

Impact of the presence of one or more multinucleated blastomeres on the developmental potential of the embryo to the blastocyst stage

A retrospective study of 5,982 embryos in 619 blastocyst-stage embryo transfer cycles revealed that detection of multinucleated blastomeres either on day 2 or 3 signifies a poor prognosis for blastocyst formation and that no good-quality blastocyst can be expected from an embryo with more than one multinucleated blastomere. Patients should be counseled regarding a poor prognosis when multiple embryos with multinucleated blastomeres are present. (*Fertil Steril*® 2005;83:243–5. ©2005 by American Society for Reproductive Medicine.)

The presence of multinucleated blastomeres (MNB) in the cleaving embryo has been known to be associated with poor embryo development and IVF outcome. Multinucleated blastomeres are not uncommon in embryos developed in vitro: their incidence has been reported to vary between 15% and 20% (1–4). A recent retrospective analysis of >10,000 cleavage-stage embryos showed an association of multinucleation with impaired cleavage, increased fragmentation, and low implantation rates (5). Single or multiple MNB can be recognized in day 2 or 3 embryos. The developmental capacity of multinucleated embryos can vary. Alikani et al. (6) reported that multinucleated embryos developed blastocysts at a lower rate. In this study, we evaluated the impact of different parameters, such as number of MNB, blastomere number, and developmental stage of the embryo at first detection of multinucleation, on blastocyst formation and quality.

A retrospective analysis was performed on 5,982 embryos from 619 blastocyst transfer cycles. The mean female age in the study group was 32.7 years (range, 19–39 years).

Three different criteria were recorded at the first detection of multinucleation: [1] stage of embryo development (day 2 or 3), [2] blastomere number (two or fewer, three or four, five or more), and [3] number of MNB (single or multiple).

The mean number of M-II oocytes retrieved was 13.8, and the fertilization rate was 76%. Of the 5,982 day-2 and 4,043 day-3 embryos, MNB were detected in 1,568 (26.2%) and 464 (11.4%), respectively. The incidence of MNB decreased from day 2 to day 3. In the majority of the embryos, only one MNB was detected.

Cleavage and blastocyst formation rates were analyzed according to the day of detection and number of MNB.

These were compared with 4,414 embryos having no MNB. The results are summarized in Table 1.

The cleavage rate of embryos without any MNB was 91.6%. This declined to 56.0% and 36.4% if the embryo contained one or more MNB, respectively ($P<.0001$). The presence of MNB compromises further development of the embryo: the 51.0% blastocyst formation rate in embryos without MNB declined to 11.4% and 6.5% if the embryos contained one or more MNB, respectively ($P<.0001$). Blastocyst quality was also affected when MNB were present. The chance of having a good-quality blastocyst derived from a day 2 embryo with MNB was <5%.

Among the groups, three- to four-cell GrI-II embryos with a single MNB showed the highest rate of cleavage and blastocyst formation. More than 50% developed into good-quality day-3 embryos, and 22.4% developed into good-quality blastocysts. Three- to four-cell cell GrI-II embryos with multiple MNB also showed comparable cleavage and blastocyst formation, albeit at a lower rate. None of the multinucleated three- to four-cell GrIII-IV embryos developed into good-quality blastocysts.

The blastocyst formation rates of GrI-II day-2 embryos with five or more blastomeres that contained single or multiple MNB were <10% (8.3% and 7.7%, respectively). More than 60% of these embryos arrested during cleavage. Similar to the other groups, none of the multinucleated five-or-more-cell GrIII-IV embryos developed into good-quality blastocysts.

The blastocyst formation rate of day-3 embryos with single or multiple MNB was 3.2% and 0.9%, respectively. No good-quality blastocysts developed from an embryo when MNB appeared on day 3. Only 10.7% of GrI-II day-2 embryos with a single MNB developed into blastocysts, and this rate declined to 3.2% if they contained multiple MNB. None of the multinucleated two-cell GrIII-IV embryos developed good-quality blastocysts. More than 60% of these embryos arrested during cleavage.

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TABLE 1

Cleavage and blastocyst formation rates according to the first day of detection and number of MNB.

Groups	n	Cleavage on day 3 (%)	Blastocyst formation (%)	BG1-2 blasts (%)
Embryos without MNB	4,414	4,043 (91.6)	2,062 (51.0)	1,241 (30.7)
Day-2 embryo with 1 MNB	643	360 (56.0)	41 (11.4)	17 (4.7)
2-cell GrI-II	211	150 (71.1)	16 (10.7)	7 (4.7)
2-cell GrIII-IV	194	70 (36.1)	3 (4.3)	0
3-4-cell GrI-II	68	58 (85.3)	13 (22.4)	9 (15.5)
3-4-cell GrIII-IV	82	38 (46.4)	6 (15.8)	0
≥5 cell GrI-II	33	24 (72.7)	2 (8.3)	1 (4.2)
≥5 cell GrIII-IV	55	20 (36.3)	1 (5.0)	0
Day-2 embryo with >1 MNB	461	168 (36.4)	11 (6.5)	4 (2.4)
2 cell GrI-II	101	62 (61.4)	2 (3.2)	1 (1.6)
2 cell GrIII-IV	194	30 (25.7)	2 (6.7)	0
3-4 cell GrI-II	22	19 (86.4)	3 (15.8)	2 (10.5)
3-4 cell GrIII-IV	38	18 (47.4)	2 (11.1)	0
≥5 cell GrI-II	20	13 (65.0)	1 (7.7)	1 (7.7)
≥5 cell GrIII-IV	86	26 (30.3)	1 (3.8)	0
Day-3 embryo with 1 MNB	248	—	8 (3.2)	0
Day-3 embryo with >1 MNB	216	—	2 (0.9)	0

Note: χ^2 test, Fisher exact test $P < .0001$.

Yakin. Multinucleated embryos and blastocyst formation. *Fertil Steril* 2005.

Among 7,982 cleavage-stage embryos, 1,750 (21.9%) showed an early cleavage. Multinucleated blastomeres were detected in 105 of these embryos (6.0%), and 92 (87.6%) of them arrested at different stages of development, whereas 6 hatched, and 7 collapsed blastocysts developed on day 5 of culture.

Among all cycles, none of the patients were transferred blastocysts developed only from multinucleated embryos. In 58 cycles (9.3%), blastocysts from both mono- and multinucleated embryos were transferred, yielding to an implantation rate of 44.4%. The remaining 561 cycles were transferred blastocysts developed from mononucleated embryos, and the implantation rate was 45.8%. There was no statistically significant difference between these two groups.

There have been many studies of embryos with MNB. Jackson et al. (1) analyzed 3,557 embryos in 483 IVF cycles and detected at least 1 embryo with MNB in 74% of the cycles. They reported the incidence of MNB among all embryos as 18.3%. The investigators reported a significant reduction in the cleavage rate of embryos containing MNB. Implantation, clinical pregnancy, and live birth rates were lower when embryos containing one or more MNB were transferred (1). Pelinck et al. (7) also reported poor clinical pregnancy and implantation rates in 136 cycles when embryos with MNB were transferred. Alikani et al. (6) evaluated multinucleation and blastocyst development and re-

ported that among embryos with MNB on day 2 and/or day 3, only 15.9% formed normal blastocysts.

Defects in synchronous development of karyokinesis and cytokinesis might be associated with chromosomal abnormalities in the developing embryo. Kligman et al. (8) reported that 74.5% of MNB embryos were chromosomally abnormal, compared with 32.2% of mononucleated embryos. Conversely, blastomere biopsy of embryos with MNB detected on day 4 of cleavage revealed mostly normal chromosomes (9). Staessen and Van Steirteghem (10) were more optimistic regarding the genetic constitution of the embryos with MNB, suggesting that they are not always abnormal because multinucleation might be a temporary and reversible phenomenon. They also studied the possible correlation of chromosomal abnormalities with the day of MNB appearance, number of nuclei per cell, and number of MNB per embryo but found no statistically significant correlation.

The mechanism underlying multinucleation is not clearly defined, but it seems to represent an error in the cytokinesis process. Karyokinesis in the absence of cytokinesis leads to multinucleation in the blastomere. The alternate mechanism proposed for multinucleation might be the errors of chromosome segregation, partial fragmentation of nuclei, and/or errors in packaging at mitosis through defective migration at mitotic anaphase (2, 3, 11). Significant fluctuations in temperature, suboptimal culture

conditions, or follicular underoxygenation have been proposed to be associated with the presence of multinucleation (2, 3, 11, 12). Another interesting finding was reported by Styne et al. (13): in a retrospective analysis of 424 IVF cycles, the incidence of MNB was significantly increased in cycles with high peak E₂ levels (>1,000 pg/mL) and a high number of retrieved oocytes (>15). Similarly, Jackson et al. (1) have also reported a positive correlation between MNB and elevated serum E₂ levels as well as large numbers of retrieved oocytes. These findings point out how clinicians can contribute to the outcome of assisted reproduction cycles by tailoring controlled ovarian hyperstimulation in the optimal way. Avoiding overstimulation might help reduce the incidence of embryos with MNB and improve laboratory performance of the resulting embryos.

Our study has revealed the association of multinucleation in cleavage state with poor embryo quality and development. Multinucleation was more frequent in day-2 embryos. The prognosis was worse when multinucleation appeared on day 3. Presence of multiple MNB signifies a higher risk of cleavage arrest, and the chance of developing a good-quality blastocyst out of these embryos is practically zero. The blastomere cell number when multinucleation is first noticed is also important for the prediction of its further growth. Approximately 25% of multinucleated day-2 GrI embryos with three or four cells developed good-quality blastocysts. On the other hand, slow-growing or fast-cleaving (five or more cells) multinucleated day-2 embryos failed to develop blastocyst.

A significant drawback of this study is the lack of implantation potential of multinucleated embryos. Blastocysts developed from multinucleated embryos were discarded from candidates for transfer and transferred only when there were no other embryos suitable for transfer. Because all these cycles were blastocyst transfers, very few blastocysts were developed from multinucleated embryos, and most failed to achieve good-quality scores. Of 619 transfer cycles, none of the patients had been transferred blastocysts developed only from multinucleated embryos. Blastocysts developed from embryos with and without MNB were transferred in 58 cycles (9.3%). The implantation rate in these transfers was not significantly different from that of 561 cycles in which all transferred blastocysts were developed from mononucleated embryos (44.4% and 45.8%, respectively).

We have detected MNB in 26.2% of day-2 and 11.4% of day-3 embryos. Detection of MNB either on day 2 or day 3 signifies a poor prognosis for blastocyst formation. The presence of more than one MNB in the embryo is associated with slow growth and poor-quality embryos. No good-

quality blastocyst can be expected from an embryo with more than one MNB. Therefore, the patient should be counseled regarding a poor prognosis when multiple embryos with MNB are present. Blastocyst culture is a good way to eliminate multinucleated embryos from being transferred. Furthermore, the clinicians' role in the management of controlled ovarian hyperstimulation has to be stressed to improve the laboratory outcome.

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