## Original Article

# Comparison of Immediate and Delayed Transfer of Micro- Injected Oocytes into Fallopian Tubes: A Prospective, Randomized Clinical Trial

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Abstract

**Background:** Transfer of micro-Injected oocytes into fallopian tubes is an alternative procedure for IVF-ET with a similar success rate. This could be either done immediately after ovum pick-up microinjected oocytes intrafallopian transfer (MIFT) or after a time interval zygote intrafallopian transfer (ZIFT). This study was designed to compare the outcomes of the two procedures.

**Materials and Methods:** The study population included 149 infertile patients who needed assisted reproductive technologies (ART) and fulfilled the criteria for transfer of oocytes into tubes. 2-5 injected oocytes were transferred into normal fallopian tube either immediately (Group A) or 24 hours later (Group B).

**Results:** A total of 63 (36.9%) pregnancies were achieved. There were 33 pregnancies in the immediate transfer group and 30 pregnancies in the delayed transfer group. No significant difference was found in the implantation rate and the clinical pregnancy rate.

**Conclusion:** This study demonstrates that no difference could be observed in outcome between immediate and delayed transfer groups. Therefore, immediate transfer of microinjected oocytes into fallopian tubes or MIFT is the preferred method .

***Keywords:*** ZIFT, Intracytoplasmic Sperm Injection, Infertility

## Introduction

Zygote intrafallopian transfer (ZIFT) was first introduced in 1986 (1). It was claimed to be more successful than IVF–ET or GIFT in preliminary studies, because embryos were assisted by sojourn into the oviduct (2-6). There is no consensus about the superiority of this procedure (7, 8), and for this reason, the rate of performing ZIFT has declined recently (9). However, while the rate of performing ZIFT has declined, the pregnancy rate has been reported to be between 20.9% and 52.3% (10-16).

Immediate transfer of injected oocytes into the fallopian tubes is known as rapid intra cytoplasmic sperm injection (ICSI)-ZIFT. Sahebkashaf et al. presented a large series of rapid ICSI-ZIFT with pregnancy rates of 46% (17). There had been no significant difference in clinical pregnancy between the rapid ZIFT and pronuclear stage transfer of zygotes (delayed type) (18).

Vorsselmans et al. (19) introduced a similar procedure known as microinjected oocytes intrafallopian transfer (MIFT) with an ongoing pregnancy rate and implantation rate of 24% and 11%, respectively. They demonstrated that the pregnancy rate in the MIFT procedure did not differ from intrauterine transfer in patients younger than 37 years old (29% versus 35%).

Currently, rapid ICSI–ZIFT or MIFT are the main procedures in our center (17). In our opinion, couples with male factor infertility that have adequate sperm in the semen specimen for performing ICSI, normal hysterosalpingography, and normal laparoscopy could be candidates for MIFT.

Cervical stenosis and difficult embryo transfer (ET) in previous failed cycles are other indications for MIFT. The aim of this study was to observe if there exist significant differences in the pregnancy and implantation rate between the two procedures. We wanted to know whether the pregnancy rate differs between MIFT and ZIFT in our center.

## Materials and Methods

This was a prospective study, designed as a randomized clinical trial performed in the infertility unit of Dr. Shariati Hospital (a university teaching hospital). This study was approved by Institution Review Board and ethical committee of Tehran University of Medical Sciences and carried out during January 2006 to January 2007. All patients and their

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husbands signed written, informed consent forms.

***Randomizoation***
Randomization was completed after ovarian stimulation, sperm preparation, ovum pick–up, performing ICSI, confirming the presence of least one normal tube by laparoscopy, and before transfer of the injected ova into the fallopian tube. We used the method of Block randomization, which was computer generated using sealed envelopes (20).

***Patient Selection***
All patients fulfilled the selection criteria. Inclusion criteria were: female less than 40 years old; primary infertility male factor; azospermicmales; candidates for percutaneous epididymal sperm aspiration; or testicular sperm extraction. Exclusion criteria were: basal FSH<10 and basal E2 levels<80 pg/ml at the initiation of the ovarian stimulation (21). The patients with at least two metaphase 2 (M II) normal-shaped oocytes obtained by ovum puncture were considered suitable to enter the study.

***Ovarian Stimulation***
All patients were stimulated with a standardized stimulation protocol. Down-regulation of pituitary gland was performed by long-protocol GnRH agonist analogue. After 17 days of oral contraceptive pretreatment, buserlin Sc injection (superfact, Hoechst, Frankfurt, Germany) was administrated at a daily dose of 0.5 cc beginning immediately after discontinuation of oral contraceptives until hMG initiation on the third day of next menstrual cycle. On the day of initiation of hMG the dose of buserlin was lowered to 0.2 cc until hCG injection.
After confirmation of adequate pituitary desensitization by low serum E2 and FSH levels, hMG (pergonal; serono. S.P.A; Rome, Italy) was administrated at a daily dose of 150-300 IU / day for 6–7 days. The dose was then modified according to the ovarian response: when the mean diameter of three leading follicles reached 18 mm, hCG (10.000 IU) was given IM. Oocyte pick–up was performed 35 hours after hCG injection through a transvaginal route.

***Sperm Preparation***
All specimens were collected through masturbation at the clinical andrology laboratory after an abstinence period of 48-72 hours, on the morning of ovum pick–up. In general, semen samples were produced and prepared 0.5-2 hours before performing ICST. After liquefaction, routine sperm analysis was performed to measure sperm concentration, percentage of motility and normal morphology. Swim-up procedure was completed for all specimens (22).

***MIFT Procedure***
Oocytes were prepared immediately for ICSI by one embryologist. The method of ICSI was similar to that completed by other researchers (23, 24). The only difference in the faster procedure was omission of the two hour incubation period before ICSI.

***Transfer***
Patients with at least 2 MII oocytes were divided in 2 groups by randomization protocol. Transfer of injected oocytes into fallopian tube was immediately performed for group A (Immediate transfer or MIFT group), by cannulation of tubes under laparoscopy. Transfer of injected oocytes in Group B (delayed transfer or ZIFT) was performed 24 hours after ovum pick–up using the same methodology as Group A.

***Luteal Supplementation***
The luteal phase was supplemented with a vaginal administration of 800 mg of natural progesterone (cyclogest; Hoeschst) daily, beginning 24 hours after transfer of injected oocytes and continuing until 8 weeks of gestation.

***Outcome Measures***
A pregnancy was defined by the detection of a positive serum β-hCG (>200 mIU/mol) 18-19 days after MIFT or ZIFT. Clinical intrauterine pregnancy was confirmed by detection of a gestational sac with fetal pole and fetal heart rate (FHR) in the uterus 2-3 weeks later by transvaginal ultrasound.

***Statistical Analysis***
This study was designed to have sufficient power to detect an absolute difference ($p_2$) of 20% in the clinical pregnancy rate. The clinical pregnancy rate ($p_1$) at our center in 2003 for MIFT has been 41% (unpublished data). Therefore, p1 was estimated to be 0.41. The value of $p_2$ was chosen to be 0.2.
Because of the limited number of cases that potentially met the inclusion criteria, it was calculated that 80 patients in each group would have an adequate number to achieve an 80% power of detection of differences at a significance level (alpha) of 0.05, using a two-sided Z-test.
Differences between groups were evaluated using ANOVA. The P-value was set at 0.05. Data analysis was carried out using statistical package for the social sciences (spss 11.0; spss, Inc, Chicago; IL).

## Results
This study included 160 couple who were randomized into two groups. The mean characteristics are shown in Table 1.

**Table 1: Patient characteristics**

| Characteristics | Immediate Transfer (n=80) | Delayed Transfer (n=80) | P-value |
|---|---|---|---|
| **Age (y)** | 29.8 | 30.94 | 0.179(NS) |
| **Duration of infertility (y)** | 7.81 | 8.97 | 0.225(NS) |
| **Stimulation Period (d)** | 15.88 | 15.34 | 0.061(NS) |
| **No. of hMG Ampoules** | 38.58 | 38.33 | 0.929(NS) |
| **No. of Oocytes retrieved** | 7.86 | 8.94 | 0.188(NS) |
| **No. of injected Oocytes transferred** | 4.14 | 4.42 | 0.152(NS) |

**NS = Not Statistically Significant**

**Table 2: Outcome of Treatment.s**

| Outcome | Immediate Group (n=80) | Delayed Group (n=80) | P-value |
|---|---|---|---|
| **Implantation Rate n(%)** | 38/320 (11.8) | 32/320 (10) | 0.13 |
| **Clinical Pregnancy Rate n (%)** | 33/80 (41.2) | 30/80 (37.5) | 0.11 |

**NS = Not Statistically Significant**

Table 1 lists the patients' characteristics in each group. No significant difference was found in the mean age, duration of infertility, stimulation period, number of hMG ampules, number of oocytes retrieved and number of injected oocytes transferred into fallopian tubes, in two groups.

A total of 63 (39.3%) clinical pregnancies were reported. In the immediate group, 33 (41.2%) clinical pregnancies (29 single, 3 twins, 1 triplets), and in the delayed group 30 (37.5%) clinical pregnancies (28 single, 2 twins) were achieved. No significant difference was observed in the implantation rate and also in clinical pregnancy rate (Table 2) (25).

## Discussion

ZIFT has been considered as an alternative to *in vo-tro* fertilization (IVF)-ET. Although this procedure is not cost-effective, it has its own indications. Unexplained infertility (3), male factor infertility (4), repeated failure of implantation in IVF-ET (14), tubal factor infertility with repeated failure of implantation in IVF-ET (15), and cervical factor (26) are some indications cited in the literature for ZIFT. Transfer of zygote in ZIFT is commonly performed 24-40 hours after ovum pick–up when pronucleous is observed, but intrafallopian transfer of ova immediately after ICSI (Rapid ICSI-ZIFT or MIFT) is performed without the classic period of waiting for observation of pronucleous.

Severe male factor infertility has been introduced as one of the ZIFT indications. In a study conducted by Kashaf et al, the clinical pregnancy rate of ZIFT in severe male factor infertility was 45% (27). In a large multicenter study the success of his method for male factor infertility was 46% (17).

This method is suitable for patients with a history of difficult intra uterine transfer, and we believe it might be suitable for patients with proven fertilization and failed ART cycles.

Effect of failed fertilization following immediate transfer has always been a point for consideration. Therefore, the aim of this study was to evaluate the effect of immediate transfer when occurrence of fertilization is not evident, to delayed transfer when signs of fertilization are definite. Therefore, the results of our study suggest no significant differences were observed in pregnancy and implantation rate between immediate and delayed transfer (28-30).

## Conclusion

Based on our search, there is no similar study in the literature suggesting any difference between the two procedures. Thus, immediate transfer of injected oocytes into fallopian tube can offer distinct advantages over delayed transfer. The advantages include, less time consumption and less expensive; since there is only one episode of anesthesia and operation, and in addition there is less laboratory effort. Therfore we propose immediate transfer or MIFT instead of delayed transfer or ZIFT.

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