Medical Journal of the
Islamic Republic of Iran

Volume 11
Number 3
Fall 1376
November 1997

Original Articles

INTRACYTOPLASMIC SPERM INJECTION FOR THE TREATMENT OF MALE FACTOR INFERTILITY—THE FIRST PRELIMINARY REPORT FROM IRAN

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ABSTRACT

The latest micromanipulative technique, intracytoplasmic sperm injection (ICSI), has been very successful in cases of severe male factor infertility. The main objective of this study is to report the results of our first experience with ICSI in cases of male factor infertility, as well as patients who had failed their previous cycles with conventional *in vitro* fertilization (IVF). Normal fertilization occurred in 30.9% of oocytes retrieved from 99 patients. Embryo transfer was done for 67.7% of the patients, and a total of 12 (12.12%) clinical pregnancies were achieved. The success rate was noticeably lower in patients greater than 35 years old, and in patients with severely abnormal sperm parameters (e.g., oligoasthenoteratozoospermia). This study suggests that ICSI is a method of choice to treat couples with severe male factor infertility. Patients' age, as well as sperm parameters, can affect the success rate of infertility treatment with ICSI.

Keywords: Infertility, IVF.

MJIRI, Vol. 11, No. 3, 181-185, 1997.

INTRODUCTION

Infertility treatment is a rapidly growing field, with new techniques and advancements in the existing techniques. *In vitro* fertilization (IVF) is usually compromised when an insufficient number of spermatozoa are available.[14] However, recently, several micromanipulation techniques have been developed as assisted fertilization tools in cases of low and/or abnormal spermatozoa. These include zona drilling (ZD),[14] partial zona dissection (PZD),[6] subzonal insemination (SUZI),[4,16] and most recently, the advancing technique of direct intracytoplasmic sperm injection (ICSI).[10,11,20] This technique is aimed at patients who have impaired spermatozoa and in which IVF and perhaps some other infertility treatments have already failed.

Micromanipulation with ICSI involves the injection of only one immobilized, but vital, spermatozoon directly into the ooplasm of the oocyte.[11,20] Alternatively, this will initiate the biochemical processes necessary for oocyte

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activation.[8,10] The very important advantage of ICSI as compared to other micromanipulation techniques is the absolute certainly that only one selected sperm enters the ooplasm in order to initiate the fertilization of the oocyte.[8] This will certainly prevent polyspermia, which has been reported from other techniques. In addition, the sperm will easily pass the thick barrier, the zona pellucida, which surrounds the oocyte.[1,8]

For the first time in Iran, the micromanipulation technique of ICSI was established as part of our infertility treatment program in early February 1995 for two specific groups of infertile patients: (1) couples who had undergone earlier standard IVF, but had failed, and (2) couples in whom the male was responsible for infertility (male factor infertility), because of severely abnormal and/or low count spermatozoa. The aim of this preliminary report is to present our experience with ICSI in cases of male factor infertility and previously failed IVF treatments; ours being not only the first research and clinical center for infertility established in Iran, but also the first to use ICSI.

MATERIALS AND METHODS

Patient selection

A total of 107 patients were offered assisted fertilization by ICSI during the period from March 20 to July 20, 1995 (4 months). Criteria for the selection of these couples were either sperm characteristics which were too abnormal for them to be included in standard IVF treatment, or the lack of fertilization in previous IVF cycles. Overall, the mean ages of the female and male partners were 32 (range 17-40 years) and 34 years (range 25-55 years), respectively. No restrictions were applied with regard to patient's ages. Since our center was the first to offer the micromanipulation treatment, patients were referred from all over the country. The mean duration of infertility was 9.5 years (range 1-27 years). The infertile couples were screened for hepatitis B antigen (HBsAg) and human immunodeficiency virus (HIV).

Using the World Health Organization guidelines for normal semen (>20×10^6/mL, ≥50% forward progressive motility, ≥30% normal morphology), the samples were categorized into normozoospermic, and spermatozoa with single, double, and triple defects.[21]

Semen preparation and oocyte collection

Fresh, ejaculated semen was collected in an individual sterile glass container after at least 48 h of sexual abstinence. The patients were asked to produce the sample at the center, and deliver it to the Androloy laboratory no later than a few minutes post-ejaculation. The color and volume were recorded and the sample was allowed to liquify at 37°C, for 1 h before processing. The following sperm parameters were evaluated: sperm count, motility, vitality, and morphology according to WHO standards. For sperm selection, swim-up separation method from unprocessed semen was used in Ham's F10, and centrifuged (5 min; 500 g). The supernatant was discarded and the remaining pellet resuspended with 1 mL of Ham's F10 medium. After swim-up, the sperm parameters were evaluated again. Washed sperm preparations were incubated at 37°C in an atmosphere of 5% CO_2 in air until required. In the case of severe oligoasthenozoospermia, spermatozoa were prepared by single centrifugation (2800 g for 45 min) with a mini-Percoll method.

Oocytes were recovered from ovarian follicles by ultrasonically-guided follicular aspiration in cycles stimulated with follicle stimulating hormone (FSH) and/or human menopausal gonadotropin (HMG). HMG was injected (i.m.) on the 2nd or 3rd day of the menstruation cycle. After the last HMG injection and when there were at least two to three follicles of over 18 mm in diameter, human chorionic gonadotropin (HCG), 5,000-10,000 IU, was injected. Follicular response was transvaginal-directed, and retrieval of oocytes from the follicles took place 34-36 h after the HCG injection. The retrieved oocytes were graded on a 3-point scale for shape, size, and degree of fragmentation: 1 point for "poor", 2 points for "reasonable", and 3 points for "good". Before ICSI, oocytes were cultured in Ham's F10 in a CO_2 incubator for 6 h. The cumulus oophorus and the corona radiata of oocytes were mechanically removed. Handling of the oocytes was performed with cleaned and sterilized glass pasteur pipettes.

Micromanipulation techniques

First, both holding and injecting pipettes were produced from glass capillaries. The injection pipettes were produced by pulling the capillaries in a Microelectrode puller (Model p-87; Sutter Instrument Co., CA). The holding pipette was pulled manually over a flame; an inner diameter of 20 to 30 µm was obtained by fire-polishing the pipette on a Narshige MF-9 microforge (Tokyo, Japan). The injection pipette had an inner diameter of 4 to 5 µm. The ICSI procedure was carried out on the heated stage (37°C) of an Olympus IMT-2 inverted microscope equipped with a modulation contrast system (Olympus, Japan). The injection dish contained a central sperm droplet which was a mixture of 4 µL of sperm solution. The central sperm droplet was surrounded by eight droplets of Ham's F10, each containing a single oocyte. For the injection, one oocyte was held in place on the holding pipette by slightly negative pressure. Then, a single, mechanically immobilized, living spermatozoon was aspirated into the injection pipette to be introduced into the oocyte cytoplasm at the 3 o'clock position. The motile spermatozoon was immobilized by placing the injecting pipette on the sperm tail and moving it across the tail. Then, the immobilized sperm was drawn tail-first into the injection

pipette. The spermatozoon was deposited with the smallest amount of the medium. Immature and morphologically abnormal oocytes were not injected. After ICSI, the oocytes were washed and returned to Petri dishes containing Ham's F10, to be stored in the incubator at 37°C.

Fertilization was assessed 17 to 18 h after ICSI. The oocytes that did not present the pronuclei were also assessed at 48 h, in order to evaluate any embryo development occuring as a result of delayed fertilization. The embryos were observed at low as well as high magnification on an inverted microscope. The embryos were evaluated and scored according to the following morphological criteria: type A or "excellent" were defined as embryos in which all blastomeres were of equal size with no fragmentation, type B or "good" had blastomeres of relatively equal size with a minimum of fragmentation, Type C or "intermediate" had fragmentations in 30-50% of the volume of the embryo; and type D or "poor" with unequal blastomeres, with full fragmentation. At the time of embryo-transfer (ET), the best three embryos in terms of morphology and the rate of development were selected. In a few cases, usually in older patients, four to five embryos were transferred.

Pregnancy was confirmed when serum hCG concentrations were rising around by following embryo transfer. In the case of a positive result, ultrasonography was carried out 3 weeks later to determine the viability of the gestational sacs.

RESULTS

The clinical results of the ICSI cycles are shown in Table I. A total of 746 oocytes were collected (mean 6 per cycle), of which 585 (78.7%) were suitable for micro-injection. Of microinjected oocytes, a total of 181 (30.9%) achieved fertilization. A total of eight cycles (7.5%) were cancelled due to various reasons, such as poor ovarian response or husband's inability to produce semen. Fifteen of the patients had been enrolled in one or more previous IVF programs.

A total of 5 semen samples were normozoospermic (5%), while 9 (9%) and 11 (11%) of the samples were teratozoospermic and oligozoospermic, respectively. The patients with normozoospermic semen had been enrolled in one or two previous IVF programs, but the cycles were unsuccessful. Therefore, it had been recommended that they enroll for ICSI. Asthenozoospermic samples or samples with forward progressive motility below 50% were more common (16.16%). Also, 31 (31.3%) of the husbands had severe defects of oligoasthenoteratozoospermic spermatozoa. The outcome of ICSI related to the number of sperm defects present is shown in Table II.

From the 99 patients involved in treatment cycles, 67 had embryos transferred (67.7%). Only one patient was a surrogate. Thirty-two couples (32.3%) had transfer of three embryos, whereas 24 (24.2%) and 11 (11%) had two and one embryos transferred, respectively. High pregnancy rates were observed when two/three embryos with grade A were transferred. The pregnancy rate was significantly reduced after the age of 35 (Table III). A total of 12 patients became pregnant, of whom two have recently delivered a normal healthy baby.

Table I. Clinical results of intracytoplasmic sperm injection.

No. of patients	99
Average female age	32
No. of retrieved oocytes	746
No. of injected oocytes	585
Fertilized oocytes	181
No. of embryos transferred	67
No. of pregnancy	12
Take-home baby (so far)	2
Pregnancy rate per embryo transferred	18%

Table II. Results of intracytoplasmic sperm injection in relation to semen parameter.

Semen characteristics	No.	Fertilization	Pregnancy
Normozoospermic	5	3	1
Teratozoospermic	9	5	2
Oligozoospermic	11	8	4
Asthenozoospermic	16	10	3
Oligoasthenozoospermic	17	3	1
Asthenoteratozoospermic	2	0	0
Oligoteratozoospermic	8	2	0
Oligoasthenoteratozoospermic	31	8	1

Table III. Results of intracytoplasmic sperm injection in relation to female age.

Age	No. of patients	Total oocytes retrieved	Pregnancy
≤35	85	664 (7.81%)*	11 (12.94%)*
>35	14	82 (5.86%)*	1 (7.14%)*

*Values in parentheses are percentages of the average number of oocytes retrieved and rate of pregnancy per patients.

DISCUSSION

Lately, assisted fertilization by the micromanipulation of retrieved oocytes in an attempt to achieve fertilization in those with previous failure of IVF or severe male infertility, is a common practice in many infertility centers throughout

the world.[1,2,9,10,11,22] During the last few years, several micromanipulation techniques have been introduced, although the most promising of these has been the technique of direct intracytoplasmic sperm injection which was first reported by Palermo etal. in Belgium.[10,11] A high fertilization rate along with subsequent pregnancy rates via ICSI have been reported by several investigators.[1,7,8,11,13,15,17] However, Hoshi et al.[5] recently reported a very low success rate in embryo transfer and pregnancy following ICSI. They applied microinjection using motile and immobilized spermatozoon.

The present study is the first preliminary report on pregnancies obtained after ICSI at the first infertility center established in Iran. This technique was introduced as the first micromanipulation technique during February 1995. The recent technique of subzonal insemination (SUZI)[1,8] was not practiced at this infertility center, because several outstanding investigators conducted controlled comparisons of SUZI and ICSI, and report much higher fertilization and pregnancy rates after ICSI.[1,2,8,19]

In contrast to the results of Cohen et al,[2] Palermo et al,[10,11] and Tsirigotis et al,[17] semen parameters (forward-progressive motility and morphology) seemed to correlate directly with the outcome of the assisted fertilization via ICSI. The motility with >40% forward progression certainly improved the success rate. Patients with two or more spermatozoal defects showed lower success rates when compared with the outcomes from the group with only one spermatozoal defect. As shown in Table II, triple sperm defects were significantly high in the present study (approximately one-third of the patients), and a very low success rate was achieved in them. However, only a small number of patients were used in this study; a larger series of patients are needed to be able to make the final judgment.

In light of the success rate, it seems that the fertilization/ pregnancy rates following ICSI at our center are lower when compared to results from other well-established centers. Tsirigotis et al.[17] reported fertilization and pregnancy rates of 55.2% and 36.2% per treatment cycle, respectively. Also, the Brussels group observed high fertilization (51%) and pregnancy (35.3%) rates with ICSI.[10] One major factor which might have affected the outcome of the results was the age of the female patients. Patients who were 35 or more showed a much lower success rate (Table III). A total of fourteen females were over 35 with only one on-going pregnancy. In this regard, Tucker et al.[18] reported a 31% oocyte degeneracy rate after ICSI in older patients (> 35 years old), compared with only 9% degeneracy in younger patients (≤ 35 years old). As a consequence, the pregnancy rate was also significantly lower in older patients (10.8% versus 35.3%) than in younger patients. In particular, this makes the outcome even worse when combined with severe spermatozoal defects.

In conclusion, we believe that the micromanipulation technique of ICSI is the method of choice for severe male factor infertility. Considering the short time period during which such a sophisticated technique has been in use at this center, we believe the results are satisfactory. We also however believe that careful patient selection, along with an improvement in the micromanipulation technical skill concurrent with greater experience will ICSI, will certainly improve the success rate. In addition, the enhancing effect of pentoxifylline on sperm motility and, hence, the fertilizing capacity of asthenospermic semen samples, is being currently evaluated at our center. It is now well established that a higher fertilization success rate is correlated with the improved progressively motile spermatozoa.[3,11,12]

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the excellent technical assistance of Mrs. Marjan Kazemi, Miss Fakhrozaman Moghimi, and Mr. Mehrdad Solaimani. The authors also appreciate the assistance of Mrs. Sara Thurman, M.A., M.S., for correcting this manuscript.

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