Effect of two assisted oocyte activation protocols used to overcome fertilization failure on the activation potential and calcium releasing pattern

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Objective: To assess the effect of two assisted oocyte activation (AOA) protocols with the use of two calcium (Ca^{2+}) ionophores, ionomycin and A23187 (calcimycin), on the intracellular Ca^{2+} level in mouse and human oocytes and the fertilization rates.
Design: Comparison of two Ca^{2+} ionophores, ionomycin and A23187, regarding their capacity to increase the intracellular Ca^{2+} level and to support subsequent oocyte activation and development.
Setting: University hospital research laboratory.
Patient(s)/Animal(s): Patients undergoing intracytoplasmic sperm injection (ICSI) treatment and B6D2F1 mice.
Intervention(s): Assisted oocyte activation and microinjection of mouse and human oocytes with sperm.
Main Outcome Measure(s): Measurement of the fertilizing and Ca^{2+}-releasing ability of human sperm.
Result(s): Ionomycin was more potent than A23187 in provoking Ca^{2+} increases in both mouse and human oocytes with significantly higher amplitude and area under the receiver operating characteristic curve. The oocyte activation rate was significantly higher when mouse oocytes were activated with the use of the ionomycin- rather than the A23187-based AOA protocol. Furthermore, oocyte activation rate was higher when human in vitro matured oocytes were activated with the ionomycin-based AOA protocol, but the difference did not reach statistical significance.
Conclusion(s): In both mouse and human oocytes, the AOA protocol that used ionomycin was more efficient than the one that used A23187. Bearing in mind that mammalian fertilization is successful when the total dose of Ca^{2+} released reaches a minimal threshold, the use of ionomycin for human AOA might be justified instead of the use of A23187. (Fertil Steril® 2016;105:798–806. ©2016 by American Society for Reproductive Medicine.)
Key Words: Assisted oocyte activation, fertilization failure, calcium ionophore, ionomycin, A23187

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With the advent of intracytoplasmic sperm injection (ICSI), most cases of severe male factor infertility, failed in vitro fertilization (IVF), or unexplained infertility can now be treated. Although ICSI is a remarkably efficient technique, leading to fertilization rates of 70%–80% (1, 2), total fertilization failure still occurs in ~1%–3% of ICSI cycles and can reoccur in subsequent cycles (3–5). Fertilization failure after ICSI is typically the result of an oocyte activation deficiency (2, 4, 6–8). Successful oocyte activation relies on both sperm- and oocyte-borne factors and is mediated by characteristic oscillations in cytoplasmic Ca^{2+} concentration in the oocyte which are triggered by the sperm factor phospholipase C zeta (3, 9, 10). The intracellular Ca^{2+}

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release from the intracellular stores via the inositol 1,4,5-trisphosphate (IP_3) receptor, is essential for the timely completion of all subsequent oocyte activation events, which include the cortical granule exocytosis to block polyspermy, resumption and completion of meiosis, and pronuclei formation (11). Recent studies suggest that a postacrosomal sperm protein found in the perinuclear theca, the postacrosomal WW-binding protein (PAWP), elicits Ca^{2+} oscillations similar to those seen during fertilization in mammalian and nonmammalian oocytes, and that there is a significant correlation between PAWP levels in human sperm and fertilization rates (12–15). However, a recent study failed to demonstrate Ca^{2+} oscillations in mouse oocytes in response to the injection of recombinant PAWP (16).

Heterologous ICSI models have been used to distinguish between sperm- and oocyte-related deficiencies in couples faced with failed fertilization after ICSI, with the most common being the mouse oocyte activation test (MOAT) (10, 17–20). Recently, the added value of performing mouse oocyte Ca^{2+} analysis to reveal activation deficiencies has been demonstrated (21). Although the MOAT and similar tests are considered to be reliable for oocyte activation problems, they can be routinely applied in only a few clinics owing to the need for animal oocytes, special equipment, and other technical limitations (22).

After appropriate counseling, assisted oocyte activation (AOA) is often offered to couples dealing with fertilization failure after ICSI. Several chemical, mechanical, or physical stimuli can be applied to promote oocyte activation during a subsequent ICSI cycle to overcome failed fertilization (22). Previous studies have reported an increase in fertilization rates and cleavage-stage embryos with the application of AOA protocols (23). The most commonly used AOA protocols involve the use of Ca^{2+} ionophores (19, 22, 24–27), in addition to protocols using strontium (28, 29), a modified ICSI technique (10, 30), or electric pulses (31, 32).

The most widespread AOA agents used in human assisted reproductive technology (ART) are two Ca^{2+}-selective ionophores: ionomycin and A23187 (also known as calcimycin) (3). These ionophores can induce Ca^{2+} influx by altering the plasma membrane permeability or can act directly on intracellular organelles which release Ca^{2+} (33, 34). Studies in rat liver mitochondria and starfish oocytes showed that ionomycin is a more potent and more specific Ca^{2+} ionophore than A23187 (35, 36). When ionomycin is used as an AOA agent, the reported fertilization rates are usually higher than with the use of A23187. Apart from their inherent differences, the discrepancies in the efficiency of these AOA protocols might also be due to the fertility background of the patients, the number of included patients and the specific AOA protocols used, which diverge fundamentally (18, 19, 24, 25, 27, 37–45). For example, different Ca^{2+} ionophore concentrations have been used (5 μmol/L, 10 μmol/L, or unknown), and the times and duration of ionophore exposure have differed (one- or twofold, lasting 10, 15, or 30 minutes each), as has the timing of ionophore exposure (immediately after or 30 minutes after ICSI). Additionally, some groups have described the concomitant injection of calcium chloride ($CaCl_2$) during ICSI in their AOA protocol (19, 27). Recently, a ready-to-use A23187 solution (GM508 Cult-Active; Gynemed) has been commercialized for ART use, but the exact concentration of A23187 is not disclosed (24).

The main aim of the present study was to compare the intracellular Ca^{2+} response, oocyte activation, and embryonic developmental potential following the application of ionomycin and ready-to-use A23187 solution in mouse and human oocytes.

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich (Diegem, Belgium) unless otherwise specified.

Ethical Approval

The study was approved by the local Ethical Committee of Ghent University Hospital (2009/130, 2010/808, and 2010/182) and by the Belgian Federal Ethical Committee (Adv020), and written informed consent was obtained from patients undergoing controlled ovarian hyperstimulation for ICSI treatment. All procedures involving handling and killing the animals were approved by the Ghent University Hospital Ethical Committee for Laboratory Animals (ECD no. 09/15).

Source of Mouse Oocytes

Metaphase II (MII) mouse oocytes were collected by priming 6–10-week-old B6D2F1 hybrid female mice with the use of 5 IU/mL pregnant mare serum gonadotropin (Folligon; Intervet) followed by 5 IU/mL hCG (Chorulon; Intervet) 48 hours later. MII oocytes were collected 13–14 hours after hCG injection and freed from their cumulus cells with the use of brief exposure to 200 IU/mL hyaluronidase. Culture and manipulation media were potassium simplex–optimized medium (KSOM) and HEPES-buffered KSOM (KSOM-HEPES), respectively, which were supplemented with 4 mg/mL bovine serum albumin (BSA; Calbiochem) at 37°C under 6% CO_2, 5% O_2, and 89% N_2.

Source of Human Oocytes

Immature oocytes from patients undergoing ICSI/IVF treatment at the Ghent University Hospital were used in the study. Women were administered a GnRH agonist (Decapeptyl; Ferring) or antagonist (Cetrotide; Merck Serono). Ovarian stimulation was performed with the use of hMG (Menopur; Ferring) or recombinant FSH (Gonal-F; Merck Serono) at a dose of 112.5–300 IU daily under ultrasonographic and biochemical monitoring, and ovulation was induced with the use of 5,000 IU hCG (Pregnyl; MSD). Oocytes were enzymatically denuded by means of brief exposure to 80 IU/mL hyaluronidase (Cook Medical) and then mechanically denuded. Donated oocytes that had failed to mature after controlled ovarian hyperstimulation and were at the MI stage (defined by the absence of both a polar body and a germinal vesicle structure) were cultured in Sydney IVF Cleavage medium (CC) (Cook Ireland) at 37°C under 6% CO_2, 5% O_2, and 89% N_2. Oocytes with a polar body extruded within 3 hours of