In Vitro Fertilization and Intracytoplasmic Sperm Injection for Couples with Unexplained Infertility After Failed Direct Intraperitoneal Insemination

SHIGETO TAKEUCHI,[1,2] HIROYUKI MINOURA,[1] TAKASHI SHIBAHARA,[1] XIAOXIONG SHEN,[1] NORITAKA FUTAMURA,[1] and NAGAYASU TOYODA[1]

Submitted: March 3, 2000
Accepted: April 21, 2000

***Purpose**: The objective was to determine the optimal insemination technique in patients undergoing in vitro fertilization (IVF) after failed direct intraperitoneal insemination (DIPI) and the outcome of intracytoplasmic sperm injection (ICSI) in such cases.*

***Methods**: In case–control studies, 53 couples with unexplained infertility who underwent IVF after four failed DIPI cycles were compared with 75 couples with tubal or endometriosis infertility as controls. Thirty couples with unexplained infertility after failing to conceive with DIPI and conventional IVF who underwent ICSI and 58 couples with male-factor infertility as controls also were compared. Fertilization cleavage, embryo quality, implantation, and pregnancy were compared after IVF and after ICSI.*

***Results**: There was a significant difference in fertilization rates after IVF between cases of unexplained infertility after failing to conceive with DIPI (40.4%) and patients with tubal or endometriosis infertility (67.9%). There also was a significant difference in total fertilization failure rates between the two groups (30.4% and 3.9%, respectively). There was a slight but significant difference in numbers of fertilized oocytes after ICSI between patients with low fertilization rate undergoing IVF after failing to conceive DIPI (85.8%) and patients with male factor (90.4%). Total fertilization failure was not observed in these cases.*

***Conclusions**: Couples with unexplained infertility after failing to conceive with DIPI show a failed fertilization or a low fertilization rate after IVF. However, they demonstrated a good chance of becoming pregnant after subsequent ICSI, even with statistically significant difference in fertilization rate as compared with male-factor cases.*

KEY WORDS: Intracytoplasmic sperm injection; unexplained infertility; in vitro fertilization; direct intraperitoneal insemination; failed fertilization.

INTRODUCTION

The prevalence of infertile couples without any identifiable cause (unexplained infertility) has been reported to vary between 1% and 37% (1). Intrauterine insemination (IUI), especially direct intraperitoneal insemination (DIPI), with previously reported pregnancy rates of 22.7% (2), 23.0% (3), or 35.6% (4), appears to offer the advantage of being a relatively noninvasive way of dealing with unexplained infertility and is well worth trying before moving on to more invasive and costly approaches such as gamete intrafallopian transfer (GIFT) or in vitro fertilization (IVF). Gurgan *et al.* (5) and Ruiz *et al.* (6) reported that there was a higher incidence of total fertilization failure events in couples with unexplained infertility. However, the prognosis of couples failing to conceive with superovulation and DIPI is unknown. Furthermore, it remains to be defined whether couples failing to conceive with superovulation and DIPI represent a less favorable subset of patients for subsequent treatment with more sophisticated means of assisted reproduction. The present study was designed to determine the prognosis after IVF and subsequent ICSI in couples with unexplained infertility failing to conceive with superovulation and DIPI.

[1] Department of Obstetrics and Gynecology, Mie University School of Medicine, Mie, Japan.
[2] To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Mie University School of Medicine, 2—174 Edobashi, Tsu, Mie, Japan.

1058-0468/00/1000-0515$18.00/0

MATERIALS AND METHODS

Patient Selection

The patients were divided into four groups according to the underlying indication for IVF or ICSI. The first IVF group consisted of 53 patients with unexplained infertility who had failed to conceive with four to six episodes of superovulation and DIPI. The second group consisted of 75 patients undergoing IVF because of endometriosis or a tubal factor. The third group consisted of 30 patients undergoing ICSI because of failed fertilization or a low fertilization rate in the first group. The fourth group consisted of 58 patients undergoing ICSI because of severe male factor problems according to the criteria of the World Health Organization (WHO) (7). Unexplained infertility was defined as a normal semen analysis according to the criteria of the WHO, including the absence of antisperm antibodies, and postcoital test. Endometriosis was diagnosed by laparoscopy or laparotomy.

Ovarian Stimulation and Oocyte Collection

Ovarian stimulation was carried out by a desensitizing protocol using the gonadotropin-releasing hormone agonist (GnRHa) buserelin acetate (Supurecur; Hoechst Japan) in combination with human manopausal gonadotropin (hMG) (Humegon; Organon, Japan; or Pergogreen; Seronol Japan). The response to stimulation was determined by serial estradiol determination and ovarian ultrasonograms. When the leading follicle achieved a diameter of 18 mm, human chorionic gonadotropin (hCG) (10,000 IU) was administered and transvaginal ultrasound-guided oocyte retrieval was carried out 36 hr later. hCG injection (1500 IU) on days 2 and 4 and intravaginal micronized progesterone (400 mg/day) were given for luteal support.

Preparation of Sperm

Semen samples were allowed to liquefy for 20 min, prepared with the standard swim-up technique for IVF, and processed through discontinuous Percoll gradients (40% and 90%) for ICSI. Percolled sperm pellets were washed twice and resuspended in HEPES buffered human tubal fluid (modified-HTF, Irvine Scientific, CA) before use.

Preparation of Oocytes

Oocytes were rinsed thoroughly and kept in HTF with 10% heat-inactivated maternal serum [insemination medium, (IM)] for up 5 hr at 37°C under 5% CO_2 in air. For IVF, fertilized oocytes were transferred to growth medium consisting of HTF with 15% heat-inactivated maternal serum. For preparation of ICSI, the cumulus was removed by repeated aspiration into glass pipettes in modified-HTF with 0.025% hyaluronidase type VIII (Sigma Chemical Co.). Denuded oocytes were thoroughly rinsed and returned to the IM. Only metaphase II oocytes were used for ICSI.

Intracytoplasmic Sperm Injection

The ICSI procedure was performed according to the method described by Kimura and Yanagimachi (8) with minor modifications. Briefly, several piezo-pulses (intensity 2, speed 2) were administered to advance the pipette until its tip passed through the zona pellucida. The pipette then was advanced quickly and manually through the ooplasm until it reached one third of the distance to the opposite side of the cortex of the oocyte. The oolemma was broken (as confirmed by its relaxation) using a light negative pressure without piezo-pulses.

The cover (10 mm in depth) of a plastic dish (60 × 15 mm) (Falcon Plastics, Oxford) was used as a microinjection chamber. A row consisting of two round droplets and three elongated drops was placed along the center line of the dish. The first droplet (3 μl) was formed from 10% polyvinylpyrrolidone (PVP) (Sigma) dissolved in Dulbecco phosphate-buffered saline (GIBCO BRL) and was for washing the pipette. The second droplet (3 μl) was a suspension of sperm in 10% PVP. The third elongated droplet (3 μl) was modified-HTF containing prepared sperm. The fourth and fifth elongated droplets (3 μl) were for the oocytes. These droplets were covered with mineral oil (Sigma). The dish was placed on the stage of an inverted microscope (Olympus, Tokyo, Japan) and its contents viewed with the aid of Nomarski differential interference optics. Motile sperm were aspirated from the suspension into the injection pipette, transferred to a drop containing an oocyte, and immobilized by crushing the tail between the injection pipette and the dish. Intracytoplasmic sperm injection was performed on the microscope. After injection, oocytes were transferred to growth medium consisting of HTF with 15% heat-inactivated maternal serum.

Survival, Fertilization, and Embryo Cleavage

The oocytes were deemed to have survived the ICSI procedure if they remained intact and failed to show any obvious signs of lysis or degeneration over the 2 days following injection. Oocytes were examined for fertilization approximately 16 to 18 hr after injection. Normal fertilization was considered to have occurred when two clearly distinct pronuclei (PN) were present. The number of abnormal fertilizations, such as oocytes with one PN or three PN, also was recorded. After another 24-hr in vitro culture, three or four embryos were transferred into the uterus. Embryos were classified following the Veeck classification. Clinical pregnancies were confirmed by the presence of a gestational sac on ultrasonography 4 to 6 weeks after embryo transfer.

Statistical Evaluation

Data were expressed as a mean ± SEM values. For statistical comparisons, the χ^2 test was employed. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

IVF was performed for 53 patients (53 cycles) with unexplained infertility who had failed to conceive with four to six episodes of superovulation and DIPI and 75 patients (128 cycles) because of endometriosis or tubal factor problems (Table I). Age, the dose of menotropin administration, estradiol level, progesterone level, endometrial thickness on hCG administration, the number of retrieved oocytes per patient, and surviving rate for insemination oocytes did not differ statistically significantly between the groups. The fertilization rate, when total fertilization failure was excluded from the analysis, was significantly lower for the group of patients with unexplained infertility who had failed to conceive with DIPI (52.0%, respectively) than for the endometriosis or tubal factor group (68.4%, respectively) ($P < 0.01$). The incidence of total fertilization failure was 30.2% and 3.9% in the group of patients with unexplained infertility who had failed to conceive with DIPI and the endometriosis or tubal factor group, respectively ($P < 0.01$). The cleavage rate was significantly higher in the group patients with unexplained infertility who had failed to conceive with DIPI (96.4%) than in the endometriosis or tubal factor group (89.9%) ($P < 0.01$). The morphological quality of the oocytes cleaved, mean number of embryos transferred, and embryo implantation rates were similar in both groups. The pregnancy rate per embryo transfer, when events of total fertilization failure or low fertilization rate were excluded from the analysis was similar in both groups (54.2% and 36.8%, respectively). The embryo transfer cycle cancellation rate was significantly higher in the group of patients with unexplained infertility who had failed to conceive with DIPI (57.4%) than in the endometriosis or tubal factor group (10.9%) ($P < 0.01$).

ICSI was performed for 30 patients (49 cycles) because of failed fertilization or a low fertilization rate in the first group and 58 patients (98 cycles) because of severe male factor (Table II). Age, the dose of menotropin administration, estradiol level, progesterone level, endometrial thickness on hCG administration, the number of retrieved oocytes per patient, and survived rate for inseminated oocytes did not differ statistically significantly between the groups. The fertilization rate was slightly but significantly lower in the group patients with failed fertilization or a low fertilization rate (85.8%) than in severe male factor group (90.4%) ($P < 0.05$). Total fertilization failure was not observed in either group. The cleavage rate was significantly higher in the group of patients with failed fertilization or a low fertilization rate (93.9%) than in severe male factor group (83.9%) ($P < 0.01$). The morphological quality of the oocytes cleaved, mean number of embryos transferred, embryo implantation rate, pregnancy rate per embryo transfer, and embryo transfer cycle cancellation were similar in both groups.

DISCUSSION

Different methods of assisted reproduction such as superovulation with IUI (9), fallopian tube sperm perfusion (FSP) (10), DIPI (2–4), gamete intrafallopian tube (GIFT) transfer (11), and IVF (12) have been used for the treatment of unexplained infertility. We (4) reported women with unexplained infertility who failed to conceive after repeated superovulation and IUI had a favorable outcome when treated with a combination of DIPI and GnRHa/hMG/hCG without complications. DIPI is less expensive and invasive than either IVF-ET or GIFT. Therefore, we performed DIPI on women with unexplained infertility who failed to conceive after repeated superovulation and IUI prior to more invasive assisted reproductive

Table I. Date for Tubal/Endometriosis and Unexplained Factor Couples Undergoing IVF-ET*

	Tubal/endometriosis	Unexplained	*P* value
No. of couples	75	53	
No. of retrieval cycles	128	53	
Age (years)[a]	32.8 ± 0.4	33.5 ± 0.5	NS
hMG dose (IU)[a]	1826.1 ± 57.6	1578.2 ± 124.7	NS
Thickness of endometrium (mm)[a]	10.05 ± 0.19	9.57 ± 0.21	NS
Serum estradiol value (pg/ml)[a]	1355.8 ± 84.6	1578.2 ± 124.7	NS
Serum progestrone (ng/dl)[a]	0.617 ± 0.040	0.562 ± 0.041	NS
No. of retrieved oocytes	949	416	
No. of retrieved oocytes per patient[a]	7.3 ± 0.5	7.9 ± 0.47	NS
No. of surviving oocytes (%)	932(98.2)	416(100)	NS
No. of fertilized oocytes (%)	644(67.9)[a]	168(40.4)[b]	$p < 0.01$
No. of total fertilization failures (%)	5(3.9)[b]	16(30.2)[a]	$p < 0.01$
No. of fertilized oocytes (total fertilization failure excluded) (%)	644(68.4)[a]	168(52.0)[b]	$p < 0.01$
No. of cleaved embryos (%)	579(89.9)[b]	162(96.4)[a]	$p < 0.01$
No. of good quality embryos (grade 1,2) (%)	312(48.4)[b]	82(50.6)[a]	$p < 0.01$
No. of transferred embryos	326	606	
No. of embryos per transfer cycle[a]	3.1 ± 0.1	3.1 ± 0.2	NS
No. of transfer cycles	114	25	
No. of pregnancies per transfer cycle (%)	42(36.8)	13(54.2)	NS
Implantation rate (%)	53(15.0)	14(15.1)	NS
Cycle cancellation (%)	14(10.9)[b]	29(54.7)[a]	$p < 0.01$

[a] Values are means ± SEM. NS = not significant.
* Within a row, means with different superscripts are different ($p < 0.01$).

techniques, such as IVF-ET or GIFT. It is unknown, however, whether lack of conception with such treatment options dictates a less favorable prognosis for future attempts at a more complex form of assisted conception methods such as IVF.

Our results indicate that couples with unexplained infertility who fail to conceive after four to six cycles of superovulation and DIPI have relatively good conception rates in subsequent IVF-ET. When compared with endometriosis or tubal factor patients undergo-

Table II. Date for Male Factor and Previous Failed Fertilization or Low Fertilization Rate Couples Undergoing ICSI*

	Male	Previous failed fertilization or low fertilization rate	*P* value
No. of couples	58	30	
No. of retrieval cycles	98	49	
Age (years)[a]	32.9 ± 0.5	33.8 ± 0.6	NS
hMG dose (IU)[a]	1739.4 ± 63.4	1514.6 ± 75.5	NS
Thickness of endometrium (mm)[a]	0 ± 0.25	9.69 ± 0.27	NS
Serum estradiol value (pg/ml)[a]	1718.2 ± 102.1	1764.7 ± 117.1	NS
Serum progestrone (ng/dl)[a]	0.569 ± 0.038	0.559 ± 0.048	NS
No. of retrieved oocytes	709	325	
No. of retrieved oocytes per patient[a]	9.0 ± 0.5	9.0 ± 0.7	NS
No. of survived oocytes (%)	680(96.0)	325(100)	NS
No. of fertilized oocytes (%)	641(90.4)[a]	279(85.8)[b]	$P < 0.05$
No. of total fertilization failure (%)	0(0)	0(0)	NS
No. of cleaved embryos (%)	538(83.9)[b]	262(93.9)[a]	$P < 0.01$
No. of good quality embryos (grade 1,2) (%)	254(47.2)	126(48.1)	NS
No. of transferred embryos	297	152	
No. of embryos per transfer cycle[a]	3.3 ± 0.1	3.2 ± 0.1	NS
No. of transfer cycle	90	47	
No. of pregnancies per transfer cycle (%)	36(40.0)	17(36.2)	NS
Implantation rate (%)	46(15.5)	18(11.8)	NS
Cycle cancellation (%)	8(8.2)	2(4.1)	NS

[a] Values are means ± SEM. NS = not significant.
* Within a row, means with different superscripts are different ($p < 0.05$).

ing similar treatment, however, they showed a higher incidence of total fertilization failure. Fertilization rates were not similar in two groups when events of total fertilization failure were excluded from analysis of data. Kahn *et al.* (10), Gurgan *et al.* (5), and Ruiz *et al.* (6) reported total failure of fertilization in 30.0%, 20.4%, 11.4%, respectively, of cases of unexplained infertility undergoing IVF after failure of superovulation and IUI. Audibert *et al.* (12) reported total failure of fertilization in 22.7% of cases of unexplained infertility. The rate of total fertilization failure in our study (30.2%) was slightly higher than theirs. The reason for the discrepancy may be explained by the fact that pregnancy rate per cycle with superovulation and DIPI even in patients with unexplained infertility failing to conceive after four to six cycles of superovulation and IUI is 35.6% in our data (4). Because the rate of the low or total fertilization failure with unexplained infertility was higher than tubal and endometriosis infertility, the cancellation of embryo transfer with unexplained infertility was higher than tubal and endometriosis infertility (Table I).

In the present study, although the fertilization rate obtained with ICSI was lower in patients with previous failed fertilization or a low fertilization rate than in patients with male factor, it was still 85.8%, which is higher than in other reports (13,14). The reason for the difference might be due to absent or abnormal oocyte activation (15). Similar findings also were reported by Gabrielsen *et al.* (16). In contrast to our and previous studies, Tomas *et al.* (13) and Miller *et al.* (14) reported fertilization rates obtained with ICSI to be similar in patients with previous failed fertilization or a low fertilization rate and in patients with male factor. The reason for failed fertilization after conventional IVF programs may reside in abnormal spermatozoa and/or oocytes, with lack of penetration of the zona pellucida and the ooplasmic membrane. This problem can be resolved partly by ICSI so that acceptable rates of fertilization can be achieved, as shown here.

In the present study, the implanation and pregnancy rates obtained with ICSI were similar in patients with previous failed fertilization or a low fertilization rate to those in the male factor group, in line with the findings of Ruiz *et al.* (6) and Svalander *et al.* (17). In contrast, Tomas *et al.* (13) and Miller *et al.* (14) reported implanation and pregnancy rates obtained with ICSI to be lower in patients with previous failed fertilization or a low fertilization rate than in patients with male factor. Maternal age was older in patients with previous failed fertilization or a low fertilization rate than in patients with male factor, in the reports of Tomas *et al.* (13) and Miller *et al.* (14), but in our study was similar in both groups. Implanation failure in older women could be due to aneuploidy as noted by Munne *et al.* (18). Therefore, it is possible that embryos with ICSI from older women with previous failed fertilization or a low fertilization rate may have genetic defects that prevent normal embryonic development.

In summary, the present study demonstrated that once a failed cycle has occurred in patients with unexplained infertility failing to conceive after four to six cycles of superovulation and DIPI, ICSI should be the method of choice in a second attempt to avoid failed fertilization or a low fertilization rate, because fertilization failure in couples with unexplained infertility appears to be more likely to recur than in tubal factor patients (5).

REFERENCES

1. Collins J, Crosignani P: Unexplained infertility: A review of diagnosis, prognosis, treatment efficacy, and management. Int J Obstet Gynecol 1992;39:267–275
2. Crosignani PG, Lauretis LD, Ragni G, Olivares MD, Finzi G, Perotti L: Intraperitoneal insemination in the treatment of male and unexplained infertility. Fertil Steril 1991;55:333–337
3. Turhan NO, Artini PG, Ambrogio G, Droghini F, Volpe A, Genazzani AR: Studies on direct intraperitoneal insemination in the management of male factor, cervical factor, unexplained and immunological infertility. Hum Reprod 1992;7(1):66–71
4. Minoura M, Takeuchi S, Xiaoxiong S, Shibahara T, Fumamura N, Toyoda N: Increasing the pregnancy rate after combined treatment with hMG/hCG and direct intraperitoneal insemination. J Reprod Med 1999;44(1):18–22
5. Gurgan T, Urman B, Yarali H, Kisnisci HA: The results of in vitro fertilization–embryo transfer in couples with unexplained infertility failing to conceive with superovulation and intrauterine insemination. Fertil Steril 1995;64:93–97
6. Ruiz A, Guanes PP, Remohi J, Simon C, Minguez Y, Pellicer A: The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. Fertil Steril 1997;68:171–173
7. World Health Organization. Laboratory Manual for the Examination of Human Semen and Semen Cervical Mucus Interaction, 3rd ed. New York, Cambridge University Press, 1993, 43–44
8. Kimura Y, Yanagimachi R: Intracytoplasmic sperm injection in the mouse. Biol Reprod 1995;52:709–720
9. Aboulghar MA, Mansour RT, Serour GI, Amin Y, Abbas

AM, Salash IM: Ovarian superstimulation and intrauterine insemination for the treatment of unexplained infertility. Fertil Steril 1993;60:303–306
10. Kahn JA, Sunde A, During VV, Sordal T, Molne K: Treatment of unexplained infertility, fallopian tube sperm perfusion (FSP). Acta Obstet Gynecol Scand 1993;72:193–199
11. Asch RH, Balmaceda JP, Ellsworth LR, Wong PC: Preliminary experience with gamate intrafallopian transfer (GIFT). Fertil Steril 1986;45:366–
12. Audibert F, Hedon B, Arnal F: Results of IVF attempts in patients with unexplained infertility. Hum Reprod 1989;4: 766–771
13. Tomas C, Orava M, Tuomivaara L, Martikainen H. Low pregnancy rate is achieved in patients treated with intracytoplasmic sperm injection due to previous low or failed fertilization in in vitro fertilization. Hum Reprod 1998;13:65–70
14. Miller K, Falcone T, Goldberg JM, Attaran M: Previous fertilization failure with conventional in vitro fertilization is associated with poor outcome of intracytoplasmic sperm infection. Fertil Steril 1998;69:242–206
15. Tesarik J, Sousa M, Testart J: Human oocyte activation after intracytoplasmic sperm injection. Hum Reprod 1994;9: 511–518
16. Gabrielsen A, Petersen K, Mikkelsen AL, Lindenberg S: Intracytoplasmic sperm injection does not overcome an oocyte defect in previous fertilization failure with conventional in vitro fertilization and normal spermatozoa Hum Reprod 1996;11: 1963–1965
17. Svalander P, Forsberg AS, Jakobsson AH, Wikland M: Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995;63:828–837
18. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J: Embryo morphology, developmental rates, and maternal age are correlated with chomosome abnormalities. Fertil Steril 1995;64: 382–391