Does the use of calcium ionophore during artificial oocyte activation demonstrate an effect on pregnancy rate? A meta-analysis

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Objective: To study the effect, if any, of calcium ionophore as a method of artificial oocyte activation (AOA) on pregnancy outcomes and fertilization rates.
Design: Meta-analysis of randomized controlled trials, prospective observational and retrospective trials, case reports, and a case-control trial.
Setting: University-affiliated teaching hospital.
Patient(s): Infertile couples undergoing fertilization treatment.
Intervention(s): Use of calcium ionophore during AOA.
Main Outcome Measure(s): Odds ratio (OR) as the summary statistic for binary variables was used. Both a fixed and random effects model were applied. Subgroup analysis using quantitative methodology (risk of bias, metaregression) and graphical comparison (funnel plot) assessed statistical heterogeneity.
Result(s): Fourteen studies were selected. AOA with calcium ionophore increased the overall clinical pregnancy rate (per ET; OR = 3.48; 95% confidence interval [CI], 1.65–7.37) and the live birth rate (OR = 3.33; 95% CI, 1.50–7.39). This effect of adding calcium ionophore was further demonstrated with fertilization, cleavage, blastocyst, and implantation rates. Subgroup analysis further supported our findings (studies where $n > 10$ in both arms; random and fixed effects models). A metaregression (beta = –.145) found that as the quality of the study increases, the effect of calcium ionophore is significantly more pronounced with regards to overall pregnancy rate.
Conclusion(s): AOA with calcium ionophore treatment after intracytoplasmic sperm injection (ICSI) results in a statistically significant improvement in fertilization, cleavage, blastulation, and implantation rates, as well as overall pregnancy and live-birth rates. The conclusion of this systematic review, demonstrating a strong effect of calcium ionophore use, is reassuring and promising, particularly for couples for whom ICSI alone yields poor fertilization rates. (Fertil Steril® 2017;108:468–82. ©2017 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, artificial oocyte activation, calcium ionophore, pregnancy, live birth

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The development and introduction of intracytoplasmic sperm injection (ICSI) to treat male factor infertility has transformed the field of assisted reproduction (1). This has allowed fertilization even in couples with severe male factor infertility. The method bypasses all anatomical structures and potential pitfalls by depositing a single sperm into the oocyte cytoplasm. ICSI generates high fertilization rates of up to 70%–80% (2). A small percentage of individuals, however, despite repeated ICSI, continue to face repeated fertilization failure. This is even with normal sperm parameters and a good ovarian response (3, 4). The persistent fertilization failure raises the question of an underlying cause and whether other factors, aside from simply being located within the oocyte cytoplasm, may play a role in fertilization.

The physiological agent of oocyte activation has been identified as a sperm-borne phospholipase Cz (PLCz) (5). During uncomplicated fertilization, PLCz enters the oocyte cytoplasm and cleaves membrane-bound phosphatidylinositol biphosphate (PIP_2). This yields diacylglycerol (initiates zona reaction) and inositol triphosphate (IP_3). IP3 subsequently binds to its receptors located at the endoplasmic reticulum, which causes calcium release from this internal store (6). Sperm-induced Ca^{2+} oscillations stimulate mitochondrial respiration and, in turn, the resulting adenosine triphosphate production required to maintain sperm-triggered calcium waves. Any deficiency in this pathway, leading to a reduction in these essential biochemical substances (PLCz, PIP_2, IP_3), will cause a decrease in intracellular calcium and the absence of Ca^{2+} oscillations. A physiological (7) or artificial (8) lack of calcium leads to embryo arrest or cleavage anomalies. This obvious drawback can be compensated by artificially increasing calcium in the oocyte and, thus, inducing oocyte activation.

To combat persistent fertilization failure after ICSI, modified techniques have successfully been applied to obtain fertilization (4, 9). Electrical activation is one method, although not frequently applied (10, 11). More commonly, artificial oocyte activation (AOA) is induced by a variety of chemical agents, such as 6-dimethylaminopurine, strontium chloride, or calcium ionophores, such as ionomycin and calcimycin (A23187). A deficiency in intracellular calcium can be compensated by approaches that aim for an artificial calcium entrance or release. The potential of calcium ionophore to support AOA and yield high fertilization rates was shown at the beginning of the era of ICSI (12). Since that time, a number of studies have been devised and carried out to assess the value of calcium ionophores as a method of AOA in humans.

This meta-analysis aimed to assess the effect, if any, of calcium ionophore as a method of AOA on pregnancy outcomes and fertilization rates.

MATERIALS AND METHODS

The meta-analysis was done according to PRISMA guidelines. A literature search was performed using Medline, Embase, the Cochrane Library, and Google Scholar databases for comparative studies until and including December 2016 to investigate the effect of calcium ionophore on pregnancy, live-birth, fertilization, cleavage, blastulation, top-quality embryo, implantation, and multiple pregnancy rates. The following MESH search headings were used: fertility, infertility, pregnancy, birth, miscarriage, embryo quality, calcium ionophore, A23187, calcimycin, fertility agents, female, infertility treatment, AOA, intracytoplasmic sperm injection/ICSI, and in vitro fertilization/IVF. The "related articles" function was used to broaden the search, and all citations identified were reviewed, irrespective of language. Using these strategies, studies comparing infertility patient groups who did and did not undergo AOA with calcium ionophore were identified, and data regarding the outcome of interest (pregnancy outcomes) were extracted. The search strategy and included studies are shown in Figure 1.

Data Extraction

Two reviewers (S.S. and S.M.) independently extracted the data from each study. Quantitative data were extracted as follows: logistics (first author, year of publication, study design, study period, study country); study groups (number of AOA patients with calcium ionophore vs. non-AOA patients); and the following fertility-related rates: fertilization, cleavage, blastulation, top-quality embryo, implantation, pregnancy, live birth, and miscarriage. These factors are shown in Table 1. Also added to the same table was the AOA protocol for each study. Patient characteristics were also extracted: mean age, type of infertility, sperm parameters and source, and factors specific to fertility (fertility drugs used, number of stimulated cycles, mean number of oocytes per stimulated cycle, and previous outcome). Supplemental Table 1 lists these factors. Inclusion, exclusion, and matching criteria are listed in Supplemental Table 2.

Inclusion and Exclusion Criteria

All comparative studies of "AOA with calcium ionophore" versus "no AOA" patient groups reporting the incidence of pregnancy outcomes were included. Studies that defined fertility treatment as AOA with calcium ionophore and those that used the term in a much broader sense to include also IVF and non-IVF therapy were included. The term "fertility treatment" refers here specifically to patients who were administered fertility drugs to induce multiple folliculogenesis, also known as superovulation, as part of their routine IVF treatment.

Those studies not reporting pregnancy outcome incidence either at all or separately in the AOA and non-AOA treatment groups were excluded. Finally, natural cycle IVF with no stimulation was not included.

Outcomes of Interest

The primary outcome of interest was the overall pregnancy rate incidence in all "AOA with calcium ionophore" versus "no AOA" (AOA with calcium ionophore vs. no AOA) patient groups. Overall pregnancy is defined as pregnancy at all points during gestation: from positive pregnancy test to live birth. Data are reported as per ET and as per cycle. Secondary outcomes of interest were the following incidence rates in the