Use of metaphase I oocytes matured in vitro is associated with embryo multinucleation

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Objective: To evaluate the impact of oocyte maturational stage at retrieval on embryo multinucleation.
Design: Retrospective study.
Setting: Private institution for assisted reproduction.
Patient(s): A total of 412 patients undergoing 500 intracytoplasmic sperm injection (ICSI) cycles between August 2006 and September 2010.
Intervention(s): Routine ICSI laboratory procedures.
Main Outcome Measure(s): Normal and abnormal fertilization; embryo development; arrest at pronuclear stage; failure to undergo first mitotic division; presence of embryo multinucleation; embryo quality; pregnancy, implantation, and miscarriage rates.
Result(s): A significantly lower percentage of multinucleation was found in embryos originating from metaphase II (MII) oocytes when compared with MI–II- and MI-derived oocytes. Significantly fewer multinucleated cells per embryo were observed in MII-derived oocytes. Clinical pregnancy and implantation rates were significantly higher when only embryos derived from MII oocytes were transferred.
Conclusion(s): Embryo multinucleation rate increases when in vitro–matured (2–5 hours incubation) MI (MI–II) oocytes are used instead of in vivo–matured oocytes in ICSI. Furthermore, all other ICSI outcome parameters are also compromised. The use of donated gametes does not modify these results. (Fertil Steril® 2013;99:414–21. ©2013 by American Society for Reproductive Medicine.)
Key Words: In vivo maturation, metaphase II, metaphase I, ICSI outcome, multinucleation

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During growth phase, oocytes accumulate messengers RNAs and proteins necessary for completion of meiosis, chromatin rebuilding, and activation of the cell cycle associated with fertilization, first embryo mitotic division cycles, establishment of the embryo genome, and normal mechanisms of cellular homeostasis. Any alteration in these processes should be interpreted as incorrect temporal utilization of specific products followed by delay or failure in the development of the embryo.

Developmental competence is a process that seems to be totally independent of nuclear maturation (meiotic competence), which establishes the capacity to complete first meiotic division and arrest at metaphase II stage (MII). Surprisingly, oocytes at 5 mm in diameter would already have the capacity to complete nuclear maturation, but they still have to reach 10–12 mm to obtain developmental competence (1). All transcripts accumulated during oocyte growth control embryo development until its own genome is correctly activated (2).

In assisted reproduction techniques, approximately 85% of the oocytes obtained are at MII stage; the rest remain at prophase I (11%) and metaphase I (MI) (4%) (2–5). However, not all MIIs obtained have the same capability in terms of fertilizing potential, embryo development, implantation, and normal pregnancy achievement. Furthermore, maturing MIs are usually injected during routine intracytoplasmic sperm injection (ICSI) at the same time as the "in vivo"–matured MIIs from the same patient.

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To the best of our knowledge, the literature reviewed is still controversial and/or inconclusive regarding the laboratory and clinical results obtained using MIIs, maturing MIs, and MIs with regard to ICSI outcome (3, 6–9). In addition, we have been unable to find any studies comparing percentage of normally fertilized arrested oocytes, number of embryos failing to undergo first mitotic division (arrested at syngamy stage), number of multinucleated embryos, and more notably, number of multinucleated cells in embryos derived from these oocytes categories. Furthermore, none of these parameters has been reviewed when donated or patients' gametes were used (10–12).

In view of the lack of or controversial information in the literature, the present study was undertaken to compare ICSI outcome when the three established oocyte categories (MIIs, maturing MIs, and MIs) at retrieval were individualized.

MATERIALS AND METHODS

Patient Population

All patients undergoing ICSI procedures (August 2006–September 2010) were included. A total of 500 ICSI cycles in 412 patients were performed within this period: 382 using the patients' own gametes (group A); 91 using donated oocytes (group B; normal female gametes); 15 using donated sperm (group C; normal male gametes); and 12 done with both donated male and female gametes (group D). The study protocol was approved by the Seremas institutional review board. All patients signed consent forms authorizing the procedures (ICSI, gamete donation, and cryopreservation as appropriate).

An attempt to evaluate the impact of female and male etiologies of infertility, in all the variables analyzed in the study, was made. Nonetheless, because of the small sample size it could not be done.

Ovarian Stimulation Protocol

The ovarian stimulation protocol used an initial GnRH agonist down-regulation (long or short), followed by recombinant simultaneous FSH administration. Cycle progression was monitored by daily serum E_2 levels and ultrasonographic assessment of follicle size either daily or every other day. Final follicle maturation was triggered using a single SC injection of 250 μg recombinant hCG (Ovidrel; Serono) or 5,000 IU recombinant hCG (Gonacor 5000; Ferring) administered within 36 hours after the last FSH dose, when the patient had at least one follicle $>$18 mm and half of the remaining follicle cohort was $\geq$14 mm. Oocyte retrieval was performed 36 hours after hCG administration, under general anesthesia using transvaginal ultrasound guidance.

Laboratory Procedures

For the purpose of this investigation, only ICSI procedures were included in the study to accurately evaluate oocyte maturation at the time of oocyte retrieval.

Oocytes were cultured in early cleavage medium (Irving Scientific) supplemented with 15% human serum albumin (Irvine Scientific) and covered with oil (embryo-tested mineral oil; Irving Scientific). The same medium was used for sperm samples and embryo culture. All cultures were done at 37°C in a 5% CO_2 atmosphere.

Before ICSI, oocytes were treated with hyaluronidase (20 IU/mL; Irving Scientific) approximately 30 minutes after oocyte retrieval. At that time, oocytes were classified and separated using an inverted microscope (Nikon Eclipse TE2000-S) according to nuclear maturity in MII, MI, prophase I, atretic, fracture zone, and parthenogenetic embryos. All MIIs and MIs were cultured for 2–5 hours in separated drops for further maturation, until ICSI was performed. All MIs reaching maturity at the time of injection will be referred to hereafter as MI–IIs. The ones remaining at MI stage will be referred to as MIs, and in an attempt to confirm their performance they were injected at the same time as the rest (MI group).

Sperm samples were obtained from fresh or frozen ejaculate in nonazoospermic patients and from fresh or frozen epididymal/testicular samples in patients with obstructive and nonobstructive azoospermia, respectively. Motile sperm were recovered either using direct swim-up technique or by washing the sample with early cleavage medium–15% human serum albumin, according to sperm concentration, motility, and source. Fresh and frozen–thawed samples were prepared and used the same day of oocyte retrieval.

The ICSI technique was performed by standardized techniques, and all MIIs, MI–IIs, and MIs were injected at the same time and kept in separate drops until fertilization as checked 14–18 hours later and for further evaluation of postinjection oocyte progression: [1] normal and abnormal fertilization; [2] normally fertilized oocytes arrested at two pronuclei (2PN) on day 2, [3] embryos failing to undergo first mitotic division (arrested at syngamy stage); [4] embryo quality at days 2 and 3; [5] percentage of multinucleated embryos at day 2; [6] number of multinucleated cells per multinucleated embryos at day 2; [7] embryo cell number at days 2 and 3; [8] number of arrested embryos at day 3; and [9] pregnancy, implantation, and miscarriage rates according to the origin of the embryos transferred (MII/MI–II/MI/mix) and to the presence or not of any multinucleated embryo. All results were related to the origin of the gametes used (from donors or from patients).

Fertilization

Oocytes showing 2PN and a second polar body were classified as normally fertilized. The oocytes with other than 2PN were considered abnormally fertilized. The degenerated, abnormally fertilized, or nonfertilized oocytes were discarded at that time.

Observations at Day 2 (40–48 Hours after Aspiration)

Oocytes arrested at 2PN. All normally fertilized oocytes keeping the pronuclear structure at 40–48 hours after oocyte aspiration were considered arrested and were discarded.

Embryos arrested at syngamy. All embryos failing to undergo first mitotic division at 40–48 hours after oocyte aspiration were considered arrested at syngamy and were discarded.

Embryo classification. Embryos were classified according to cell number, presence of even- or uneven-size blastomeres, and percentage of fragmentation under an inverted microscope. The degree of fragmentation was expressed as a percentage of the embryonic volume occupied by enucleated cytoplasmic fragments (13). Embryos were scored as follows: 1, good embryo quality: embryos showing at least four even cells with less than 15% fragmentation; 2, intermediate embryo quality: embryos with two to five even or uneven cells and no more than 30% fragmentation; 3, poor embryo quality: embryos with fewer than two cells and multiple fragments covering more than 50% of the embryo surface; 4, accelerated cleavage: embryos presenting more than five cells at the time of observation; 5, polyfragmented embryos: no embryonic cells were observed, only anucleated fragments present. The presence of multinucleated blastomeres was not included in this evaluation.

Embryo multinucleation. The number of multinucleated embryos and multinucleated cells (binucleated or multinucleated) per embryo were recorded. All affected embryos were further incubated in individual drops to monitor and record their development. Results were expressed as [1] number of multinucleated embryos regardless of the number of affected embryonic cells present; and [2] number of affected cells per embryo.

Embryos presenting binucleation (two nuclei of the same size and shape) were cultured in a separate drop until confirmation 2 to 3 hours later of the persistence of binucleation.

Of all 2,828 embryos, 185 (6.5%) could not be evaluated for embryo multinucleation: 42 of 185 (22.7%) had not undergone first mitotic division (arrested at syngamy stage; 1.5% of all the embryos; 42 of 2,828), and in the remaining 143 (77.3%) multinucleation could not be accurately observed owing to presence of very high percentages of fragmentation, vacuolation, or cytoplasmic inclusions (5.1% of all embryos, 143 of 2,828). Therefore, the number of embryos evaluated for the presence of multinucleation is lower than the total number of embryos obtained (2,643 of 2,828 total embryos obtained, 93.1%).

Observations at Day 3 (65–70 Hours after Aspiration)

Embryo classification. Embryos were again classified according to cell number, presence of even- or uneven-size blastomeres, and percentage of fragmentation, under an inverted microscope in the following categories: 1, good embryo quality: embryos showing at least 6 to 10 even cells with less than 15% fragmentation; 2, intermediate embryo quality: embryos with 5 to 10 even or uneven cells and no more than 30% fragmentation; 3, poor embryo quality: embryos with fewer than 3 cells and multiple fragments covering more than 50% of the embryo surface; 4, arrested embryos: embryos presenting the same number of cells as the day before; 5, accelerated cleavage: embryos presenting more than 11 cells at the time of observation; 6, polyfragmentated embryos: no embryo cells were observed, only anucleated fragments.

The number of embryos under consideration at this point is lower than at day 2, because some of them were transferred on day 2.

Embryo Transfer

Embryo transfer was performed at either 40–48 hours (day 2) or 65–70 hours (day 3) after oocyte retrieval, according to the number of oocytes obtained and the couples' decision regarding the use of cryopreservation.

In 21 cases (21 of 500 cycles performed, 4.2%) no ET could be performed because of different reasons. Fertilization failure was observed in 2 of 21 (9.5%); cryopreservation of all embryos in 14 of 21 (66.7%: in 9 cycles clinical impending ovarian hyperstimulation was detected; in 5 cycles ET could not be done owing to extreme discomfort of the patient); and in the remaining 5 cycles (5 of 21, 23.8%) ICSI was not possible because no oocytes at all were recovered or only immature ones and/or fractured zonae were produced. Overall, 479 ETs were performed (479 of 500, 95.8%): 171 of 479 (35.7%) were done at day 2 and 308 of 479 (64.3%) at day 3.

The embryos selected for transfer were then moved into a one-well Petri dish containing 1 mL of H-HTF (Irving Scientific) supplemented with 50% human serum albumin and loaded into the embryo catheter selected for each specific patient. All remaining embryos considered suitable were cryopreserved.

At ET (479 ET), record generally was made of the origin of the individual embryos according to the respective origin (pure MII, pure MI–II, pure MI, and mixed) and of the presence or not of any multinucleated embryo transferred. Nonetheless, in 13 cycles no record was available on the origin of the embryos transferred according to the maturational stage of the injected oocytes (13 of 479, 2.7%). These cycles were not considered for further evaluation of the results on pregnancy outcome. Whenever possible according to embryo quality, only MII embryos were transferred. This issue significantly reduced the number of pure MI transfers to judge clinical pregnancy rate (CPR), implantation rate (IR), and clinical miscarriage rate. Pure MI transfers were done only when no other embryos were available, and therefore statistical analysis was not always feasible. On the other hand, in 65 cases (65 of 479, 13.6%) no record was available on the previous presence of multinucleation, and they were not considered for evaluation of pregnancy, implantation, and miscarriages rate results. Once again, the best embryos were selected for transfer, trying to avoid multinucleated ones if possible, and ET with pure multinucleated embryos was very low ($n = 1$).

Preclinical Miscarriages, Clinical Pregnancies, and Clinical Miscarriages

Serum β-hCG levels were measured 12 days after ETs. If positive levels were observed, an ultrasonography was performed within a month to ascertain the presence of gestational sac(s) and cardiac activity (clinical pregnancy). A preclinical miscarriage was diagnosed when only positive values of β-hCG were obtained but clinical pregnancy was never confirmed.

An early clinical miscarriage was diagnosed when a fetus showing cardiac activity was aborted.

Statistical Analyses

Results were analyzed by the statistical relationship between the origin of injected oocytes (MIs, MI–IIs, or MIIs) with normal and abnormal fertilization, arrest at 2PN or at syngamy stage, embryo development and quality, embryo multincleation and number of multinucleated cells, and implantation and pregnancy rates. These relationships were evaluated by χ^2 and Fisher exact tests, as appropriate. Comparisons between two groups were made using the Mann-Whitney *U* test. Analysis was performed using GraphPad Prism (GraphPad Software). All tests were two-tailed, with statistical significance assessed at the $P<.05$ level.

RESULTS

Patient and Gamete Population

Several characteristics of subjects enrolled are shown in Table 1. Female age was found to be, as expected, significantly lower in the donor group. Average time of ICSI performance was 3–5 hours after aspiration (4:00 ± 1:20 hours).

Fertilization and Early Embryo Development

The percentage of normal and abnormal fertilization was significantly different in the three groups of injected oocytes. As expected, better performance was observed in the MII group (Table 2A).

When the overall population was classified according to the origin of the gametes (A: patients' own gametes; B: donated oocytes; C: donated sperm; D: both sperm and oocytes donated), significantly better results in terms of normal fertilization were obtained using MIIs in groups A, B, and C (Supplemental Table 1, available online), suggesting that the use of donated oocytes or sperm did not modify the tendency observed; that is, the MI–IIs are not equally competent as the MIIs. Because of small sample size, group D did not show statistical differences. This last group should be enlarged in the future to determine whether the same tendency is confirmed. A similar observation was obtained regarding abnormal fertilization. A significantly lower abnormal fertilization rate was observed in the MII group, and this tendency was not modified even when donated oocytes were used (group B). Because of low sample size, groups C and D could not be statistically evaluated and should be enlarged for further analysis.

Two hundred two fertilized oocytes were cryopreserved at this time (6.9%, 202 of 2,928), and the remaining 2,726 were allowed to develop further (93.1%, 2,726 of 2,928). From those that were not cryopreserved and continued their development, 3.88% (106 of 2,726) were arrested at pronuclear stage or at syngamy. As predictable, the best performance for both variables was observed in the MII group (Table 2B). Similar results were observed when the individuals were classified according to the origin of the gametes in group A. However, because of small sample size groups B, C, and D did not show statistical differences (Supplemental Table 2).

Overall, these results show that MII oocyte performance in terms of normal and abnormal fertilization and pronuclear and syngamy arrest is significantly better than that of MI–IIs and MIs, and these observations are not dependent on gamete origin.

Embryo Quality at Day 2

Embryo quality was evaluated in the total patient population and in the three categories according to the origin of the injected oocytes: MII, MI–II, and MI groups. The best embryo quality and the highest number of cleaved embryos (three to five cells) were observed in the MIIs (Table 2C). As observed in the previous section, the overall performance of MI–IIs and MIs was not as good as in MIIs, as evidenced by the percentage of fragmented embryos, the percentage of embryos with low cell numbers (two or fewer cells), and the quality of embryos (Materials and Methods). Similar results were observed when these variables were analyzed in group A. Because of low sample size groups B, C, and D did not show statistically significant differences, and these groups should be enlarged for proper analysis (Supplemental Table 3).

TABLE 1

General features of the population of patients and donors that was enrolled in this study.

Parameter	Patients (n = 397)	Donors (n = 81)	Recipients (n = 103)
Age (y)	34.52 ± 3.99[a]	26.27 ± 3.49[a]	41.74 ± 4.98
No. of oocytes	4,455	1,973	–
Average no. of oocytes obtained	11.20 ± 7.12	24.35 ± 12.99	–
MII	53.6	64.1	–
MI	24.0	15.8	–
Prophase I	18.5	16.1	–
Other	3.9	4.0	–
Average no. injected MII	5.92 ± 4.39	–	6.86 ± 3.76
Average no. injected MI–II	1.38 ± 1.58	–	1.14 ± 1.19
Average no. injected MI	1.11 ± 1.49	–	0.62 ± 0.96

Note: Results are expressed as mean ± SD or percentage, unless otherwise noted. "Other" denotes fractured zonae, degenerated oocytes, and parthenogenic embryos.
[a] Significant difference between patients and donors ($P<.0001$, Mann-Whitney *U* test).

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TABLE 2

A. Normal vs. abnormal fertilization in the three oocyte groups.

Oocyte maturity at ICSI	Normal fertilization	Abnormal fertilization
All oocytes	69.6 (2,928/4,206)	3 (127/4,206)
MII	76.1 (2,317/3,044)[a]	2.4 (72/3,044)[b]
MI–II	65.2 (436/669)[a]	3.4 (23/669)[b]
MI	35.5 (175/493)[a]	6.5 (32/493)[b]

B. Arrest at 2PN and syngamy stage at day 2 in the three oocyte groups.

Oocyte maturity at ICSI	Arrested 2PN	Syngamy stage
All oocytes	2.3 (64/2,726)	1.6 (42/2,662)
MII	1.6 (34/2,166)[a]	1.2 (25/2,134)[b]
MI–II	4 (16/397)[a]	2.6 (10/379)[b]
MI	8.6 (14/163)[a]	4.7 (7/149)[b]

C. Embryo development and quality at day 2 in the three oocyte groups.

Oocyte maturity at ICSI	Fragmented embryos	≤2 Cells	3–5 Cells	Accelerated cleavage	GEQ	IEQ	PEQ
All oocytes	0.6 (15/2,662)	20.1 (536/2,662)	63 (1,675/2,662)	0.9 (25/2,662)	75.7 (2,068/2,662)	19.8 (527/2,662)	1.2 (31/2,662)
MII	0.3 (7/2,134)[a]	17.5 (384/2,134)[b]	65.2 (1,392/2,134)[c]	0.9 (20/2,134)	81.4 (1,737/2,134)[d]	17 (361/2,134)[e]	0.7 (16/2,134)[f]
MI–II	0.8 (3/379)[a]	28 (106/379)[b]	54.9 (208/379)[c]	1 (4/379)	66.7 (253/379)[d]	28.5 (108/379)[e]	2.4 (9/379)[f]
MI	3.3 (5/149)[a]	30.9 (46/149)[b]	50.3 (75/149)[c]	0.7 (1/149)	52.3 (78/149)[d]	39 (58/149)[e]	4 (6/149)[f]

Note: Values are percentage (number). Similar superscript letters denote groups that have a statistically significant difference. $P<.05$ was considered statistically significant as analyzed by χ^2 or Fisher exact test, as appropriate. GEQ = good embryo quality; IEQ = intermediate embryo quality; PEQ = poor embryo quality. Similar superscript letters denote groups that have a statistically significant difference.

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In conclusion, significant and progressive impaired embryo quality was observed as oocyte immaturity increased.

Embryo Multinucleation at Day 2

In the global population 11.2% of the embryos showed multinucleated cells, and they represented 4.2% of the total cell number. Significantly more embryos with multinucleated cells and an increase in the total number of them were found in the MI–II and MI categories (Fig. 1).

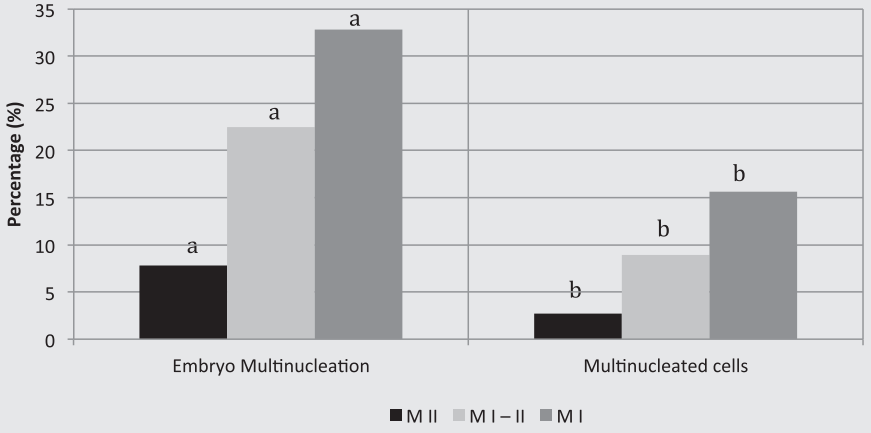
As observed in the analysis of previous variables, these results were similar when origin of the gametes was taken into account (groups A and B; Supplemental Table 4). Because of low sample size groups C and D could not be statistically evaluated.

The use of donated oocytes did not modify the performance trend already mentioned, indicating that at least oocyte quality does not play a role in the incidence of multinucleation.

Embryo Arrest at Day 3

The total embryo population exhibited 5.2% arrested embryos at day 3. When the incidence of embryo arrest was investigated, MIIs showed 4.3% (71 of 1,628), MI–IIs 7.3% (19 of 259), and MIs 13.7% (13 of 95), indicating significantly less incidence of embryo arrest in the MIIs ($P<.0001$). When gamete origin was taken into account, group A showed 4.9% arrested embryos, group B 5.4% (26 of 477), group C 9.2% (6 of 65), and group D 7.1% (2 of 28). No

FIGURE 1



Embryo multinucleation and multinucleated cells in the three established oocyte categories. Values are percentages. $P<.05$ was considered statistically significant as analyzed by χ^2 test or Fisher exact test, as appropriate. [a]$P<.0001$; [b]$P<.0001$.

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significant differences were found in the number of embryos arrested at day 3 when groups A and B were statistically compared ($P=.8965$).

When oocyte maturity was considered in group A, similar results as in the global population were found. These results showed once again that as immaturity of the original oocytes increases, the incidence of embryo arrest at day 3 also increases, and at least the fact of using only the couples' gametes did not modified these observations. Groups B, C, and D must be enlarged for further analysis.

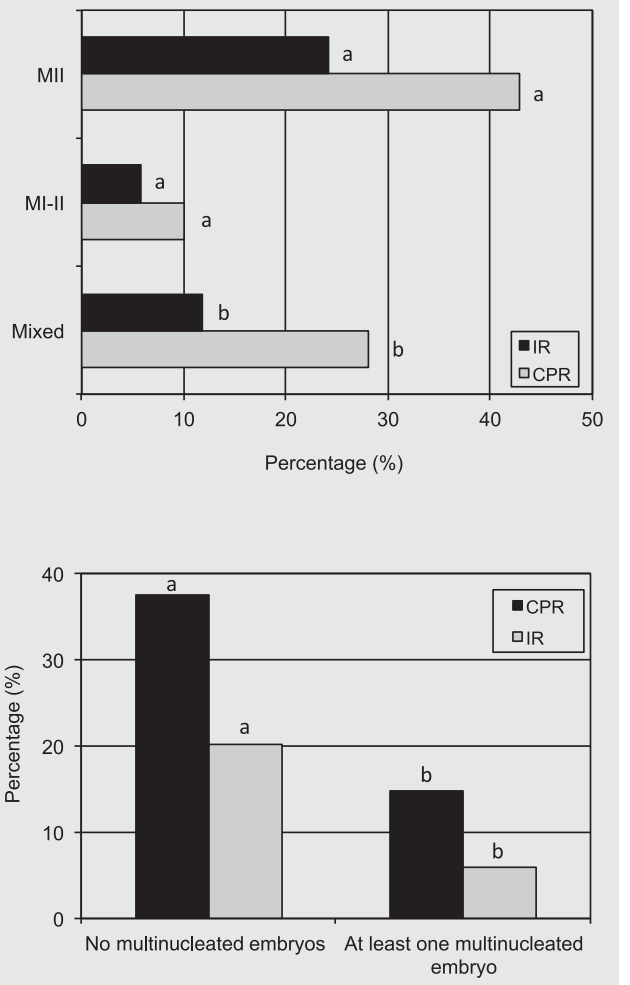
Pregnancy Outcome

A total of 479 ETs were performed (95.8% of the cycles, 479 of 500). Overall CPR per transfer was 37.4% (179 of 479), preclinical miscarriage rate was 8.6% (41 of 479), IR was 19.6% (266 of 1,355), and clinical miscarriage rate was 17.9% (32 of 179), regardless of the origin of the embryos transferred (originated from MIIs, MI–IIs, or MIs) and of the presence or absence of multinucleation.

When results were analyzed according to embryo origin, a total of 466 ETs were evaluated (Materials and Methods), and 1,320 embryos were considered: MII-derived embryos were transferred in 63.9% of the cases (306 of 466; 850 embryos transferred); 2.1% (10 of 466; 17 embryos) received only MI–II-derived embryos; 0.8% (4 of 466; 6 embryos) had MI-derived embryos exclusively; and 30.4% (146 of 466; 447 embryos) received embryos of combined origin (MII-, MI–II-, and MI-derived embryos). In this last group embryo distribution was as follows: 56.1% (251 of 447) from MIIs; 31.1% (139 of 447) from MI–IIs, and 12.8% (57 of 447) from MIs. When oocyte maturity was taken into account, significantly better CPR and IR per transfer ($P<.0001$) were obtained in the MII group. A clear tendency of achieving lower pregnancy rate and IR as immaturity increases was observed. The combined group, in which the majority of the embryos derived from MIIs, shows rates in between the MII and MI–II groups (Fig. 2, top), which are consistent with the proportion of embryos that were present in the mixture at ET. In the MI group only four ETs were done, and no pregnancies resulted. Miscarriage rates could not be compared in the different types of ETs because of low sample size.

On the other hand, results were analyzed according to the presence or absence of multinucleated embryos at the time of transfer, and 414 ETs were analyzed (Materials and Methods). Fifty-three multinucleated embryos were transferred, representing 4.3% of all embryos at ET. Overall, 47 of 414 transfers (11.3%) had at least one multinucleated embryo present. When presence or absence of multinucleated embryos at transfer was considered in the whole population, significantly better CPR and IR were obtained in the group with no multinucleated embryos present ($P<.001$ and $P<.0001$, respectively; Fig. 2, bottom), and no differences were observed regarding miscarriage rate. Additionally, this could also be explained because patients with at least one multinucleated embryo transferred had a significantly lower proportion of MIIs and a significantly higher proportion of MIs at retrieval (data not shown). This resulted in time, as evidenced by the results presented here, in a higher proportion of multinucleated embryos, forcing the fact of having to transfer at least one of them.

FIGURE 2



Top: Pregnancy and implantation rates according to the origin of embryos: derived exclusively from MIIs, derived only from MI–IIs, or mixed from the three oocyte groups. *Bottom*: Pregnancy and implantation rates according to presence or absence of multinucleated embryos at transfer. Values are percentages. $P<.05$ was considered statistically significant as analyzed by χ^2 test or Fisher exact test, as appropriate. [a]$P<.001$; [b]$P<.0001$.

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DISCUSSION

During routine assisted reproduction techniques, despite using hCG injection to trigger final nuclear maturation, not all the oocytes reach the desire MII stage. Furthermore, not all MIIs behave in terms of embryo development as expected (7, 14). It is a common scenario to have some MIs at retrieval and to inject the ones maturing within 2–5 hours at the same time as the in vivo–matured ones.

Some work has been done on embryo development when MIs were used in ICSI procedures, demonstrating a high incidence of chromosomal abnormalities in embryos

originating from them (15–21). Nonetheless, there is some controversy or even lack of evidence regarding the performance of maturing MIs within routine culture time and conditions.

Blastomere multinucleation has been negatively associated with embryo development and pregnancy outcome. It has been proposed that this nuclear abnormality has its origin in deficiencies in oocyte maturation that produce spindle defects, nuclear fragmentation, or defective migration at mitotic anaphase (14).

We have demonstrated in our study not only that multinucleation, when present, is associated with poor embryo potential to implant, but that its presence is more frequently observed in embryos derived from oocytes that at retrieval time were at MI stage, regardless of whether they matured into MIIs at ICSI. Furthermore, we demonstrated that embryo multinucleation does not correlate with the origin of the gametes used for ICSI, meaning that the use of donated sperm or oocytes did not modify the observation that as immaturity of the original oocytes increases, the incidence of embryo multinucleation also increases. Another notable issue is the evidence that the number of multinucleated cells per multinucleated embryo also increases significantly ($P<.0001$) with the use of MI–IIs and MIs, reflecting a deeper disarrangement in embryo development.

To the best of our knowledge, this is the first work comparing results obtained in the three oocyte categories (MII, MI–II, and MI) using the patients' own gametes and donated oocytes and sperm. The use of donated gametes from fertile donors did not modify the observed trend in all the variables evaluated.

Infertile women may have, among other problems, alterations in oocyte maturation that in time may result in higher percentages of multinucleation and lower pregnancy rates, but the use of donated oocytes or sperm did not modify this tendency. Other causes may contribute to the presence of high percentages of multinucleation in MI–II-derived embryos.

The fact of maturing in vitro could be the cause of observing a higher incidence of multinucleation even when donated gametes were used. This culture usually involves 2–5 hours' incubation before injecting at the same time MIIs and MI–IIs. Despite this short time, maturing MIs seemed to render a significantly higher incidence of multinucleation when compared with the in vivo–matured MIIs kept in the same culture condition as the maturing MIs.

In summary, stimulation protocols may play an important role in the incidence of embryo multinucleation in ICSI if a high proportion of MIs is obtained, regardless of whether they mature to MII stage (MI–II) at ICSI. It must be considered, as confirmed in this work, that MI–IIs behave quite differently in terms of normal and abnormal fertilization, percentage of arrested pronuclear stage, failure to undergo first mitotic division, embryo development, and pregnancy and implantation rates. Furthermore, they show a significant higher incidence of embryo multinucleation and multinucleated cells per embryo at day 2 than in vivo–matured MIIs. These in time may imply diminished embryo development potential and an evident failure to implant, as evidenced in the results presented here, and transfer of MI–II- and MI-derived embryos should be avoided whenever possible.

In addition, these results could help clinicians, if no pregnancy is achieved, when planning a second attempt for a specific patient. Perhaps a delay in the day of hCG and/or in the interval between hCG administration and aspiration time to allow in vivo maturation (22) could be an option to improve ICSI outcome and pregnancy by diminishing the rate of MIs obtained.

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SUPPLEMENTAL TABLE 1

Comparison of normal and abnormal fertilization in the different types of injected oocytes among the different groups.

Group of cycles (n = 500)	Oocyte maturity at ICSI	Normal fertilization, 69.6 (2,928/4,206)	Abnormal fertilization, 3 (127/4,206)
Subgroup A (n = 382)	Total	68.2 (2,166/3,177)	3.4 (107/3,177)
	MII	74.9 (1,677/2,237)[a]	2.6 (58/2,237)[b]
	MI–II	65.3 (340/521)[a]	3.8 (20/521)[b]
	MI	35.6 (149/419)[a]	6.9 (29/419)[b]
Subgroup B (n = 91)	Total	74.9 (590/788)	1.4 (11/788)
	MII	80.3 (510/635)[c]	0.9 (6/635)[d]
	MI–II	62.4 (63/101)[c]	2 (2/101)[d]
	MI	32.7 (17/52)[c]	5.8 (3/52)[d]
Subgroup C (n = 15)	Total	73.6 (103/140)	5.7 (8/140)
	MII	79 (79/100)[e]	7 (7/100)[f]
	MI–II	66.7 (20/30)[e]	3.3 (1/30)[f]
	MI	40 (4/10)[e]	0 (0/10)[f]
Subgroup D (n = 12)	Total	68.3 (69/101)	1 (1/101)
	MII	70.8 (51/72)[g]	1.4 (1/72)[h]
	MI–II	76.5 (13/17)[g]	0 (0/17)[h]
	MI	41.7 (5/12)[g]	0 (0/12)[h]

Note: Values are percentage (number). $P<.05$ was considered statistically significant as analyzed by χ^2 or Fisher exact test, as appropriate.

[a] $P<.0001$.
[b] $P<.0001$.
[c] $P<.0001$.
[d] $P<.05$.
[e] $P<.05$.
[f] Not statistically evaluated.
[g] Not significant.
[h] Not statistically evaluated.

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SUPPLEMENTAL TABLE 2

Pronuclear and syngamy arrest according to the maturity stage of injected oocytes in the different groups of cycles.

Group of cycle (n = 500)	Oocyte maturity at ICSI time	Arrested 2PN, 2.3 (64/2,726)	Syngamy stage, 1.6 (42/2,662)
Subgroup A (n = 382)	Total	2.5 (51/2,009)	1.6 (31/1,958)
	MII	1.5 (24/1,556)[a]	1.2 (18/1,532)[b]
	MI–II	4.8 (15/314)[a]	2.7 (8/299)[b]
	MI	8.6 (12/139)[a]	3.9 (5/127)[b]
Subgroup B (n = 91)	Total	1.9 (11/562)	1.4 (8/551)
	MII	1.8 (9/494)[c]	1 (5/485)[d]
	MI–II	1.9 (1/52)[c]	3.9 (2/51)[d]
	MI	6.2 (1/16)[c]	6.7 (1/15)[d]
Subgroup C (n = 15)	Total	0 (0/102)	1 (1/102)
	MII	0 (0/78)[e]	0 (0/78)[f]
	MI–II	0 (0/20)[e]	0 (0/20)[f]
	MI	0 (0/4)[e]	25 (1/4)[f]
Subgroup D (n = 12)	Total	3.8 (2/53)	1.9 (2/51)
	MII	2.6 (1/39)[g]	5.3 (2/38)[h]
	MI–II	0 (0/10)[g]	0 (0/10)[h]
	MI	25 (1/4)[g]	0 (0/3)[h]

Note: Values are percentage (number). $P<.05$ was considered statistically significant as analyzed by χ^2 or Fisher exact test, as appropriate.

[a] $P<.0001$.
[b] $P<.05$.
[c] Not significant.
[d] Not significant.
[e] Not statistically evaluated.
[f] Not statistically evaluated.
[g] Not statistically evaluated.
[h] Not statistically evaluated.

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SUPPLEMENTAL TABLE 3

Embryo development and quality at day 2 according to the maturity stage of injected oocytes in the different groups of cycles.

Group of cycles (n = 500)	Oocyte maturity at ICSI	Fragmented embryos	≤2 Cells	3–5 Cells	Accelerated cleavage	GEQ	IEQ	PEQ
Subgroup A (n = 382)	Total	0.6 (12/1,958)	24 (470/1,958)	60.5 (1,184/1,958)	1 (19/1,958)	75.2 (1,473/1,958)	22.2 (435/1,958)	1.2 (24/1,958)
	MII	0.3 (4/1,532)[a]	21.3 (327/1,532)[b]	62.7 (961/1,532)[c]	1 (15/1,532)	79.3 (1,215/1,532)[d]	19.1 (292/1,532)[e]	0.7 (11/1,532)[f]
	MI–II	1 (3/299)[a]	31.4 (94/299)[b]	54.5 (163/299)[c]	1 (3/299)	64.5 (193/299)[d]	30.8 (92/299)[e]	2.3 (7/299)[f]
	MI	4 (5/127)[a]	38.6 (49/127)[b]	47.2 (60/127)[c]	0.8 (1/127)	51.2 (65/127)[d]	40.1 (51/127)[e]	4.7 (6/127)[f]
Subgroup B (n = 91)	Total	0.5 (3/551)	7.8 (43.551)	72.2 (398/551)	0.9 (5/551)	85.3 (470/551)	12.2 (67/551)	1.1 (6/551)
	MII	0.6 (3/485)	7 (34/485)	73.8 (358/485)[g]	0.8 (4/485)	86.8 (421/485)[h]	11.1 (54/185)[i]	1 (5/485)
	MI–II	0 (0/51)	17.6 (9/51)	56.9 (29/51)[g]	2 (1/51)	78.4 (40/51)[h]	15.7 (8/51)[i]	2 (1/51)
	MI	0 (0/15)	0 (0/15)	73.3 (11/15)[g]	0 (0/15)	60 (9/15)[h]	33.3 (5/15)[i]	0 (0/15)
Subgroup C (n = 15)	Total	0 (0/102)	26.5 (27/102)	60.8 (62/102)	1 (1/102)	80.4 (81/102)	17.6 (18/102)	1 (1/102)
	MII	0 (0/78)	25.6 (20/78)	64.1 (50/78)	1.3 (1/78)	84.6 (66/78)	15.4 (12/78)	0 (0/78)
	MI–II	0 (0/20)	30 (6/20)	50 (10/20)	0 (0/20)	70 (14/20)	25 (5/20)	5 (1/20)
	MI	0 (0/4)	25 (1/4)	50 (2/4)	0 (0/4)	50 (2/4)	25 (1/4)	0 (0/4)
Subgroup D (n = 12)	Total	0 (0/51)	21.6 (11/51)	60.8 (31/51)	0 (0/51)	84.3 (43/51)	13.7 (7/51)	0 (0/51)
	MII	0 (0/39)	25.6 (10/39)	59 (23/39)	0 (0/39)	89.7 (35/39)	7.7 (3/39)	0 (0/39)
	MI–II	0 (0/9)	0 (0/9)	66.7 (6/9)	0 (0/9)	66.7 (6/9)	33.3 (3/9)	0 (0/9)
	MI	0 (0/3)	33.3 (1/3)	66.7 (2/3)	0 (0/3)	66.7 (2/3)	50 (1/2)	0 (0/2)

Note: Values are percentage (number). $P < .05$ was considered statistically significant as analyzed by χ^2 or Fisher exact test, as appropriate. GEQ = good embryo quality; IEQ = intermediate embryo quality; PEQ = poor embryo quality.

[a] Not statistically evaluated.

[b] $P < .0001$.

[c] $P < .001$.

[d] $P < .0001$.

[e] $P < .0001$.

[f] $P < .0001$.

[g,h,i] Not significant; all the other values could not be statistically evaluated owing to small sample size.

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SUPPLEMENTAL TABLE 4

Comparison of embryo multinucleation and multinucleated cells in the different types of oocytes injected among the different groups of cycles.

Group of cycles (n = 500)	Oocyte maturity at ICSI	Embryo multinucleation, 11.2 (292/2,605)	Cell multinucleation 4.2 (386/9,190)
Subgroup A (n = 382)	Total	12.3 (236/1,915)	4.7 (308/6,543)
	MII	8.5 (129/1,510)[a]	3 (159/5,238)[b]
	MI–II	22.6 (65/288)[a]	9.1 (85/938)[b]
	MI	35.9 (42/117)[a]	17.4 (64/367)[b]
Subgroup B (n = 91)	Total	8.3 (45/540)	3 (63/2,106)
	MII	6.1 (29/477)[c]	2.1 (40/1,864)[d]
	MI–II	30.6 (15/49)[c]	11.2 (21/187)[d]
	MI	7.1 (1/14)[c]	3.6 (2/55)[d]
Subgroup C (n = 15)	Total	6.9 (7/101)	2.5 (9/356)
	MII	5.1 (4/78)[e]	1.8 (5/274)[f]
	MI–II	10 (2/20)[e]	4.2 (3/72)[f]
	MI	33.3 (1/3)[e]	10 (1/10)[f]
Subgroup D (n = 12)	Total	8.2 (4/49)	3.2 (6/185)
	MII	5.6 (2/36)[g]	2.3 (3/131)[h]
	MI–II	10 (1/10)[g]	2.3 (1/44)[h]
	MI	33.3 (1/3)[g]	20 (2/10)[h]

Note: Values are percentage (number). $P<.05$ was considered statistically significant as analyzed by χ^2 or Fisher exact test, as appropriate.

[a] $P<.0001$.
[b] $P<.0001$.
[c] Not statistically evaluated.
[d] $P<.0001$.
[e,f,g,h] Not statistically evaluated.

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