

## Efficacy of rescue ICSI after total fertilization failure in conventional IVF

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### Abstract

Partial or complete fertilization failure after IVF was reported in 5-10% of cycles. Total fertilization failure in patients with normal sperm parameters is rare but very stressful incident after IVF. To rescue the IVF cycles after total fertilization failure, ICSI of unfertilized oocytes after initial insemination has been reported. We reviewed a 2 years experience of rescue ICSI and analyzed the cycles outcomes. Cycles with total fertilization failure using mature oocytes with normal morphology, which had no evidence of fertilization 24 hours after IVF were selected for this study and reinsemination by ICSI was performed. 15 rescue ICSI cycles were included in our study. After rescue ICSI fertilization rate per cycle was 54.32%. Also  $5.53 \pm 4.15$  embryos were obtained and  $2.54 \pm 0.34$  embryos were transferred in each cycles. But no pregnancy was reported after fresh embryo transfer. This study showed that day1 rescue ICSI is not a good option for prevention of complete fertilization failure after IVF procedure.

**Key words:** Rescue ICSI, conventional IVF, oocyte, fertilization failure.

### Introduction

In vitro fertilization (IVF) is the standard insemination method. While Intracytoplasmic sperm injection (ICSI) is an assisted fertilization procedure that involves injection of single spermatozoa into a mature oocyte (1), and is usually performed in patients with severe male factor infertility.

Conventional IVF is much less effective when the semen characteristics, including concentration, motility or morphology are grossly below the standard values (2). ICSI is also performed when fertilization rate in previous IVF cycle has been low (3). The fertilization rate through standard IVF is about 60%-70%. Nonetheless, partial or complete failure of fertilization after IVF occurs in around 5-10% of cycles (1).

To overcome this barrier of fertilization failure, some investigators suggest intracytoplasmic sperm injection on day 1 of unfertilized mature oocytes, also known as "rescue ICSI" (1-4). ICSI is an assisted fertilization procedure that involves injection of single spermatozoa into a mature oocyte (5). However routine use of ICSI does not improve

fertilization, and so is not recommended (in non male factor infertility or in normozoospermia patients (5)). Until now, there are many studies that have showed positive effects of rescue ICSI on fertilization rate, embryo quality and pregnancy rate. Reports described that by using this procedure success rate is from 28% to 60% (2,6,7,8). Although the published data showed low pregnancy rate and only 3 pronucleus (PN) formation after rescue ICSI (6,7,9).

To rescue the IVF cycles after total fertilization failure, ICSI of unfertilized eggs after early insemination has been reported (6-9). The aim of this work was to evaluate the fertilization and the subsequent cleavage characteristics of oocytes treated by conventional reinsemination or ICSI after failure to fertilize through the standard IVF and ICSI procedures. We reviewed a 2 years experience of rescue ICSI and analyzed the cycle outcome.

In this study, we reviewed a 2 years experience of rescue ICSI and analyzed the outcomes of cycles.

### Methods and materials

This study was approved by ethics committee of Research and Clinical Center for infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran.

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All of infertile women, aged less than 38 years presenting to our Research and Clinical Center for Infertility between April 2011 and September 2012 were enrolled in the study after signing an informed consent form. Couples with sperm count >10 million/mL, normal morphology >8% (krugers strict criteria) and progressive motile sperm > 40% were included in this study. Women with basal FSH >10 IU/mL were excluded from the study.

Controlled ovarian stimulation was done using down-regulation with gonadotropin-releasing hormone (GnRH) agonist protocol or GnRH antagonist protocol. When at least two follicles reached a mean diameter of 18 mm, by transvaginal ultrasonography, 10000 IU HCG was administered. Oocytes retrieval was programmed 36 hours after the hCG injection. Total retrieved oocytes were treated by conventional IVF and were inseminated 4 hours after oocytes retrieval with 60000 motile sperm in 1 ml of IVF medium. Fertilization was evaluated 18 hours after IVF. Normal fertilized zygotes were defined by the development of two pronuclei (2PN).

Only cycles with total failure of fertilization were chosen for rescue ICSI. For this reason, mature oocytes with normal morphology, which showed no evidence of fertilization 24 hours after IVF were selected. A single motile spermatozoa with apparently normal morphology was microinjected into the ooplasm at the 3 o'clock position. Fertilization was evaluated 16-18 hours after ICSI. Zygotes with 2PN were cultured and embryos were transferred using a Labotect catheter (Labotect, Gottingen Germany). No more than 3 embryos were transferred in fresh cycles, and all of the embryos with 30% fragmentation were cryopreserved by vitrification methods.

Luteal phase support was started with progesterone in oil (Progesterone, Aburaihan Co., Tehran, Iran) 100 mg daily IM on the day of oocyte retrieval and was continued until the documentation of fetal heart activity by ultrasound.

Chemical pregnancy was defined by positive beta-hCG 14 days after embryos transfer. Clinical pregnancy was identified as observation of fetal heart activity by transvaginal ultrasonography that was performed 3 weeks after positive beta-hCG.

## Results

Fifteen rescue ICSI cycles were performed in this study. Basic demographic characteristics of patients are shown in table 1.

After rescue of ICSI fertilization rates per cycles was 54.32% and 5.53±4.15 embryos was obtained

per cycles and 2.54±0.34 embryos were transferred per cycles (table 2).

Embryo transfer was cancelled in 8 cycles. In 3 cycles because of no embryo was obtained, in one patient due to risk of OHSS and 4 couples, because endometrium was not appropriate (Table 3).

No pregnancy was reported after fresh embryo transfer. only 2 of patient returned for frozen-thawed cycles, and clinical pregnancy was reported in one of them (table 2).

Table 1. Basic characteristics of patients

Parameters	Result
Female age (years)	29.53±4.83
Duration of infertility (years)	7.53±2.23
Basal FSH (IU/L)	4.93±1.22
Etiology of infertility:	
Tubal, n (%)	5 (33.3)
Unexplained, n (%)	4 (26.7)
Total, n (%)	6 (40)
Mean±SD	

Table 2. Outcome of rescue ICSI

Parameters	Result
No of MII oocytes	10.06±4.92
Fertilization rate (%)	54.32
No of obtained embryo	5.5±4.15
No of transferred embryos	2.54±0.52
Chemical Pregnancy rate (fresh cycles) NO (%)	0 (0)
Chemical pregnancy rate (frozen cycles) NO (%)	2 (50)
Clinical pregnancy rate (frozen cycles) NO (%)	2 (50)
Mean±SD	

Table 3. Cancellation rate per cycles.

Cancellation rate	NO	(%)
Total no cancellation cycles	7	46.66
Total cancellation cycles:	8	53.33
Inappropriate endometrium	4	26.66
OHSS risk	1	6.66
no of transferred embryos	3	20

## Discussion

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technique that has been introduced in 1992. Conventional IVF contains standard insemination and ICSI involves injection of single spermatozoa into a cytoplasm of mature oocyte (14-18).

The safety of ICSI is still unknown and seems the unnecessary ICSI maybe effect on embryo formation and its quality, however ICSI is one of the best method for patient with sever male factor. (19-21). The possibility of complete fertilization failure after conventional IVF was assessed 12.5% in normozoospermia and tubal factor infertility, 16.7% in unexplained infertility and up to 50% in astenozoospermia (22-24). In our center IVF is performed when sperm parameters is within normal range with sperm count >10 million/mL, normal morphology >8% (krugers strict criteria) and progressive motile sperm > 40%, also ICSI routinely is done in cycles with less than 5 retrieved oocytes and in patients with history of total fertilization failure. Only 15 cycles with total fertilization failure after IVF were included in this study.

Fertilization is a process involves many steps, including sperm-zona binding, gamete fusion and egg activation and any sperm defects disturbances in sperm oocyte interaction and oocyte abnormality have been proposed as possible causes of failed fertilization after IVF (25,26).

Fertilization of oocytes by rescue ICSI shows that intrinsic potential of sperm for fertilization is intact (27).

In this study fertilization rates after ICSI was 54.32% however the pregnancy rates after transfer of embryos obtained by rescue ICSI, 24 hours after IVF failure was poor. Only one pregnancy after transfer of cryopreserved embryo was reported. Very poor outcomes in terms of pregnancy shows that some intrinsic or developmental defect of oocytes has been occurred after rescue of ICSI. Aging of oocytes is the most important cause for poor outcome (28). The time interval between oocyte retrieval and fertilization is critical for good embryo development. Prolonged culture of oocytes can resulted to zona hardening, increased parthenogenesis and impaired embryo development (29). Wang et al. showed that spindle of most aged oocytes was partially or completely disassembled. Chromosomal analysis of failed fertilized oocytes after IVF showed that about one-third of the oocytes had an abnormal karyotype. Premature chromosome condensation was present in about one-third of unfertilized oocytes after sperm entering (30).

Chen et al. in their retrospective study analyzed outcome of rescue ICSI after conventional IVF failure. They showed that normal fertilization rate of rescue ICSI performed 6 hours after failed IVF significantly increased compared to late ICSI 22 hours after failed IVF they showed that clinical pregnancy rates was significantly higher when rescue of ICSI performed 6 hours after failed IVF (31).

Nagy et al. in their study demonstrated that Rescue ICSI of unfertilized oocytes 6 hours after insemination and 9 hours after oocyte retrieval lead to normal fertilization, embryo development and pregnancy rates (29).

The earliest sign of fertilization is extrusion of the second polar body. It has been demonstrated that following ICSI 22% of oocytes release their second polar body during 2-4 hours and by 8 hours all oocytes released their second polar body if they have been fertilized. 80% of oocytes have two pronuclei by 8 hours (32). Performing rescue ICSI on oocytes that are in the process of fertilization could resulted to 3PN zygotes results from 2 sperm participating in fertilization. This risk can greatly decrease by performing rescue ICSI 8 hours after initial insemination.

Synchronization between the embryo development and endometrium is very important factor in implantation after ART (33). Previous studies has shown that endometrial maturation on the day of the oocytes retrieval in IVF cycles is 2-4 days more advance compared to normal cycles and no ongoing clinical pregnancy reported if endometrial advancement exceed 3 days after ovarian hyperstimulation (34,35). The procedure of day 1 reuse ICSI is worsening this phenomenon by delaying the time of transfer. Sermondade et al. in their study showed better implantation rates after freeze of embryos obtained after rescue ICSI and transfer in a frozen thawed cycles in compared to fresh embryo transfer (36). This may be due to better synchronization between endometrium and embryos in frozen-thawed embryo transfer cycles. Here we confirm previous reports in our study, the only clinical pregnancy was reported after transfer of cryopreserved embryos.

However our study was Cross-sectional and number of patients that included in the study was limited but this was the first study that evaluated the fertilization failure oocytes after conventional IVF and rescues the oocytes by late ICSI in Iran.

## Conclusion

This study showed that day1 rescue ICSI is not a good option for prevention of complete fertilization

failure after IVF. According literature review it seems that earlier rescue ICSI and transfer in frozen –thawed cycles is better option to prevent complete fertilization failure. Another suggestion is to perform ICSI in at least a number of oocytes even in presence of normal sperms.

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