

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

Intracytoplasmic sperm injection (ICSI) is the method of choice for achieving fertilization and pregnancies in cases of severe oligoastheno-teratozoospermia. These patients often have no or very low fertilization rates using conventional IVF. Couples with unexplained infertility after superovulation and intrauterine insemination undergoing IVF have an 11%–23% chance of fertilization failure that can be overcome easily by using ICSI (1–3). The overall fertilization rate after ICSI is between 64% (4) and 71% (5). However, in 3% of the cycles, none of the injected oocytes became fertilized (6, 7).

Total fertilization failure after ICSI may be explained by different factors related to oocyte

(low number and poor quality) or semen characteristics. The most important semen factors are total immotile or rounded-headed spermatozoa. Follow-up ICSI treatment has been shown to achieve some fertilization in ≤90% of cases (6, 7). In two thirds of cases with total immotile sperm, there are motile spermatozoa available for injection in subsequent ICSI cycles (8).

When all the spermatozoa display round heads (100% globozoospermia), the fertilization rate after ICSI is 0–37% (6, 9, 10). Activation of human oocytes using calcium ionophore after ICSI increases the fertilization rate, the number of embryos, and the ongoing pregnancy rate for couples with low fertilization

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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 $52\text{--}100 \times 10^6/\text{mL}$ 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 ordinary IVF on eight oocytes. Motile sperm with no obvious abnormal morphology were injected into eight metaphase II (MII) oocytes. None of the oocytes were fertilized or divided during the next 48 hours.

Subsequent electron microscopy and quantitative ultra-morphological sperm analysis of the spermatozoa revealed mild teratozoospermia, with 5% of the motile sperm ultra-morphologically normal (laboratory standard $\geq 4\%$ [13]). Both the male and the female partners' karyotypes were normal.

Seventeen oocytes were retrieved in the second cycle, 16 of them MII. Motile spermatozoa were obtained by the Isolate method (Irvine, Santa Ana, CA). Oocytes were injected with a motile sperm using the routine ICSI procedure. Within 1 hour of injection, 6 oocytes were exposed to 10 $\mu\text{mol/L}$ of ionophore A23187 (Sigma, St. Louis, MO) in IVF-50 medium (Scandinavian IVF Science, Gothenborg, Sweden) for 7 minutes at 37°C in 5% CO₂. The oocytes were

then washed free of the ionophore through 10 drops of fresh culture medium and incubated further as usual. The couple was briefed about the known effects of calcium ionophore on intracellular Ca²⁺ levels and on oocyte activation. They also were informed about the limited data in the medical literature on the application of this pharmaceutical product in this manner. They signed a written consent for the procedure. A fertilization check was performed 18 hours after ICSI. Four of the ionophore-treated but none of the ICSI-treated oocytes were fertilized. The patients signed a written consent for embryo transfer. Three two-cell embryos were transferred 48 hours after oocyte retrieval; however, pregnancy did not occur.

In the third assisted reproductive techniques attempt, 17 oocytes were retrieved. Sixteen MII oocytes were treated with ICSI, followed by calcium ionophore oocyte activation. Five oocytes were fertilized. Three six- to eight-cell embryos, with minimal fragmentation in two of them, were transferred 72 hours after oocyte retrieval. The patient conceived. The pregnancy was uneventful until the 15th week, when a large omphalocele and no heartbeats were detected. No other anomalies could be found in the abortus, and its karyotype was 46XY.

In the fourth assisted reproductive techniques cycle, 20 MII oocytes were treated 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 sheet 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 omphalocele and the second in three normal newborns. No other chromosomal or morphological anomalies could be found in the abortus. It has been shown that round-headed sperm do not carry structural or numeric chro-

mosomal 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

1. Aboulghar MA, Mansour RT, Serour GI, Sattar MA, Amin YM. Intracytoplasmic sperm injection and conventional in vitro fertilization for sibling oocytes in cases of unexplained infertility and borderline semen. *J Assist Reprod Genet* 1996;13:38–42.
2. Ruiz A, Remohi J, Minguez Y, Guanes PP, Simon C, Pellicer A. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril* 1997;68:171–3.
3. Hershlag A, Paine T, Kvapil G, Feng H, Napolitano B. In vitro fertilization-intracytoplasmic sperm injection split: an insemination method to prevent fertilization failure. *Fertil Steril* 2002;77:229–32.
4. Tarlatzis BC, Bili H. Intracytoplasmic sperm injection. Survey of world results. *Ann NY Acad Sci* 2000;900:336–44.
5. Van Steirteghem A, Nagy P, Joris H, Janssenswillen C, Staessens C, Verheyen G, et al. Results of intracytoplasmic sperm injection with ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa. *Hum Reprod* 1998;13 Suppl 1:134–42.
6. Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 1995;10:626–9.
7. Flaherty SP, Payne D, Matthews CD. Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection. *Hum Reprod* 1998;13 Suppl 1:155–64.
8. Vandervorst M, Tournaye H, Camus M, Nagy ZP, Van Steirteghem A, Devroey P. Patients with absolutely immotile spermatozoa and intracytoplasmic sperm injection. *Hum Reprod* 1997;12:2429–33.
9. Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 1997;68:118–22.
10. Rybouchkin AV, Van der Straeten F, Quatacker J, De Sutter P, Dhont M. Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity. *Fertil Steril* 1997;68:1144–7.
11. Tesarik J, Sousa M. More than 90% fertilization rates after intracytoplasmic sperm injection and artificial induction of oocyte activation with calcium ionophore. *Fertil Steril* 1995;63:343–9.
12. Adams CA, Anderson LS, Wood SH. Activation of human oocytes using calcium ionophore after ICSI increases fertilization and number of embryos for couples with low ICSI fertilization rates. Abstracts of the 15th Annual Meeting of the European Society of Human Reproduction and Embryology. Tours, France. [abstract no. O-059]. *Hum Reprod* 1999;14:32.
13. Reichart M, Eltes F, Soffer Y, Zigenreich E, Yogev L, Bartoov B. Sperm ultramorphology as a pathophysiological indicator of spermatogenesis in males suffering from varicocele. *Andrologia* 2000;32:139–45.
14. Tesarik J. Oocyte activation after intracytoplasmic sperm injection of mature and immature sperm cells. *Hum Reprod* 1998;13 Suppl 1:117–27.
15. Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. *Nature* 1996;379:364–8.
16. Stone S, O'Mahony F, Khalaf Y, Taylor A, Braude P. A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. *Hum Reprod* 2000;15:139–41.
17. Zeyneloglu HB, Baltaci V, Duran HE, Erdemli E, Batioglu S. Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI: case report. *Hum Reprod* 2002;17:1833–6.
18. Kim ST, Cha YB, Park JM, Gye MC. Successful pregnancy and delivery from frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. *Fertil Steril* 2001;75:445–7.
19. Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhont M. Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. *Hum Reprod* 1996;11:2170–5.