Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection

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Objective: To describe a successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection (ICSI).

Design: Case report.

Setting: In vitro fertilization unit in a university affiliated medical center.

Patient(s): A couple with 5 years of unexplained primary infertility who had repeated failed fertilization after ICSI.

Intervention(s): Controlled ovarian hyperstimulation, oocyte pick-up, ICSI, assisted oocyte activation with calcium ionophore, embryo culture, and ET.

Main Outcome Measure(s): Fertilization rate, implantation, pregnancy, and delivery.

Result(s): Assisted oocyte activation with calcium ionophore A23187 after ICSI resulted in reasonable fertilization rates in three cycles (4/6, 5/16 and 7/20 oocytes). Two pregnancies were achieved; the first ended with second trimester miscarriage due to fetal anomaly and the second with a delivery of three healthy babies.

Conclusion(s): Calcium ionophore oocyte activation seems to be a useful method in cases of repeated failed fertilization after ICSI. (Fertil Steril ©2003;79(Suppl 3):1656 – 8. ©2003 by American Society for Reproductive Medicine.)

Key Words: Calcium ionophore, failed fertilization, ICSI, oocyte activation

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rates, particularly in cases of 100% globozoospermia (10–12). We present a couple with prolonged unexplained infertility with repeated total ICSI fertilization failure, despite good ovarian response and normal sperm parameters. Successful pregnancy and delivery have been achieved after calcium ionophore oocyte activation.

CASE REPORT

A 28-year-old woman and her 30-year-old husband presented with primary infertility of 5 years duration. Both husband and wife were healthy and physically normal. There was no history of infertility or chronic illnesses within their families. They were not receiving any medications or drugs. The fertility workup of the wife was completely normal, including day 3 and 21 hormonal profiles, hysterosalpingography, pelvic ultrasonography, laparoscopy, and hysteroscopy. Semen analyses revealed volume of 2.5–4.5 mL, sperm counts of 52–100 × 10^6/mL with 40%–70% motility (most graded as 3–4, with grade 4 being excellent), and 12%–15% normal forms (strict criteria). None of the semen analyses revealed globozoospermia.

Previous treatment included four cycles with clomiphene citrate and four cycles with menotropins superovulation combined with IUI.

A diagnosis of extended unexplained infertility requested us to attempt assisted reproductive techniques. The patient was treated with the long down-regulation protocol consisting of Decapeptyl (0.1 mg/d SC; Ferring LTD, Herzliya, Israel) starting in the midluteal phase and a daily dose of 300 IU of pure FSH (Metrodin; Teva, Petah Tikva, Israel). Seventeen oocytes were retrieved, all with morphologically mature oocyte–cumulus complex. Our policy in cases of prolonged unexplained infertility and good ovarian response is to perform ordinary IVF and ICSI split, to eliminate fertilization failure. ICSI was performed on nine oocytes and four embryos were transferred at 72 hours, but the patient did not conceive. In the fourth assisted reproductive techniques cycle, 20 MII oocytes were fertilized with ICSI, followed by calcium ionophore oocyte activation. Seven oocytes were fertilized, and four embryos were transferred at 72 hours, but the patient did not conceive. In the fifth cycle, 5 of 16 MII oocytes had fertilized using the ICSI–calcium ionophore method. Four six- to eight-cell embryos were transferred. The patient conceived. At 6 weeks, four gestational sacs, all with fetal heartbeats, were demonstrated. At 8 weeks, only three heartbeats were detected. The patient was hospitalized at 26 weeks because of premature contractions and had a cesarean section at 31 weeks. Three healthy babies were born, two girls and a boy, who weighed 1,110, 1,220, and 1,240 g, respectively.

In all, the couple underwent five cycles of IVF. A total of 18 MII oocytes underwent ICSI, of which none fertilized and one egg had one pronucleus (1PN). A total of 57 MII oocytes underwent ICSI followed by calcium ionophore, of which 17 (37%) showed 2PN and 5 showed 1PN. All 2PN zygotes cleaved, 11/17 (65%) reaching six cells or more at 72 hours after insemination.

DISCUSSION

The data on human oocyte activation suggest that the spermatozoa activate the oocyte both by acting at a receptor on the oocyte surface and by releasing a soluble factor into the ooplasm (see Tesarik [14] for review). The latter can eventually assume the totality of the task, provided that it is aided by artificial stimuli brought about by the ICSI procedure itself. After penetration by the fertilizing spermatozoon,
an acrosomal-derived soluble factor (oscillogen, oscillin) activates the repetitive calcium ion transient release (15). Activation of the oocyte results in extrusion of the second polar body, decondensation of a haploid set of chromosomes, and the formation of a nuclear membrane around these chromosomes as the female pronucleus. The activated M-phase promoting factor (MPF), which characterized the mature metaphase II oocyte, is deactivated. This is accompanied by sperm head decondensation and the formation of nuclear membrane around the male pronucleus.

Round-headed spermatozoa (globozoospermia) lack the acrosomal membrane and acrosin contents. Therefore they not only lack the capacity to penetrate oocytes but also are deficient in their oocyte-activating capacity (14). However, fertilization by ICSI and normal pregnancies could be achieved in most patients with globozoospermia, although with lower fertilization and implantation rates than usual (6, 16, 17). Calcium ionophore oocyte activation, followed by normal pregnancies and deliveries, has been described in cases of 100% globozoospermia with low fertilization rate (18, 19). The case presented here is of infertility due to absence of oocyte activation capacity. To the best of our knowledge, this is the first report of a successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after ICSI. The female partner had a good ovarian response on many occasions. No specific abnormality could be found in the oocytes or sperm, including routine and electron-microscopic evaluation. Both female and male karyotypes were normal.

Sperm from patients with globozoospermia lack the acrosome and postacrosomal sheath and may be deficient in the sperm-associated oocyte-activating factor(s). Calcium ionophore, which induces a calcium transient in ooplasm, bypasses oscillin function. We speculate that our male patient has a deficiency or has defective oscillin or other sperm-associated oocyte-activating factor, despite normal acrosome morphology. Another option could be that the female partner has a defective response to oscillin or related factors. We could not identify whether a sperm and/or oocyte defect exist because the couple are Orthodox Jews and therefore had religious objections to using their gametes for fertilization with or by those of another person. The activation of the oocyte by calcium ionophore, which bypassed these defects, allowed normal completion of meiosis, male and female pronuclear formation, and full embryonic and fetal development.

Two pregnancies were achieved in our patient, the first resulting in an ultrasound and the second in three normal newborns. No other chromosomal or morphological abnormalities could be found in the abortus. It has been shown that round-headed sperm do not carry structural or numeric chromosomal abnormalities (18). No fetal anomalies have been reported after calcium ionophore oocyte activation in cases of total globozoospermia (18, 19). Therefore, we assume that the malformation was coincidental.

References