ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Artificial oocyte activation with calcium ionophore does not cause a widespread increase in chromosome segregation errors in the second meiotic division of the oocyte

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Objective: To study the effect of artificial oocyte activation (AOA) on chromosome segregation errors in the meiotic divisions.
Design: Prospective cohort study with historical control.
Setting: Private/academic IVF centers.
Patient(s): Fifty-six metaphase II oocytes were donated from 12 patients who had undergone IVF between June 2008 and May 2009.
Intervention(s): Oocytes were activated by 40 minutes' exposure to 100 μM calcium-ionophore. The activated oocyte was tubed and analyzed by array comparative genomic hybridization and/or single-nucleotide polymorphism genotyping and maternal haplotyping (meiomapping). A control sample of embryos derived from normally fertilized oocytes was included for comparison.
Main Outcome Measure(s): Incidence of chromosome segregation errors in artificially activated and normally fertilized oocytes in relation to pronuclear evaluation.
Result(s): Of 49 oocytes that survived the warming procedure, thirty-nine (79.6%) activated. Most activated normally, resulting in extrusion of the second polar body and formation of a single or no pronucleus (2PB1PN: 30 of 39, 76.9%; or 2PB0PN: 5 of 39, 12.8%). Twenty-seven of these were analyzed, and 16 (59.3%) were euploid, showing no effect of AOA on meiotic segregation. Single-nucleotide polymorphism analysis of normally activated oocytes confirmed normal segregation of maternal chromosomes. No difference in the proportion of meiosis II type errors was observed between artificially activated oocytes (28.6%; 95% confidence interval 3.7%–71.0%) compared with embryos obtained from normally fertilized oocytes (44.4%; 95% confidence interval 13.7%–78.8%). The abnormally activated oocytes, with $\geq$2PN (4 of 39, 10.3%) were diploid, indicating a failure to coordinate telophase of meiosis II with polar body extrusion.
Conclusion(s): From this preliminary dataset, there is no evidence that AOA causes a widespread increase in chromosome segregation errors in meiosis II. However, we recommend that it be applied selectively to patients with specific indications. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)

Key Words: Calcium ionophore, chromosome segregation, fertilization failure, meiosis, oocyte activation

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Failure of fertilization resulting in few or no embryos for transfer continues to be a significant clinical challenge for a minority of patients undergoing IVF. The introduction of intracytoplasmic sperm injection (ICSI) in the early 1990s significantly improved the clinical outcome for patients with male factor infertility, particularly those with low sperm counts, who could not achieve normal levels of fertilization with conventional IVF (1). Typical fertilization rates with ICSI average 70% for most patients, including those with poor semen parameters or surgically retrieved sperm. However, a significant proportion of ICSI cycles still results in fertilization rates below 50% (2), with between 1% and 4% resulting in total failed fertilization (TFF) (3–5). Although ICSI is invasive, and operator-dependent factors may contribute to the proportion of oocytes not fertilizing normally, most commonly the failure of an oocyte to fertilize after sperm injection is failure of oocyte activation (6–8). In most mammals the mature ovulated oocyte is arrested in metaphase of the second meiotic division (meiosis II) until fertilization by a sperm. Sperm binding with the oolemma activates the oocyte, triggering a series of pulsatile increases in intracellular calcium concentration, which in turn results in the resumption and completion of meiosis II, extrusion of the second polar body (PB2), and the initiation of preimplantation development (9). Phospholipase C, zeta 1 (PLCζ), a sperm-specific phospholipase, is considered the trigger for the molecular pathway within the oocyte, resulting in the release of calcium stores from the endoplasmic reticulum (10). Recent studies have shown that fertilization failure after ICSI can be linked to sperm devoid of PLCζ or sperm with abnormal PLCζ function (11–13). It has also been demonstrated that oocyte factors as well as sperm factors are involved in failed fertilization after ICSI (14).

Artificially increasing intracellular calcium with a variety of stimuli, from a brief exposure to low concentrations of ethanol to calcium ionophore exposure to allow the influx of calcium ions from the medium, triggers oocyte activation in several mammalian species (15, 16). Artificial oocyte activation (AOA) can be induced by electrical stimulation (17, 18) and a variety of chemical substances. Most commonly, AOA is induced by chemical agents, including 6-dimethylaminopurine, strontium chloride, or calcium ionophores, such as ionomycin and calcimycin. Exposure to a medium containing a calcium ionophore is the most commonly used method for AOA in clinical trials.

In assisted conception, AOA with calcium ionophore has been used clinically in cases of failed fertilization after ICSI, resulting in completion of normal fertilization in a significant proportion of oocytes and live births after ET (19). Indeed, there is evidence to show that AOA can overcome both oocyte- and sperm-related failed fertilization (20).

However, information on the effect of AOA and its biosafety is limited to clinical follow-up of a small number of children conceived using the technique, which demonstrated that their early development is within the expected normal range (21). Additionally, because of the abnormal, sustained increase in intracellular calcium concentration, which may have effects on downstream molecular events, it has been argued that AOA should only be used in failed fertilization cases and not as a routine adjuvant to ICSI (22) or when a specific indication is present, such as globozoospermia (23) or PLCζ deficiency (11–13).

To address the limited information on biosafety, particularly in terms of potential genetic effects, here we have investigated the effect of AOA with calcium ionophore on the incidence of female meiotic errors resulting in abnormal chromosome copy number, or aneuploidy, in the activated oocytes. Chromosome copy number was analyzed by array comparative genomic hybridization (aCGH) and combined with genome-wide single-nucleotide polymorphism (SNP) genotyping of the oocyte donors and oocytes to identify the meiotic origin of any chromosome segregation errors, with a specific focus on errors occurring in the second meiotic division (meiosis II). Because all of the oocytes in the study had completed the first meiotic division (meiosis I), extruded the first polar body (PB1), and were arrested in metaphase of meiosis II, before activation, any effect of exposure to calcium ionophore should only affect the segregation of chromosomes at anaphase of meiosis II after resumption of meiosis.

MATERIALS AND METHODS

Patients and Ethical Approval

All oocytes for the study were obtained from 12 patients who had undergone IVF treatment at the Center for Reproductive Medicine GENERA in Rome between June 2008 and May 2009 (Table 1). According to Italian law at the time of the patient's IVF cycles, a maximum of three oocytes could be inseminated per patient, and any surplus mature oocytes were vitrified. Surplus vitrified oocytes were later recruited for the study after informed consent was obtained from the patients. Consent was also obtained from all donors to obtain buccal cell swabs for genotyping. The study was approved by the institutional review board of the Clinica Valle Giulia, where the oocytes were stored and processed for the study.

Cleavage-stage embryos derived from normally fertilized oocytes and analyzed with the same meiomapping method (24) were used for comparison with chromosome analysis of artificially activated oocytes. The limited number of oocytes available for activation and restrictions on creating embryos for research purposes in their country of origin prevented analysis of a normally fertilized control group using oocytes from the same donors.

Oocyte Collection, Vitrification, and Warming

Ovarian hyperstimulation was achieved using long down-regulation agonist or standard antagonist protocols, and transvaginal oocyte collection was performed 35 hours after hCG administration. The vitrification and warming procedures were performed according to a published protocol (25), using commercially available vitrification and warming kits (Kitazato BioPharma). Vitrification was performed a maximum of 40 hours after hCG administration, and the oocytes were stored on Cryotop vitrification tools (Kitazato BioPharma) in liquid nitrogen.

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