parameter should help practitioners to further increase success rates by better selecting embryos with a greater capacity to lead to live births. Even if we can imagine that, in the near future, genetic screening will be used in an extended manner and authorized in countries in which it is currently not allowed, we have to develop noninvasive, observational approaches to determine embryo quality, such as time-lapse image analysis. This technology, which combines cytokinetic and mitotic parameters in the first two cleavage divisions, is crucial to assess transitory embryonic events such as nuclear status.

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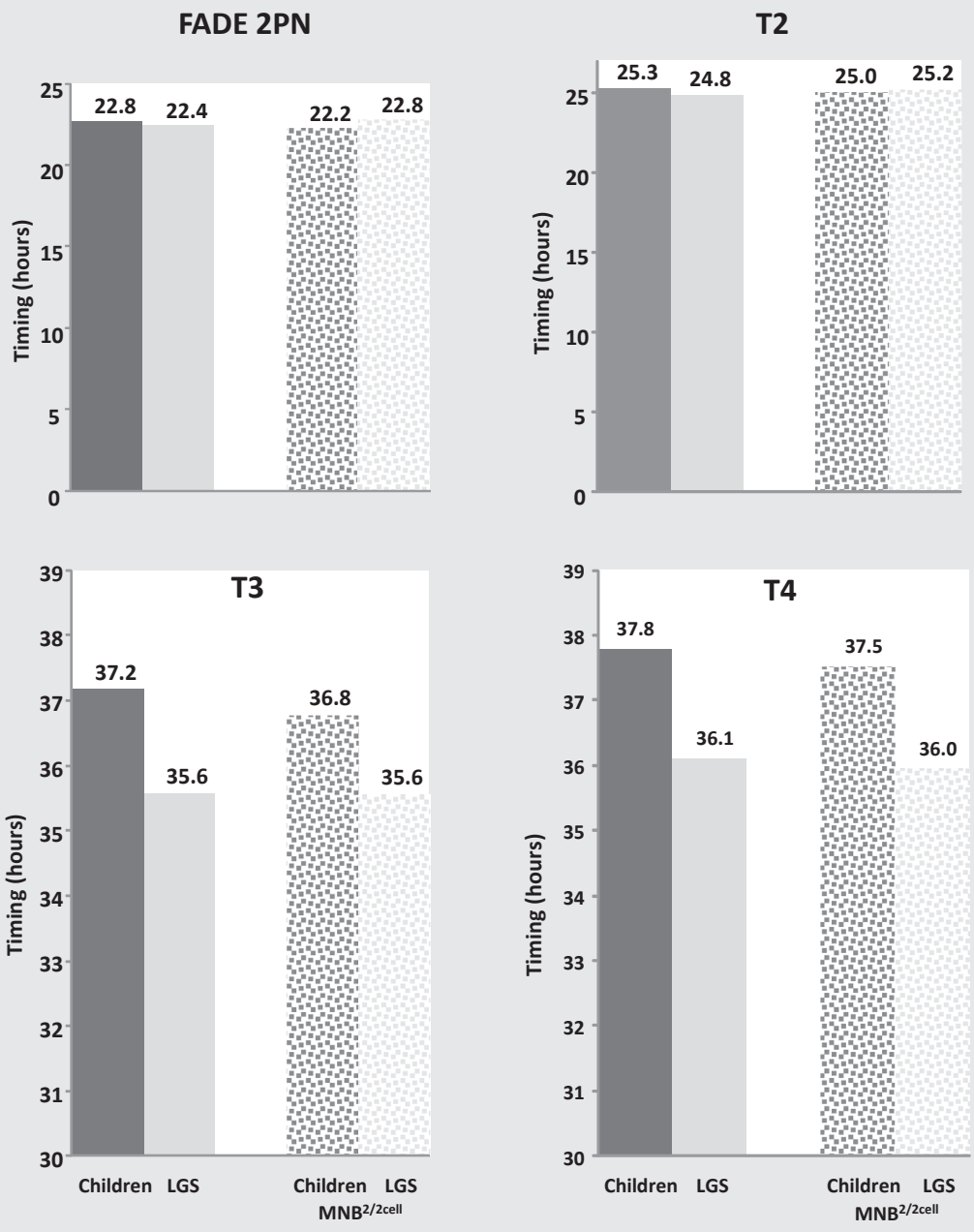
SUPPLEMENTAL FIGURE 1



Kinetics of implanted MNB^{2cell} embryos. Kinetics (tPNf, T2, T3, T4) of implanted embryos according to the nuclear status at the two-cell stage and their outcomes. Values are expressed in hours after ICSI. LGS = loss of gestational sac; MNB^{2cell} = embryo multinucleated at the two-cell stage; MNB$^{2/2cell}$ = both blastomeres multinucleated at the two-cell stage; tPNf = stage of the two pronuclei fading; T2 = time to appearance of two cells; T3 = time to appearance of three cells; T4 = time to appearance of four cells.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

SUPPLEMENTAL TABLE 1

Patient and embryo characteristics.

Characteristic	Without-MNB^{2cell}	With MNB^{2cell}	*P* value
Embryo transfers (n)	235	185	
Male age (y)	35.4 ± 6.4	35.3 ± 5.8	.836
Female age (y)	32.6 ± 4.8	33.1 ± 4.9	.212
Male BMI (kg/m^2)	25.6 ± 4.4	25.1 ± 3.8	.315
Female BMI (kg/m^2)	23.7 ± 4.8	23.2 ± 4.7	.283
Basal FSH (IU/L)	7.6 ± 2.6	7.6 ± 3.1	.934
AMH (ng/mL)	3.6 ± 2.9	3.3 ± 3.1	.335
AFC	18.2 ± 11.1	16.8 ± 9.5	.529
Primary infertility (%)	83.0	86.5	.324
Female smoking (%)	19.0	21.1	.606
Antagonist protocol (%)	20.2	28.4	.070
Total gonadotropin dose (IU)	1,710 ± 1,321	1,842 ± 1,329	.291
Embryos of TOP grade (%)	86.5	86.1	.891
Transfers at day 3 (%)	7.8	7.2	.781
Cryopreserved embryos (%)	15.8	13.0	.342

Note: Values are mean ± SD unless otherwise noted. AFC = antral follicle count; AMH = antimüllerian hormone.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

SUPPLEMENTAL TABLE 2

Implantation and live birth rates (IR, LBR) according to nuclei morphology.

Morphology	IR	*P* value	LBR	*P* value
$MNB^{1/2cell}$				
1BI	31.9		24.2	
1MN	20.0	.132	20.0	.571
$MNB^{2/2cell}$				
2BI	17.4		17.4	
2MN	22.2		13.9	
1BI/1MN	13.0	.668	8.7	.683

Note: BI = binucleated; MN = multinucleated; $MNB^{1/2cell}$ = only one multinucleated blastomere at the two-cell stage; $MNB^{2/2cell}$ = both blastomeres multinucleated at the two-cell stage.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.

SUPPLEMENTAL TABLE 3

Times of two-cell (T2) and four-cell (T4) formation according to nuclei morphology.

Morphology	T2, mean (SD)	*P* value	T4, mean (SD)	*P* value
$MNB^{1/2cell}$				
1BI	25.3 (2.5)		38.0 (3.6)	
1MN	25.6 (2.6)	.533[a]	38.4 (3.7)	.776[a]
$MNB^{2/2cell}$				
2BI	26.0 (3.2)		38.3 (4.0)	
2MN	26.1 (3.6)		38.0 (4.1)	
1BI/1MN	25.5 (2.4)	.101[b]	37.4 (3.3)	.523[b]

Abbreviations as in Supplemental Table 2.
[a] Mann-Whitney test.
[b] Analysis of variance.

Desch. Two-cell multinucleation and outcomes. Fertil Steril 2016.