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

Sabrina De Vincentiis, M.S.,[a] Evelyn De Martino, M.S.,[a] Mariano G. Buffone, Ph.D.,[a,b] and Santiago Brugo-Olmedo, M.D.[a]

[a] Centro Médico Seremas, Buenos Aires, Argentina; and [b] Instituto de Biología y Medicina Experimental, National Research Council of Argentina (CONICET), Buenos Aires, Argentina

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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Reprint requests: Sabrina De Vincentiis, M.S., Centro Médico Seremas, Arenales 1954, 1st Floor (C1124AAN), Buenos Aires, Argentina (E-mail: sdv@seremas.com).

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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.