

Electrical activation of oocytes after intracytoplasmic sperm injection: a controlled randomized study

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Objective: To evaluate the electrical activation of oocytes in patients with previously failed or limited fertilization after intracytoplasmic sperm injection (ICSI) and in patients with possible failure of fertilization.

Design: Prospective randomized study.

Setting: A private IVF center in Egypt.

Patient(s): Two hundred forty-six patients with severe oligoasthenospermia or nonobstructive azoospermia with total teratospermia or totally immotile spermatozoa were selected for the study. Patients who previously had total failure or limited fertilization after ICSI also were included.

Intervention(s): Sibling oocytes were randomly divided after ICSI into two groups: the study group ($n = 1,640$) was subjected to electroactivation, and the control group ($n = 1,435$), to no electroactivation. Electroactivation was performed by using a double-square direct-current pulse. Embryo transfer was performed with the best available embryos.

Main Outcome Measure(s): Fertilization rate, degeneration rate, and pregnancy outcome.

Result(s): Two hundred forty-one ICSI cycles were included in the study. The fertilization rate was statistically significantly higher in the electroactivated group as compared with in the control group (68% vs 60%, odds ratio = 1.397, 95% confidence interval = 1.197 to 1.629). The oocyte degeneration rate was not statistically significantly different between the two groups (5.9% vs 4.9%, odds ratio = 0.96, 95% confidence interval = 0.73 to 1.26). In total, 112 clinical pregnancies resulted (pregnancy rate = 46.5%). Total fertilization failure occurred in 5 cycles in the control group, and none failed in the study group.

Conclusion(s): Oocyte electroactivation after ICSI significantly improved the fertilization rate in severe oligoasthenoteratospermia and nonobstructive azoospermia. (Fertil Steril® 2009;91:133–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: ICSI, fertilization, electrical activation, total fertilization failure

Intracytoplasmic sperm injection (ICSI) has become the most effective therapeutic treatment for male-factor infertility (1). However, total failure of fertilization still occurs in some cases, such as globozoospermia (2), teratozoospermia (3), immotile spermatozoa (4), and even unexplained cases (5). Total fertilization failure is a rare event in cases with normal oocytes and spermatozoa, but it can even occur repeatedly (6). It is estimated that failed fertilization occurs in 2%–3% of ICSI cycles (7, 8).

In ICSI, because the spermatozoa is injected inside the ooplasm, failure of pronuclear formation and division most probably is the result of the failure of oocyte activation. Many investigators have tried different techniques for oocyte activation after ICSI to overcome this problem. Ionophore treatment for oocyte activation after ICSI resulted in the birth of a healthy baby in a case of previously failed fertilization due to globozoospermia (2). Intracytoplasmic sperm injection followed by electrical oocyte activation resulted in the delivery of healthy twins for a couple with previously failed

fertilization after ICSI (9). Recently, it was demonstrated that oocyte activation enabled normal fertilization and pregnancy in sperm and oocyte-related fertilization failure (8). The aim of this study was to estimate the value of the electrical activation of oocytes in patients with previously failed or limited fertilization after ICSI, as well as in patients with the possibility of failed fertilization as a result of teratozoospermia.

MATERIALS AND METHODS

On the basis of the experience of other researchers (8, 9), we decided to test electroactivation in a pilot study first. Electroactivation of oocytes after ICSI was performed for 10 patients in our center who had had previous total failure of fertilization in 11 ICSI cycles. After the encouraging results of these 10 cases, approval was obtained from the institutional review board of the Egyptian IVF-ET Center to perform oocyte electroactivation in cases with expected poor fertilization in a randomized controlled study. The study included 241 ICSI cycles for infertile couples with severe oligoasthenoteratospermia (OAT) or azoospermia. Poor fertilization or failure of fertilization was expected because of 100% abnormal sperm morphology or totally immotile sperms. The patients were counseled and signed consent forms. The female partners were <40 years of age and had normal hormonal profiles

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and no pelvic pathology. The oocytes for each patient were randomly divided after ICSI into two groups: the study group (electroactivation) and the control group (without electroactivation). The embryologists who allocated the oocytes and those who followed them afterward were blinded to the randomization.

Ovarian Stimulation and Oocyte Pickup

Controlled ovarian stimulation was performed by using the long GnRH-agonist down-regulation protocol. GnRH agonist (Decapeptyl, 0.1 mg SC per d; Ferring Pharmaceuticals, Copenhagen, Denmark) was given starting on day 20 of the cycle. After 2 to 3 weeks, when the down-regulation had been confirmed by E_2 levels of <50 pg/mL, hMG was started. The starting dose of hMG was 150–300 mIU/d, according to the patient's age and body weight, as well as the ovarian response in previous cycles. Monitoring was started on day 7 of hMG stimulation, with daily E_2 measurements and vaginal ultrasonography.

When at least three follicles reached 18 mm in mean diameter, hCG (Pregnyl, 10,000 IU; NV Organon, the Netherlands) was given. Oocyte pickup was scheduled 36 hours after hCG injection.

Semen Processing Before ICSI

After complete liquefaction, the semen was washed twice with tissue culture media, and the final pellet was resuspended in 0.2 mL of medium. Then it was layered carefully in the surrounding ring of a Tea tube (10), filled with tissue culture media, and incubated until the time of injection. In cases of azoospermia, testicular tissues were prepared as described elsewhere (11). Testicular biopsy was obtained under optical magnification of $\times 6.5$.

The ICSI procedure was performed as described elsewhere (12).

After injection, the oocytes for each patient were randomly divided in a 1:1 ratio into two groups: group A (with activation) and group B (without activation). After ICSI, one embryologist randomly allocated the oocytes into two dishes. Another embryologist allocated the dishes for each patient into a control dish and an electroactivation one. The embryologist who checked for signs of fertilization was blinded to the allocation.

Oocyte Electrical Activation

Oocyte electrical activation was performed 30 minutes after ICSI. The time of inducing electrical oocyte activation was recommended to be as soon as possible after ICSI (13), because nuclei swelling and chromosome fragmentation was observed in 51% of unfertilized oocytes after ICSI (14). The oocytes were suspended in 0.3 M glucose drops, with pH at 7.3, and placed between two parallel electrodes (2 mm apart) in an electric slide chamber (BTX micro slide P/N 450, 0.5-mm gap; BTX, San Diego, CA). A double-square direct-current pulse (130V, 50 μ s apart) was generated by using an electro cell manipulator (BTX) to achieve the desired

field strength of 2.6–2.8 kv/cm. The activation setting was adjusted as follows: mode, HV (99 per sec per 3 KV); voltage, 130–140 V; pulse length, 50 μ s; and number of pulses, 2.

The electrically stimulated oocytes immediately were transferred back to the tissue culture media to be rinsed, then they were incubated under oil in 5% CO_2 in air, at 37°C.

The oocytes were checked 16–18 hours after injection to determine the presence of pronuclei. Embryo transfer was performed 48–72 hours later, replacing the three best available embryos. A β -hCG test was performed 2 weeks after the embryo transfer, and ultrasonography was performed 3 weeks later for positive cases.

The primary outcome measures were defined to be the oocyte fertilization rate and oocyte degeneration rate. The secondary outcome measures were the pregnancy rates and pregnancy outcome.

Stratification of data was performed to take in account the cycle effect. Mantel-Haenszel estimates of both odds ratio and χ^2 statistics were averaged across 241 strata.

Sample-Size Calculation

It was assumed that the fertilization rate in difficult ICSI cases, with nonobstructive azoospermia and severe OAT and with previously failed fertilization, would be around 50% per metaphase II oocytes. To demonstrate an increase of 10% using the electroactivation technique, the sample size needed for a study power of 95% was 660 metaphase II oocytes in each group (total = 1,320). Assuming that a mean of 12 metaphase II oocytes was retrieved, we needed 110 patients. Our study included 241 patients.

RESULTS

For the pilot study, 10 patients with previous total failure of fertilization in 11 ICSI cycles were subjected to ICSI and then electroactivation. All 10 patients achieved fertilization, and 1 had extra fertilized oocytes that were cryopreserved. All 10 patients reached the embryo transfer stage, and 4 of them achieved pregnancy, resulting in the delivery of four healthy babies. Data from the 10 cycles are shown in Table 1.

The randomized controlled trial included 241 ICSI cycles that were performed in our center between August 2005 and January 2006 (Fig. 1). The mean age of the female partners was 29.41 ± 4.79 years. The mean period of infertility was 8.5 ± 4.2 years. Spermatozoa were obtained from ejaculated semen in 190 cycles and from testicular biopsies in 51 cycles. The sperm count ranged from only a few spermatozoa to 25×10^6 /mL, with a mean of 3.75 ± 2.51 . Motility ranged from zero to 30%, and abnormal forms ranged from 90% to 100%. The fertilization rate was significantly higher in the electroactivated group, as compared with the case in the control group (68% vs. 60%; odds ratio = 1.397; 95% confidence interval = 1.197 to 1.629; $P < .0001$). There was no

TABLE 1

Results of oocyte electroactivation in 10 ICSI cycles for patients with previous total failure of fertilization.

Case no.	Oocytes retrieved	Metaphase II oocytes	Two-pronuclei oocytes	No. of embryos transferred	No. of embryos cryopreserved	Pregnancy result
1	13	8	5	3	—	-ve
2	4	4	1	1	—	-ve
3	18	10	2	2	—	-ve
4	12	7	3	3	—	Single
5	14	8	3	2	—	-ve
6	12	6	2	1	—	-ve
7	4	4	2	2	—	-ve
8	12	9	7	3	4	Single
9	6	5	2	2	—	Single
10	8	5	5	3	2	Single
Total	103	66	31	22	6	4 Healthy babies

Note: -ve = negative.

Mansour. Electrical activation of oocytes after ICSI. Fertil Steril 2009.

significant difference in the oocyte degeneration rates in both groups (5.9% vs. 4.9%, odds ratio = 0.96, 95% confidence interval = 0.73 to 1.26; Table 2). Embryo transfer was performed with the best-quality available embryos (≤ 3 embryos). In 34 cycles, the embryos used for ET were derived from the electroactivated group, resulting in 15 pregnancies (pregnancy rate = 44%). Three ended in miscarriage, and of the remaining 12, 4 sets of twins and 8 singletons were delivered (9 healthy girls and 7 healthy boys), as shown in Table 3. In 69 cycles, the embryos used for ET were derived from the control group, resulting in 33 pregnancies (pregnancy rate = 48%). Three ended in miscarriages and one ectopic pregnancy, and of the remaining 29, 6 sets of twins and 23 singletons were delivered (1 stillbirth, 20 healthy boys, and 14 healthy girls). In the majority of cycles (138), the embryos used for ET were derived from both groups, resulting in 64 pregnancies (pregnancy rate = 46.4%). Six patients miscarried, and 58 delivered (1 set of triplets, 10 sets of twins, and 47 singletons, for a total of 30 healthy girls and 40

healthy boys). In five cycles (2%), there was a total failure of fertilization in the control group (without electroactivation); consequently, these five patients would have lost their chance of embryo transfer and possible pregnancy if no electroactivation had been performed. Embryo transfer was performed for these five patients by using the embryos that resulted from the electroactivated oocytes. Clinical pregnancies occurred in 2 patients, and 3 babies were delivered (1 singleton and 1 set of twins).

DISCUSSION

Although ICSI has overcome the most difficult male-factor infertility cases, poor fertilization and a total failure of fertilization still can occur (15) and was estimated to be around 1%–3% (7, 16, 17). In our center, total fertilization failure after ICSI occurs in about 1.5% to 2% of the cases, and in this study of 241 cycles, it occurred in 5 cycles (2%) in the control group oocytes.

TABLE 2

The effect of electroactivation on the fertilization and rates of sibling oocytes of 241 ICSI cycles with expected poor or failure of fertilization.

Parameter	Electroactivated oocytes	Control oocytes
No. (mean \pm SD) of metaphase II oocytes	1,640 (6.8 \pm 2.48)	1,435 (5.95 \pm 2.9)
No. (mean \pm SD) of 2-pronuclear oocytes	1,116 (4.63 \pm 2.3)	872 (3.62 \pm 1.96)
Fertilization rate (%) ^a	68	60
No. (mean \pm SD) of degenerated oocytes	98 (1.73 \pm 1.2)	70 (1.39 \pm 0.79)
Degeneration rate (%) ^b	5.9	4.9

^a Odds ratio = 1.397, 95% confidence interval = 1.198 to 1.63, $P < .001$.
^b Odds ratio = 0.96, 95% confidence interval = 0.73 to 1.26, $P = 0.821$.

Mansour. Electrical activation of oocytes after ICSI. Fertil Steril 2009.

TABLE 3

Pregnancy rates and outcomes.

Parameter	Embryos for transfer derived from electroactivated group	Embryos for transfer derived from control group	Embryos for transfer derived from both groups
No. of ET procedures	34	69	138
No. (%) of clinical pregnancies	15 (44)	33 (48)	64 (46.4)
No. (%) of miscarriages	3 (20)	3 (9)	6 (9.4)
Ectopic pregnancy	—	1	—
No. of deliveries	12 (4 sets of twins, and 8 singletons, totaling 16 healthy babies [9♀ + 7♂])	29 (6 sets of twins and 23 singletons, totaling 34 healthy babies [14♀ + 20♂] and 1 stillbirth)	58 (1 set of triplets, 10 twins, and 47 singletons, totaling 70 healthy babies [30♀ + 40♂])

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Oocyte activation is manifested by the extrusion of the second polar body, decondensation of the haploid set of chromosomes in the oocyte, and the formation of a nuclear membrane around these chromosomes, forming the female pronucleus (18). It is not yet fully understood how the spermatozoa activate the oocytes. It may be attributed to some proteins in spermatozoa that initiate activation (19). Oocyte activation after ICSI appears to be induced by non-membranous factors in the spermatozoon, because ICSI bypasses gamete membrane interaction (20). The sperm-borne oocyte activating factors are present in the perinuclear material at least partially (21). Excessive sperm immobilization before ICSI significantly improves the fertilization rate (22).

The failure of fertilization after ICSI may result from either the lack or deficiency of activating factors in spermatozoa or may result from the inability of the oocyte to respond to the injected sperm (9) and from the lack of ooplasmic factors triggering sperm chromatin decondensation (23).

Total teratozoospermia is the most common cause of fertilization failure after ICSI in our center. Structural abnormalities may be associated with the deficiency of oocyte-activating factors (9). However, it is not yet clear whether sperm morphology has a direct relation to DNA damage (24, 25). Total acrosin activity and acrosome reaction play an important part in fertilization (26, 27). Reduced oocyte activation and first-cleavage rate after ICSI was reported in cases of chromosomal translocations associated with OAT (28). Abnormal semen analyses often are associated with abnormal chromatin. However, 8% of men with normal semen analyses have abnormal sperm DNA (29, 30).

Total failure or very limited fertilization after ICSI in cases of normozoospermia also has been reported (18, 31–33). Finally, partial or total failure of fertilization also can be a result of transient biological reasons or technical imperfections (8).

Calcium ionophore activation (18, 33) and electrical activation (9) were reported to be successful in cases of normozoospermia with previously failed fertilization after ICSI. Chemical activation also was shown to induce parthenogenetic development of oocytes (34). Three successful pregnancies and deliveries were reported after oocyte activation by strontium chloride in six patients (35).

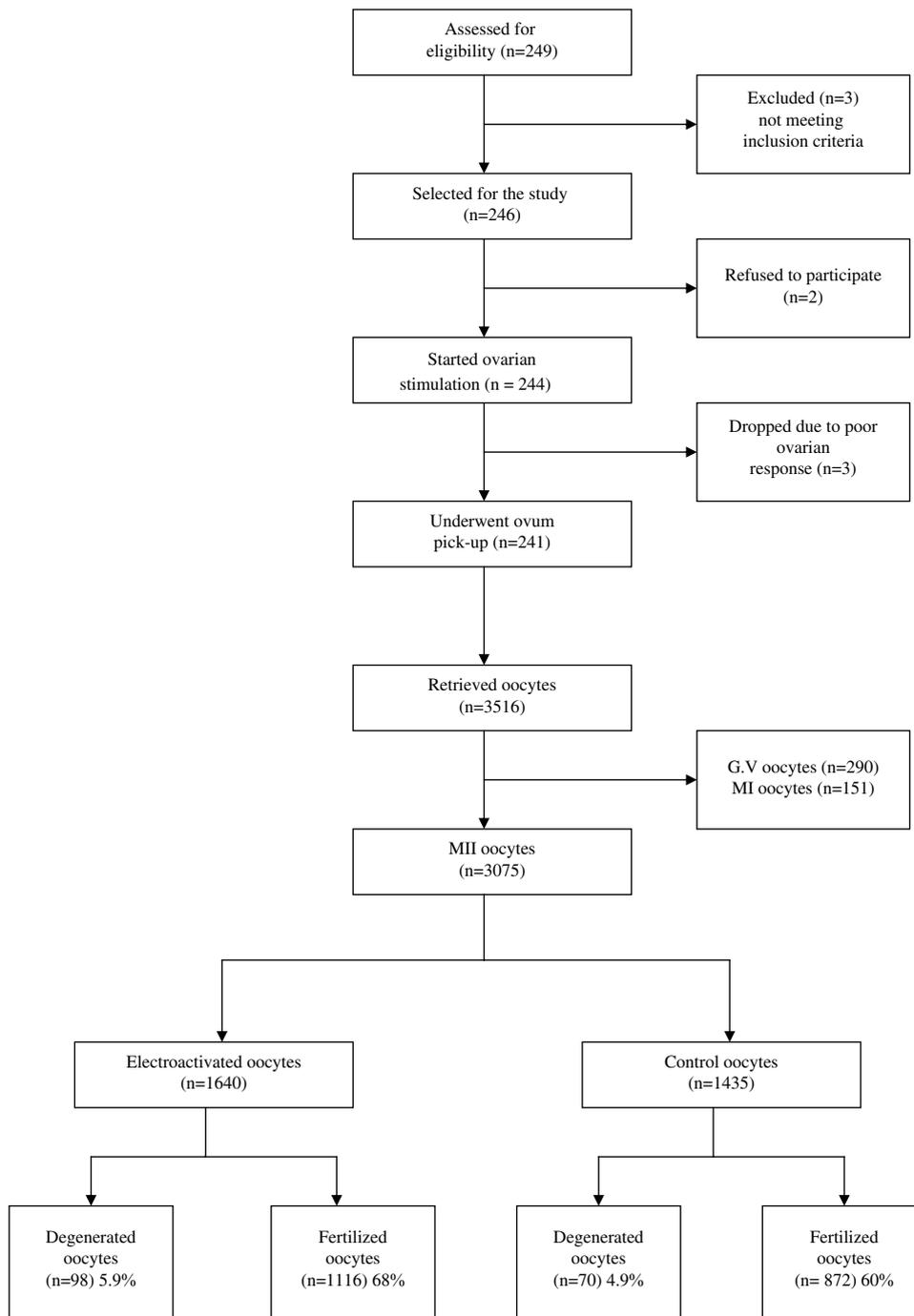
Oocyte activation also can be induced by many artificial reagents or conditions. The key event of oocyte activation is a temporal rise in the intracellular Ca^{2+} concentration (36–39). The typical pattern in a Ca^{2+} rise is repetitive until the pronuclear stage (38, 39). A single long-lasting Ca^{2+} rise is usually induced by some artificial agents such as ethanol, calcium ionophore, or a single electric pulse (2, 9, 38, 40–44).

Calcium ionophore treatment has been reported to induce successful assisted oocyte activation (2, 18, 33, 40, 42). It has been used for oocyte activation after ICSI in 17 patients with previously failed fertilization and has proven efficient in normalizing the fertilization rate after ICSI and obtaining pregnancies (8). The combination of calcium ionophore with

FIGURE 1

Consolidated Standards of Reporting Trials statement flow diagram. GV = germinal vesicle; MI = metaphase I; and MII = metaphase II.

CONSORT statement flow diagram



Mansour. Electrical activation of oocytes after ICSI. *Fertil Steril* 2009.

puromycin was used to salvage unfertilized oocytes after ICSI (45). The potential toxic effects of ionophores on oocytes and embryos have not yet been estimated sufficiently in clinical ICSI.

The modification of the ICSI technique by performing cytoplasmic aspiration near the region of the mitochondria with a high inner mitochondrial membrane potential (46) was found to overcome complete oocyte activation failure (6).

Artificial activation after ICSI may rescue oocytes that do not produce two pronuclei. It has been reported that about 70%–80% of unfertilized oocytes after ICSI responded to electroactivation and formed two pronuclei (9, 44). Spermatozoa from three patients who failed to fertilize their female partners' oocytes after ICSI were injected into unfertilized mouse oocytes by ICSI (as a test), followed by electrical stimulation (9). Activation was successful in the mouse test for the three patients. Electroactivation then was used for these three patients and resulted in successful fertilization for all and in the delivery of healthy twins for one patient. Electroactivation was used after spermatid injection, achieving a 15% pregnancy rate (35).

As was demonstrated elsewhere, electroactivation results in a rapid rise in Ca^{2+} inside the oocyte, which decreases gradually to the original level in about 300 seconds (13, 47). Comparing this method with the use of ethanol or calcium ionophore, it has not yet been proven that electrical oocyte activation is the most efficient and safest method for oocyte activation in human beings. However, in rats, chemical (SrCl₂) activation was compared with the use of two direct-current pulses in ICSI with round spermatid injection. These results indicated that direct current was better than SrCl₂ for oocyte activation, in terms of two-cell embryos developing to blastocysts and live births (48).

In our study, electrical stimulation resulted in a significant improvement in the fertilization rate after ICSI, in cases with previous total failure of fertilization or limited fertilization. Encouraged by the results of electroactivation of other researchers, we first experimented with the procedure in 10 ICSI cycles for patients who previously had total failure of fertilization (one patient failed twice) with ICSI. In all 10 patients, fertilization was successful, and pregnancy was achieved in 4 patients. These results were the basis for the design of this randomized controlled trial to evaluate the efficiency of the procedure in cases of severe OAT and azoospermia. The study included a large number of cycles ($n = 241$) and a large number of oocytes (1,640) that were subjected to electroactivation. The fertilization rate was significantly higher in the electroactivated group (68%) as compared with in the control (60%). Most important, a total failure of fertilization occurred in five cases in the control group; consequently, these patients would have lost their chance of embryo transfer and possibility of pregnancy if no electroactivation had been performed. Since the commencement of this randomized controlled trial, we have been performing routine electroactivation for at least half of the oocytes in cases of severe OAT and azoospermia with 100% abnormal morphology or rare motile sperms, in which we expect poor or no fertilization. Long-term follow-up studies are needed to ensure safety. There was a high miscarriage rate in the electroactivated group (5 of 15 pregnancies compared with the control [3 of 33]). However, the number of these was too small to draw any conclusions.

In conclusion, electroactivation of oocytes after ICSI significantly improved fertilization. However, more studies are

needed to evaluate the clinical significance and safety of this technique. It is recommended in cases of previous failure of fertilization or limited fertilization, as well as in cases of severe oligoasthenospermia or azoospermia with 100% abnormal forms or zero motility.

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