NATURAL CONCEPTION FOLLOWING TOTAL FERTILIZATION FAILURE WITH INTRACYTOPLASMIC SPERM INJECTION IN A COUPLE WITH UNEXPLAINED INFERTILITY: A CASE REPORT

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NATURAL CONCEPTION FOLLOWING TOTAL FERTILIZATION FAILURE WITH INTRACYTOPLASMIC SPERM INJECTION IN A COUPLE WITH UNEXPLAINED INFERTILITY: A CASE REPORT (Abstract): With the introduction of intracytoplasmic sperm injection (ICSI), couples with severe male factor infertility have been able to achieve fertilization and clinical pregnancy rates comparable to other in vitro fertilization patients. However, despite the utilization of microsurgical sperm injection techniques, failure of fertilization still occurs in a few patients. How such fertilization failure after ICSI might impact later ICSI treatments is less understood. We report a case of total fertilization failure after ICSI using sperm from a normozoospermic husband of a patient with unexplained infertility. Six months after the cancelled cycle, the couple conceived naturally. Unfortunately, it was an ectopic pregnancy, which required laparoscopy and surgical removal of the right fallopian tube. This case shows that a failed ICSI cycle, therefore, does not imply a hopeless prognosis for future ICSI treatment. Moreover, in cases with unexplained ICSI failure, natural conception can subsequently occur. The aim of this study is two-fold: to discuss a rare case of a spontaneous pregnancy after total fertilization failure with ICSI and to develop counseling material for patients and doctors who are faced with such a rare situation. To our knowledge, this is the first report of a case of spontaneous pregnancy after total fertilization failure with ICSI. Keywords: TOTAL FERTILIZATION FAILURE, INTRACYTOPLASMIC SPERM INJECTION, UNEXPLAINED INFERTILITY.

Intracytoplasmic sperm injection (ICSI) is a method of micromanipulation used to deposit a spermatozoon directly into the oocyte cytoplasm. When all other forms of assisted fertilization are unsuccessful, ICSI is the method of choice to overcome male infertility. ICSI has enabled fertilization of oocytes from patients whose partners have extremely low numbers of viable sperm and a very low probability of achieving fertilization after conventional spermoocyte incubation in vitro. ICSI is possible with spermatozoa obtained from ejaculate, percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm extraction (MESE), or testicular sperm extraction. In addition, indications for ICSI include unexplained infertility and previous conventional in vitro fertilization (IVF) failure. Although the fertilization rates...
after ICSI are relatively high (1), some injected oocytes fail to fertilize despite the presence of a spermatozoon in the cytoplasm and the absence of immaturity or degenerative alterations. For all ages and with all different sperm types, the fertilization rate after ICSI is reported to be approximately 70–80% (2). This fertilization failure after ICSI may occur because of the following reasons. First, the injected oocyte may fail to initiate the biochemical processes necessary for oocyte activation (3). Second, the biochemical processes are initiated, but they may not occur normally, thus leading to incomplete activation. Third, the spermatozoon may remain poorly accessible to oocyte factors required for chromatin decondensation and formation of the male pronucleus (4). Finally, the procedure used for ICSI may induce subtle structural changes in the organelles of the oocytes, which are undetectable by light microscopy. Although failed fertilization in some of the injected metaphase II (MII) mature oocytes is quite common, total fertilization failure (TFF) after ICSI is distressing for the infertile couple as well as for the fertility professionals.

TFF is a rare event in cases with normal oocytes and spermatozoa (5), and few patients may experience repeated TFF despite normal sperm parameters and a good ovarian response (6). In such cases, the primary reason for failed fertilization after ICSI is the lack of oocyte activation (7). Oocyte activation involves a complex series of events that result in the release of cortical granules, activation of membrane ATPase, meiotic resumption, and the formation of the male and female pronuclei with the extrusion of the second polar body. The oocyte is activated when the spermatozoon comes in contact with the oolemma and penetrates the ooplasm, intracellular calcium oscillation occurs, and this increase in calcium concentration induces oocyte activation and the initiation of zygote development (8). Several authors have proposed different techniques of artificial oocyte activation. Some assisted activation treatments, such as puromycin (9), promote an increase in the intracellular free calcium concentration by releasing calcium from cytoplasmic stores; other activation treatments, such as electrical stimulus (10), promote the influx of calcium from the extracellular medium. Positive results have been obtained by using a combination of calcium ionophore and puromycin (11). Similar to other new assisted reproductive procedures, the impact of either electrical or chemical oocyte activation on the health of the embryo must be evaluated by conducting larger studies, before they can be considered for routine clinical purposes.

For the typical patient who fails to achieve fertilization with ICSI, models proposed to explain why fertilization failed may be less helpful than the counseling requested regarding future treatment. Unfortunately, the efficacy of subsequent infertility treatments following TFF after ICSI remains largely unknown. Therefore, one aim of this study was to develop information that would be useful in counseling patients who experienced TFF with ICSI.

**CASE REPORT**

A 39-year-old woman and her 45-year-old husband were referred to our clinic for unexplained infertility; they had been married and trying to conceive for more than 2 years. Her physical and gynecological ex-
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Examinations revealed normal results, including hysterosalpingography and routine blood tests. Her menses were regular. However, her anti-mullerian hormone serum concentration (0.82 ng/ml) and antral follicle count (6) were indicative of low ovarian reserve. Other blood hormone concentrations were within the normal ranges (day 3 follicle-stimulating hormone (FSH) 7.01; thyroid-stimulating hormone 1.1 mUI/ml; prolactin 22.8 ng/ml). The mean sperm parameters were as follows: volume 2.7 ml; count 50.53 × 10⁶/ml (1 specimen had relatively low numbers); motility (A + B) 42.0%, and normal morphology 7.0%.

First, we advised them regarding the optimal timing for intercourse, and, after 3 months, we administered 4 cycles of stimulated intrauterine insemination (IUI), which were unsuccessful. Subsequently, after obtaining informed consent, we offered IVF to the couple. In May 2012, the couple underwent their first IVF cycle. A long GnRH agonist protocol was applied at a daily low dose of 0.05 mg (Gonapeptyl®; Ferring, Switzerland) starting from day 21 of the previous cycle. Ovarian stimulation was achieved using a combination of recombinant FSH and recombinant luteinizing hormone (rLH), to a total dose of 300 IU FSH and 75 IU LH per day (Gonal F® and Pergoveris®; Serono, Switzerland). An injection of 250 mcg (6500 IU) of recombinant human choriionic gonadotropin (hCG, Ovitrelle®; Serono, Switzerland) was administered when the leading follicle reached a mean diameter of 20 mm. Transvaginal ultrasound-guided needle aspiration of oocytes was performed 36 h after the hCG injection. All oocyte-handling procedures were conducted on warm stages (37°C) using conventional methods. We retrieved 10 oocytes: 8 were mature (MII) and 2 were immature. We performed ICSI for all 8 MII oocytes after a 4-h incubation period. The injected oocytes were cultured for several hours in Cook MINC incubators, which provide a special low-oxygen environment for the embryos; the incubators are supplied by a triple gas mixture containing 5% O₂, 6% CO₂, and 89% N₂, as opposed to the standard air supply, which contains 5% or 6% CO₂ and 20% O₂. Fertilization was assessed 18 h after ICSI by 2 embryologists: none of the 8 injected oocytes exhibited pronuclei, and all were judged unfertilized after a final assessment conducted 6 h later (Fig. 1).

![Fig. 1. Unfertilized oocytes (final assessment 24 h after ICSI)](image_url)
All possible explanations responsible for TFF were analyzed, and all external factors, such as contamination of culture media and improper gamete micromanipulation, were excluded. The lack of fertilization was attributable to the sperm’s inability to activate the oocytes, or to the inability of the oocytes to decondense the spermatozoa. However, it was impossible to determine whether the oocytes or spermatozoa were defective as the couple did not agree to an experimental protocol to overcome their complete fertilization block of unknown causes (testing the ability of the husband’s sperm to activate mouse oocytes and/or treatment of the patient’s oocytes with calcium ionophore in a subsequent ICSI cycle were proposed). After 6 months, the patient asked for a consultation for 6-weeks amenorrhea and a positive pregnancy test. The transvaginal ultrasound examination revealed an ectopic gestational sac, and the patient underwent operative laparoscopy. Surgical findings were as follows: small amount of blood in the peritoneal cavity, bloated right fallopian tube, normal uterus and ovaries, and normal left fallopian tube. After the surgical removal of the right fallopian tube, the patient’s recovery was uneventful. A subsequent histological examination of the removed block confirmed the diagnosis of a tubal pregnancy.

DISCUSSION

In this contribution, we describe the case of an infertile couple with unexplained TFF with ICSI. As all 8 oocytes were mature, with no morphological anomalies, and for the ICSI procedure only motile spermatozoa were used, concerns regarding possible technical errors were raised. The risk of oocyte damage by the ICSI procedure is low in humans and may be related to both the skill of the person performing the injection procedure and to the quality and quantity of the gametes used during the procedure. The embryologist conducting the ICSI procedure is a significant predictor of fertilization, whereas laboratory conditions such as the type and model of incubators and culture of oocytes (individually versus grouped) do not affect the fertilization rates (12).

Although ICSI is now considered a routine procedure, it remains a very demanding technique to master, partly due to its inherent technical difficulty and partly due to the heterogeneity of the cases. It is generally agreed that the ICSI procedure is subject to a learning curve. One common technical failure may result in the spermatozoon not being deposed within the cytoplasm of the oocyte. In this situation, the oocyte membrane may not have been broken while attempting to aspirate the ooplasm into the ICSI needle. Thus, the spermatozoon is then deposited next to the oocyte membrane, so that when the oolemma returns to its original position, the spermatozoon is pushed out into the perivitelline space (Fig. 2A), or is trapped inside a sac formed by the membrane (Fig. 2B). The spermatozoon may also adhere to the tip of the injection needle or remain within the injection needle and be inadvertently pulled out upon withdrawal of the needle from the cytoplasm. The degeneration of oocytes after ICSI is often a result of a faulty ICSI technique, e.g., use of an injection pipette that is too large or not sharp enough. Aspiration of the ooplasm is always performed to ensure that the oocyte membrane is broken during injection. However, if excessive ooplasm is aspirated, the oocyte frequently degenerates (Fig. 2C).
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Fig. 2. Damaged oocytes during ICSI (A-spermatozoa in perivitelline space; B-spermatozoa trapped inside a membrane sac; C-degenerated oocyte after excessive cytoplasm aspiration)

None of the previously described aspects were found in this case. When fertilization failure occurs in most or all of the injected oocytes, despite the procedure being performed by experienced practitioners using normal spermatozoa, TFF is attributable to oocyte dysfunction, oocyte activation failure, or inability of the spermatozoon to be decondensed and processed by the oocyte.

Despite frequent use and success of IVF treatment for infertile couples, cases of TFF continue to be encountered. Their consequences are devastating to the patients, with lost resources, incurred costs, and much distress. Understanding the etiology of FF is, therefore, of critical importance to assist patient counseling and optimize treatment.

The incidence of TFF after conventional IVF using sperm of normal quality has been reported to range from 5% (13) to as high as 15–20% (14). While ICSI has overcome many fertilization problems, it does not completely eliminate TFF. In a randomized clinical trial comparing outcomes after ICSI or IVF for cases of non-male factor infertility, Bhattacharya et al. (2001) documented a TFF rate of 2% versus 5% for ICSI and IVF, respectively (15). Indeed, several large studies using ICSI for a variety of infertility diagnoses reported TFF at rates ranging from 1.3% in 1779 cycles (16) to 3% in 2732 cycles (17). The possible etiologies underlying TFF are complex and may relate to the cycle-specific parameters, yield and quality of oocytes, availability of motile sperm, and/or to severity of sperm defects (18, 19, 20). Sperm abnormalities may result in abnormal sperm decondensation and/or aberrant pronuclear development, migration, apposition, and first mitosis (21). Some ICSI oocytes fail to activate, which, under some circumstances, has been successfully overcome by manipulating intracellular calcium levels in oocytes by using calcium ionophore (22) or electric pulses (23). Several early studies concerning gamete ultrastructure (24) and staining of DNA or whole chromosomes (25) led to the identification of abnormal chromatin patterns and/or chromosome numbers, of either paternal or maternal origin, in non-fertilized oocytes. Recent studies of microtubules also revealed abnormal spindle and interphase microtubules, indicating that defects in cytoplasmic components of oocytes may be the main cause of failed fertilization (26).

This case was extremely challenging
from a counseling perspective. For couples experiencing TFF after ICSI, little data exists for patient counseling, especially with respect to the efficacy of continued therapy and the future hope of a successful live birth. To date, only 2 studies have analyzed the outcome of a subsequent conventional ICSI cycle after a previous TFF. In 1 study (published in 2003), TFF reoccurred only once in 23 repeated ICSI cycles; the mean cycle fertilization rate in that population was 69.2%, and the clinical pregnancy rate per transfer was 45.4% (27). In the other study (published in 1995), TFF reoccurred in 5 of the 27 repeated cycles (17). The differences between fertilization rates remain unexplained, although fluctuations in gamete quality may play a contributory role. A history of failed fertilization may be related to a gamete abnormality that may be modified or corrected during the next cycle. Pretreatment endocrine assays and semen analyses have proven to be of little value in predicting failed fertilization. Very subtle improvements in semen parameters and/or oocyte yield/quality may result in fertilization during a subsequent ICSI attempt. Otherwise, the options of donor sperm insemination, donated oocytes or embryos, adoption, and remaining childless should be discussed with the couple (28).

CONCLUSIONS

Despite the important advances in assisted reproductive technologies, successful fertilization for every patient is not guaranteed, even with ICSI. In such cases, new laboratory techniques can aid a subsequent ICSI cycle. However, from a practical point of view, especially in patients with normal sperm parameters, conventional ICSI can be considered a good alternative. It is important to note that no unified theory exists to explain the phenomenon of TFF, since it might be the result of multiple, and, as our case shows, sometimes, temporary anomalies. Therefore, for couples with unexplained infertility (normal sperm parameters and optimal ovarian response) and TFF after ICSI, a subsequent treatment cycle with conventional IVF or split ICSI/IVF may be recommended.

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REFERENCES


### SCHIZOPHRENIA AND VIRUS-GENES INTERACTION

Schizophrenia is a severe mental disorder, which affects approximately 1% of the global population. The disease is caused by genetic and environmental factors as well as gene–environment interactions. An international study conducted by Borglum and colleges show that interaction between genes and cytomegalovirus (CMV) influences the risk of developing schizophrenia. Women that have been infected by the virus will have an increased risk of giving birth to a child who later develops schizophrenia, if the child has the gene variant associated with this mental disorder. By using single nucleotide polymorphisms (SNP), the researchers have showed a strong association between schizophrenia and a number of loci such as: SNP rs4757144, located in an intron of the circadian rhythm-associated gene *ARNTL* on 11p15, SNP rs8057927 in the intron of gene *CDH13* on 16q23 (both genes previously linked to schizophrenia or other psychiatric disorders) and, for first time, one region at chromosome 10p11 overlapping the gene *ZEB1*. This study also identified a significant interaction between maternal CMV infection and *CTNNA3* of the progeny, a gene that has not previously been associated with schizophrenia. The results emphasize the importance of environmental factors in disease development. These results may suggest also that a vaccine against CMV may help to prevent schizophrenia (Borglum AD, Demontis D, Grove J, Pallesen J *et all*. Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. *Mol Psychiatry*. 2013 Jan 29).

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