Intracytoplasmic Sperm Injection and Conventional In Vitro Fertilization Are Complementary Techniques in Management of Unexplained Infertility

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Purpose: To evaluate the role of ICSI in unexplained infertility.

Methods: In 125 cycles with six or more oocytes retrieved per cycle, sibling oocytes were randomly allocated to IVF or ICSI (group A). In 74 cycles with less than six oocytes retrieved per cycle, cycles were allocated to IVF or ICSI (group B).

Results: In group A, ICSI fertilization rate of 61% per allocated oocyte was higher than IVF fertilization rate of 51.6% ($P < 0.001$). Complete fertilization failure occurred in 19.2 and 0.8% of cycles in IVF and ICSI, respectively ($P < 0.001$). In group B, fertilization rate in IVF cycles was 53.3% as compared to 60.7% per allocated oocyte in the ICSI cycles ($P = 0.29$). Complete fertilization failure was higher ($P = 0.02$) in conventional IVF (34.3%) than ICSI cycles (10.3%).

Conclusions: Allocation of sibling oocytes to IVF and ICSI in the first cycle minimizes risk of fertilization failure. For patients with limited number of oocytes, ICSI technique is recommended.

KEY WORDS: ICSI; IVF; sibling oocytes; unexplained infertility.

INTRODUCTION

Assisted reproductive techniques offer a unique way of treating infertility by in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). In vitro fertilization is indicated in most of the female-related infertility, whereas ICSI is mostly employed in couples with male-factor infertility (1–3) or with a history of fertilization failure (4). A small percentage of IVF cycles results in total fertilization failure. However, general use of ICSI for

all cases of infertility is not recommended in assisted reproduction (5).

Intracytoplasmic sperm injection is more invasive, more costly and more time-consuming than IVF, and safety issues have not been fully studied (6). In patients with tubal-factor infertility, ICSI and IVF resulted in similar fertilization rates of sibling oocytes (7). This was further shown by a prospective randomized trial (8), in which cycles were allocated randomly to either conventional IVF or ICSI. Similar conclusions were reached in a prospective randomized study (9), and another one involving non-male-factor infertility in general (10).

Unexplained infertility counts for approximately 15% of infertility cases, and is defined as failure to conceive with no known reason when routine fertility examinations show no abnormality in either partner (11). These patients are first treated with

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controlled ovarian hyperstimulation combined with intrauterine insemination up to three cycles (12). Patients who fail to become pregnant are referred to assisted reproduction.

Total fertilization failure or lower fertilization rates in unexplained infertility after IVF has been a concern (13–16). Performing ICSI and IVF on sibling oocytes has been recommended to prevent total fertilization failure and diagnose the sperm fertilizing ability for the management of future cycles (17–19). However, it is not always possible to split the oocytes due to low number of oocytes available. The purpose of this study was to further evaluate usefulness of splitting oocytes between IVF and ICSI when there were at least six oocytes in a cycle, and to determine whether IVF or ICSI would result in better fertilization in low responders (less than six retrieved oocytes) with unexplained infertility.

MATERIALS AND METHODS

Patients

One hundred and ninety-nine patients with unexplained infertility at their first cycle were included in this analysis between June 1997 and April 2002. Couples with six or more oocytes (group A, $n = 125$) were subjected to both IVF and ICSI of their sibling oocytes. Couples with less than six oocytes (group B, $n = 74$) were treated with IVF until March 2000, and thereafter, ICSI was utilized for such patients, because a previous study (20) showed that this patient group was at higher risk of total fertilization failure.

Unexplained infertility definition was based on at least two normal semen analyses (sperm concentration $\geq 20 \times 10^6/\text{mL}$, motility $\geq 30\%$, and normal morphology $\geq 14\%$ according to Kruger's strict criteria), normal sexual history, normal female reproductive organs, ultrasound evidence of ovulation with serum progesterone more than 5 nmol/L, normal hysterosalpingogram and laparoscopy, and failure to conceive for at least 5 years before assisted reproduction.

Patient Stimulation and Oocyte Retrieval

Ovarian suppression was performed by gonadotropin releasing hormone (GnRH) agonist long protocol. Patients were given 3.75 mg Lupron Depot (Abbott Laboratories, Chicago, IL) on day 3 of the cycle. Twenty-six days after Lupron injection, ovarian stimulation with hMG (Pergonal, Serono Laboratories) started along with subcutaneous Suprefact

(400 μg , Hoechst UK Limited, Middlesex, UK) until the day of hCG. Ten thousand IU of hCG were given IM when the leading follicle size reached 18 mm and estradiol levels were appropriate. Oocyte retrieval was performed with transvaginal ultrasound guidance using an aspiration needle (Swemed Lab, Billdal, Sweden) under intravenous sedation 36 h after hCG injection. The day of oocyte retrieval was designated as day 0. The follicular aspirate was poured into 60-mm Falcon dishes and cumulus-oocyte complexes were transferred into another dish with Medi-Cult flush medium (Medi-Cult, Jillinge, Denmark). Each complex was evaluated for maturity based on cumulus-corona cell morphology. Cumulus-oocyte complexes were transferred into 100 μL IVF medium (Medi-Cult) under mineral oil (Sigma, St. Louis, MO). They were incubated in 5% CO_2 in air with saturated humidity until the time of insemination for IVF or removal of cumulus cells for ICSI.

Semen Processing, Insemination, ICSI, and Fertilization Check

All the semen samples were allowed to liquefy for 15–30 min. Sperm count and motility analyses were performed, and the motile sperm fraction was enriched by using discontinuous Percoll (95 and 47.5%, Pharmacia, Uppsala, Sweden). One half to 2 mL of raw semen was layered over the Percoll, and preparations were centrifuged at 300g for 20 min. At the end of centrifugation, the pellets from each tube were collected into 5 mL culture medium and centrifuged another 10 min at 300g for IVF and 1800g for ICSI. Each drop of medium under oil containing 3–4 oocytes was added with approximately 100,000 motile spermatozoa in the drop. Intracytoplasmic sperm injection was performed as described previously (21). Fertilization was confirmed by checking for the presence of two pronuclei and two polar bodies 15–18 h after insemination or ICSI. All other types of outcome (no fertilization, one pronucleus, polyspermia or degeneration) were also recorded.

Embryo Culture, Embryo Transfer, and Luteal Support

Zygotes were cultured in IVF medium (Medi-Cult) until transfer. The number of blastomeres, the degree of fragmentation, and evenness of blastomere size for each embryo were recorded on days 2 and 3. Embryos were graded as good, fair, and poor. Good embryos were defined as embryos

with less than 10% fragmentation. Fair embryos had 10–30% fragmentation and poor embryos were heavily fragmented (>30%). Up to three best quality embryos were transferred by using Wallace embryo transfer catheter (Simcare, West Sussex, UK) under ultrasound guidance.

Patients were supplemented with progesterone (100 mg/daily IM, Steris Laboratories Inc., Phoenix, AZ). Pregnancy was confirmed first with Tandom Icon urine hCG test (Hybritech, San Diego, CA) and serum β -hCG levels 13 days, and with ultrasound 5 weeks after embryo transfer, respectively.

Statistical Analysis

Comparisons between groups were done by using the Fisher exact test ($n < 100$) or Chi-squared analysis. A P value of ≤ 0.05 was considered as significant.

RESULTS

Group A consisted of 125 patients. The average age of patients was 31 years. A total of 1661 oocytes were obtained and they were randomly allocated to either ICSI or IVF right after oocyte collection. The fertilization rate for IVF was lower than fertilization rates for ICSI ($P < 0.001$, Table I). There were more cycles with total fertilization failure in IVF group (19.2%) compared to ICSI group (0.8%, $P < 0.001$, Table I). A total of 42 (33.6%) pregnancies were established. The study design did not allow us to compare pregnancy rates from ICSI and IVF since the best embryos were chosen to be transferred regardless of the insemination technique.

In group B, exclusively IVF or ICSI were performed for 35 and 39 patients, respectively, because there was not enough oocytes to randomize (<6). Fertilization rates were 53.3% for IVF cycles and 60.7% for ICSI

Table I. Outcome of Sibling Oocytes in the First Assisted Reproductive Cycle in 125 Patients with Unexplained Infertility

	IVF	ICSI	P value
No. of oocytes	754	907	
No. of injected oocytes (%)	NA	764 (84.2)	
No. of fertilized oocytes	389	553	
Percent fertilization/oocytes	51.6	61.0	<0.001
Percent fertilization/ injected oocytes	NA	72.4	
Total fertilization failure (%)	24 (19.2)	1 (0.8)	<0.001

Note. IVF: In vitro fertilization; ICSI: Intracytoplasmic sperm injection.

Table II. Outcome of Cycles with Low Number of Oocytes (<6)

	IVF	ICSI	P value
No. of cycles	35	39	
Average female age (years)	32.7	32.1	0.67
No. of oocytes	120	135	
Average number of oocytes/cycle	3.43	3.46	
No. of injected oocytes (%)	NA	116 (86)	
No. of fertilized oocytes	64	82	
Percent fertilization/oocytes	53.3	60.7	0.29
Percent fertilization/ injected oocytes	NA	70.7	
Total fertilization failure (%)	12 (34.3)	4 (10.3)	0.02
No. of embryo transfers	23	33	
No. of pregnancies	4	11	
Percent pregnancies/retrieval	11.4	28.2	0.09
Percent pregnancies/transfer	17.4	33.3	0.23

Note. IVF: In vitro fertilization; ICSI: Intracytoplasmic sperm injection.

cycles. There was more fertilization failure in IVF cycles (34.3%) compared to ICSI (10.3%, $P = 0.02$). Pregnancy rates per oocyte retrieval were 11.4 and 28.2% for IVF and ICSI cycles, respectively ($P = 0.09$, Table II).

DISCUSSION

The results of the present study showed a significantly higher complete fertilization failure in conventional IVF (19%) when compared to ICSI in patients with unexplained infertility in their first cycle. Similarly, complete fertilization failure ranging from 11 to 22.7% in conventional IVF has been reported in unexplained infertility patients (17–19). This finding has two major impacts; first, nearly one fifth of unexplained infertility patients would have lost the chance for embryo transfer if ICSI were not done on sibling oocytes. Second, we were able to counsel patients that gamete dysfunction could contribute to their unexplained infertility and we recommended for ICSI in their future cycles. Repeated nature of complete fertilization failure in unexplained infertility patients has been reported earlier (16,22). Lipitz *et al.* (22) showed the incidence of recurrent complete fertilization failure of 51, 27.2, and 33.3% in second, third, and fourth IVF cycles, respectively. Gurgan *et al.* (16) also showed that 62% of unexplained infertility patients had a complete fertilization failure in their second cycle. A very low rate of complete fertilization failure in IVF has been reported in a subgroup of couples with unexplained infertility in a recent randomized controlled trial (10). It is difficult to compare this report

to the present study since the demographic and other cycle-specific data are not presented for detailed analysis. Moreover, excluding patients with history of poor fertilization and including patients with previous cycle might have eliminated some of the complete fertilization failure.

Fertilization rates after ICSI in this study was higher than conventional IVF. Hershlag *et al.* (19) also observed significantly higher fertilization rates in ICSI. This higher fertilization rate by ICSI is a function of higher total fertilization failure in IVF oocytes rather than superiority of ICSI technique. When total fertilization failure cycles in IVF were excluded in the present study, fertilization rates were similar to ICSI (62% for IVF and 61% per retrieved oocytes for ICSI). In a recent prospective randomized multicenter study (10), fertilization rate per retrieved oocyte was significantly higher in IVF than ICSI cycles including a subgroup of unexplained infertility patients (61% vs. 50%). Again, the study design (excluding patients with history of poor fertilization and including patients with previous cycle) might be in favor of increasing IVF fertilization rates.

Reports addressing routine use of ICSI in low responders with unexplained infertility are not available. Moreno *et al.* (23) in a prospective randomized study between IVF and ICSI in low responders without male factor concluded that routine use of ICSI is not indicated. However, this could not be extrapolated to unexplained infertility patients. Our results in low responders showed that conventional IVF resulted in an acceptable fertilization rate of 53.3%, but a 34.3% rate of complete fertilization failure was disturbing. This prompted us to switch to routine use of ICSI in low responders. Intracytoplasmic sperm injection was superior in terms of significantly lower rate of complete fertilization failure than conventional IVF. Pregnancy rates per oocyte retrieval from IVF and ICSI in low responders were better in ICSI cycles although the difference did not reach significance probably due to low number of cases. Overall fertilization rates of oocytes by IVF or ICSI from low responders were similar to fertilization rates of normal responders in the present study. However, the total fertilization failure rates were higher in low responders probably due to uneven distribution of fertilization among patients.

In conclusion, this study further stresses the routine use of IVF and ICSI of sibling oocytes in first cycle of patients with unexplained infertility. The routine use of ICSI is indicated in low responders with unexplained infertility to avoid high rate of total fer-

tilization failure. Our results indicate that for every 100 ICSI cycles we were able to salvage 20 cycles from complete fertilization failure. This result is both statistically and clinically significant.

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