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Oocyte activation by calcium ionophore and congenital birth defects: a retrospective cohort study

Q2 Netanella Miller, M.D.,[a] Tal Biron-Shental, M.D.,[a,b] Rivka Sukenik-Halevy, M.D.,[a,b,c] Anat Hershko Klement,[a,b]
Q1 Reuven Sharony, M.D.,[a,b] and Arie Berkovitz, M.D.[a,b,d]

[a] Department of Obstetrics and Gynecology, Meir Medical Center, Kfar-Saba; [b] Sakler School of Medicine, Tel Aviv University, Tel Aviv; [c] Genetic Institute, Meir Medical Center, Kfar-Saba; and [d] Assuta Medical Center, Tel Aviv, Israel

Objective: To evaluate the safety of oocyte activation by calcium ionophore in cases of failed fertilization after intracytoplasmic sperm injection (ICSI) procedure with respect to birth defects.
Design: A retrospective cohort of pregnancies achieved by oocyte activation with calcium ionophore after ICSI (ICSI-Ca) and routine ICSI between the years 2006 and 2014.
Setting: Not applicable.
Patient(s): The cohort included a total of 793 pregnancies: 66 (8%) were lost to follow up and 49 (6%) were ongoing pregnancies at the time of data collection. Out of the 678 available cases for analysis, 595 treatments were ICSI alone (88%) and 83 were ICSI-Ca (12%).
Intervention(s): None.
Main Outcome Measure(s): Pregnancy and neonatal outcome including birth defects were compared.
Result(s): On the basis of a cohort of 595 ICSI pregnancies and 83 ICSI-Ca pregnancies, we found no difference in birth defects rate for singletons or for twins. Additionally, no significant difference was found between defect type (chromosomal aberration or structural malformations) and malformation type (heart, urogenital, and limb), between the ICSI and ICSI-Ca groups. Moreover, no significant differences were found regarding birth weight, gestational week at time of delivery, and fetal gender for singleton or twin pregnancies.
Conclusion(s): Ca ionophore oocyte activation should be considered as a legitimate option for cases of failed or low fertilization by ICSI. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Intracytoplasmic sperm injection, ICSI, artificial oocyte activation, AOA, congenital defects

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Intracytoplasmic sperm injection (ICSI) is a well-known technique in which a single sperm is injected directly into the cytoplasm of a mature oocyte. This procedure provides an effective method for assisting fertilization in males with suboptimal semen parameters (1). The average fertilization rate after ICSI is approximately 70%, while total fertilization failure occurs in 3% of ICSI cycles (2–4). Failed fertilization after ICSI procedure can be related to failure of oocyte activation or abnormal morphology of the oocyte (5).

Oocyte activation is a crucial process after sperm-oocyte fusion. It is assumed that calcium oscillations that induce a rise in the intracellular calcium levels in the oocyte, in vivo, are responsible for the cytological changes in fertilized oocytes (6). Progressive decline and eventual termination of calcium oscillations typically occur when pronuclei are formed (7). Thus, calcium is recognized as essential for triggering all downstream nuclear and cytoplasmic changes in fertilized oocytes, leading to successful oocyte activation and the onset of embryogenesis (8).

One of the artificial oocyte activation (AOA) methods uses calcium ionophore to induce oocyte activation. Montag et al. found that AOA using calcium ionophore increases fertilization rates and clinical pregnancy rates per ET, especially in patients with a compromised fertilization rate <30% in a standard ICSI cycle (9). Vanden Meerschaut et al. in a prospective study showed that ICSI-AOA is highly efficient in some patients with a suspected oocyte-related activation deficiency after previous conventional ICSI (10).

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Reprint requests: Netanella Miller, M.D., 59 Tsharnichovski, Kfar Saba, Israel (E-mail: millerne@me.com).

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An important issue regarding the AOA methods concerns its safety. There is a paucity of data regarding the possible adverse effects of calcium ionophore on postimplantation embryo and neonatal outcomes. One retrospective study found no congenital defects in 38 children conceived after ICSI and AOA procedures (11). Takisawa et al. also found no difference regarding growth and health parameters of 10 babies at birth between both AOA protocols compared with ICSI (12). A recent study assessed the long-term neurodevelopmental outcome of 21 children ages 3–10 years who had been born after AOA. The children were assessed on neurodevelopmental, intelligence, language, and social communication tests, and for all parameters the mean outcomes lay within the expected range (13).

However, all these aforementioned studies were based on a relatively small number of cases. Furthermore, none of these studies included an evaluation of the presence and type of birth defects during the course of pregnancy or their effect on pregnancy outcome.

The primary aim of our study was to evaluate the safety of AOA with the calcium ionophore after ICSI (ICSI-Ca) procedure compared with the routine ICSI procedure regarding pregnancy outcome and congenital birth defects. Data collection was done during 8 consecutive years. To the best of our knowledge, this is the largest study performed in an attempt to assess the safety of calcium ionophore for oocyte activation regarding birth defects. Furthermore, this is the first study to include an assessment of the specific types of birth defects per procedure, both structural malformations and chromosomal aberrations.

MATERIALS AND METHODS

Study Design

A retrospective cohort of pregnancies achieved by oocyte activation with ICSI-Ca and routine ICSI between the years 2006 and 2014 was evaluated.

Patients

Data were collected from a single outpatient fertility IVF clinic's records (Assuta Medical Center). All couples in our cohort had primary infertility, and before treatment initiation they received genetic counseling according to the Israeli Ministry of Health recommendations. Couples presenting male factor infertility were routinely offered micromanipulation of the ova according to the following criteria: failed fertilization or <20% fertilization in a previous IVF cycle or a post-wash total motile count of <1.5 million. In these circumstances, the patients were offered ICSI.

Patients who had failed fertilization after one ICSI procedure in the presence of at least five mature oocytes without oocyte abnormality (e.g., fragmented polar bodies, vesiculated cytoplasm, wide previtelline space, or debris) or had <10% fertilization rate were offered the AOA ICSI-Ca procedure.

As part of the clinic policy and according to the Israeli Ministry of Health regulations, patients undergo routine follow-up until the day of delivery. Each patient was contacted by phone, and admission summary letters were requested from all patients who had a live birth and also from all patients who experienced pregnancy outcomes other than a live birth including termination of pregnancy (TOP), miscarriage, intrauterine fetal death (IUFD), intrapartum death, and selective termination in a multiple embryo pregnancy. Relevant information was collected from these letters including date of birth, number of newborns, birth weight(s), and any hospitalization required during pregnancy as well as any malformations detected during the pregnancy or after delivery or other medical problems.

All cycles resulting in a pregnancy between January 2006 and December 2014 were included. We compared fetal/birth defect rates, malformations, and chromosomal abnormalities between patients who were treated by ICSI and patients treated by ICSI-Ca during the study period. The analysis combined fetal defects (defects that were detected during the pregnancy by ultrasound or by amniocentesis) and congenital abnormalities detected after birth. We excluded cases of egg donation and sperm donation, since investigation of these cases is limited, due to lack of full access to the donor's medical background.

Ethics Approval

The study was approved by the institutional ethicsreview board.

ICSI, ICSI-Ca

The ICSI procedure has been discussed elsewhere (14). ICSI-Ca is a procedure in which immediately after ICSI, the injected eggs are exposed to 10 μM of Ca++ ionophore A23187 (Sigma Chemical) in culture medium for 10 minutes in an incubator at 37°C and 5%–6% CO_2. Then the oocytes are washed thoroughly in flushing or culture medium (Origio).

In both ICSI and ICSI-CA groups, cleaved embryos, none at blastocyst stage, were transferred on day 3.

Stimulation Protocols

Two protocols were used for egg stimulation in both the ICSI and ICSI-Ca procedures: the GnRH agonist triptorelin (Decapeptyl) long protocol or GnRH antagonist protocol.

Fetal and Birth Defects and Chromosomal Aneuploidy

Malformations are defined as defects of organs or body parts due to an intrinsically abnormal developmental process. In this process, a structure is not formed, is partially formed, or is formed in an abnormal fashion. Chromosomal aberrations are either numerical abnormalities or structural defects in the chromosomes. Abnormal chromosomal analysis can be straightforward in terms of interpretation and prognosis, while in some cases it requires an additional work-up in order to provide more accurate counseling.

Data regarding malformations reported in our cohort and any chromosomal aberrations detected in this population were collected through patients' reports as well as from