Relationship Between the Sperm Motility Index Assessed by the Sperm Quality Analyzer and the Outcome of Intracytoplasmic Sperm Injection[1]

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Purpose: *Intracytoplasmic sperm injection (ICSI) has been validated as a useful treatment in severe male-factor patients who could not achieve fertilization and live births by conventional in vitro fertilization treatment. To examine the impact of male factors on ICSI outcome, clinical laboratory data were retrospectively analyzed.*

Methods: *One hundred two cycles of ICSI treatment indicated by severe male-factor infertility were entered into this study. Sperm parameters including sperm motility, sperm concentration, and sperm motility index assessed by the Sperm Quality Analyzer were evaluated.*

Results: *Five hundred seventy-six metaphase II oocytes retrieved were manipulated. The normal fertilization (2 PN) rate per oocyte was 64.9 ± 26.0% (mean ± SD). Of the 99 transfers, 31 clinical pregnancies were obtained, yielding an average pregnancy rate of 31.3% per transfer. The mean sperm motility, sperm concentration, and sperm motility index were 20.3 ± 16.1% (range, 0 to 50%), 18.2 ± 25.1 × 10^6/ml (range, <1 to 150 × 10^6/ml), and 31.2 ± 45.0 (range, 0 to 220), respectively. Sperm concentration did not have a significant impact on fertilization rate by ICSI. In four cases, ICSI was performed using totally immotile sperm and the fertilization rate was 43.5%, which was significantly lower than that of some of the other sperm motility groups, and no pregnancy could be achieved. In 14 cases in which the sperm motility index assessed by the Sperm Quality Analyzer was 0, the fertilization rate (50.0%) was significantly lower than in most of the other sperm motility index groups.*

Conclusions: *These findings suggest that in severe male-factor cases with totally immotile sperm or a sperm motility index of 0, the selection of good-quality sperm should be verified before injection.*

KEY WORDS: intracytoplasmic sperm injection; male-factor infertility; fertilization; Sperm Quality Analyzer.

INTRODUCTION

A recent breakthrough in the field of assisted fertilization was the introduction of intracytoplasmic sperm injection (ICSI) (1–3) for the treatment of severe male-factor infertility. ICSI has resulted in both higher fertilization and pregnancy rates. A large number of pregnancies have now been achieved throughout the world using this method (4) and the technique is also being used to study basic aspect of fertilization in humans.

The development of ICSI gives rise to some concern, especially with regard to the possibility of abnormal fetal development during pregnancy and an increased risk of congenital abnormalities in children. In a study with a small number of prenatal diagnoses, a high rate of sex chromosomes abnormalities was reported in pregnancies by ICSI (5). However, their observations were not supported by other authors who studied a larger number of prenatal diagnosis (6–8). A prospective follow-up study of 877 children born after ICSI by Bonduelle *et al.* (9) also revealed that there seemed to be no higher incidence of congenital malformations. Since October 1995, ICSI has been used successfully in our department for the treatment of severe male-factor infertility. To date we have not detected any sex chromosomal abnormalities in the fetuses resulting from ICSI (10).

Although high success rates have been reported following ICSI using ejaculated semen samples with very

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poor conventional sperm parameters (2,3), total fertilization failure rates have still been reported as between 3 and 7% in the injected cycles (11–14). It was concluded that total fertilization failure after ICSI was caused mainly by the poor viability of the spermatozoa used for injection (12).

The objective of this study was to determine the efficacy and factors affecting outcome of ICSI in patients with severe male-factor infertility. The sperm motility index assessed by the Sperm Quality Analyzer (SQA; United Medical Systems Inc., Santa Ana, CA), sperm motility, and sperm concentration were retrospectively analyzed and correlated with normal fertilization rates and pregnancy outcome in ICSI patients.

MATERIALS AND METHODS

Subjects

The study period lasted from October 1995 to December 1997 at the Department of Obstetrics and Gynecology, Hyogo College of Medicine, and included 102 ICSI treatment cycles using fresh ejaculated sperm. The criterion for patients to receive ICSI treatment was either (a) a total failure of fertilization or less than a 10% fertilization rate in a conventional in vitro fertilization–embryo transfer (IVF-ET) attempt or (b) a sperm penetration index below 15 evaluated by the zona-free hamster egg penetration test (HEPT) (15).

Ovulation Stimulation Protocol

The patients were stimulated using a combination of a gonadotropin releasing hormone (GnRH) agonist started in the luteal phase (suppression protocol) followed by gonadotropins as we reported previously (16–18).

Routine Semen Analysis

Fresh ejaculated semen samples of 102 men for ICSI treatment were obtained by masturbation immediately after retrieving oocytes from their wives. After liquefaction, semen quality analysis was performed according to the standardized protocols as stipulated by the World Health Organization (19). In this study the sperm motility and sperm concentration were examined using a hemocytometer and compared with the outcome of ICSI. The SQA was performed simultaneously as described below.

Measurement of the Sperm Motility Index

Sperm motility index determinations were performed simultaneously on the same semen samples using the SQA. Semen was applied by capillary force into a thin glass capillary tube (internal dimensions: depth, 0.3 mm; width, 3 mm; length, 50 mm). The sample was processed by the SQA according to the manufacturer's instructions. Using this method, each sperm motility index reading represents the mean of four 10-sec measurements of the analog signal. The photoelectric cell in the SQA detects variations in the optical density (OD) caused by the motility of spermatozoa. The frequency of the analog wave-like electrical signal was counted to provide, on analog-to-digital conversion, the sperm motility index value of the tested sample. All measurements were performed at room temperature and the values of sperm motility index were classified into three categories: "poor" ($0 \leqq$ sperm motility index $\leqq 80$), "medium" ($81 \leqq$ sperm motility index $\leqq 160$), and "good" (sperm motility index $\geqq 161$).

ICSI Procedure

ICSI was performed on the cover of an organ tissue culture dish (Falcon 3037; Becton Dickinson Labware, Lincoln Park, NJ). Sperm with apparently normal morphology and motility were chosen and introduced into a microdroplet of 10 μl human tubal fluid (HTF) containing 10% polyvinylpyrrolidone (PVP). A selected sperm was then immobilized by cutting the tail using the micropipette for injection (20) purchased from Humagen Fertility Diagnostic, Inc. (10-MIC; Charlottesville, VA) and ICSI was performed in HTF medium. The oocyte was held by a slight negative pressure on the holding pipette with the polar body at a 90° angle to the pipette. The sperm was then inserted deeply into the cytoplasm of the oocyte. After injection, oocytes were washed with B2 medium (Menezo, Paris, France) briefly, transferred to 1 ml of the same medium in a petri dish, and then kept in an incubator containing 5% CO_2, 5% O_2, and 90% N_2. On the morning following microinjection, oocytes were examined for the presence of pronuclei (PN) and cultured for another 24 hr to allow for cleavage. A maximum of three embryos with the highest morphology score was selected for transfer to the patient on the second day after oocyte retrieval, and extra preembryos, if available, were cryopreserved according to the standard methods (21). In some younger women, only two fresh embryos with excellent morphology

were selected for transfer to prevent high-rank multiple pregnancy (22,23) because any pregnancy complications are related to multiple pregnancies after ICSI (8).

Statistics

Statistical analysis of the data was carried out by χ^2 analysis using Statview 4.5 (Abacus Concepts, Berkeley, CA) for Macintosh, and $P < 0.05$ was defined as representing a significant difference. Pearson's correlation coefficient was used to analyze the predictability of the ICSI fertilization outcome by the semen characteristics and sperm motility index.

RESULTS

Overall ICSI Results

ICSI was carried out in a total of 102 cycles. Five hundred seventy-six of 710 (81.1%) oocytes retrieved were in metaphase II stage and were manipulated. The normal fertilization (2 PN) rate per oocyte was 64.9 ± 26.0% (mean ± SD). Embryo transfer could not be performed in 3 of 102 patients because no embryo was available. Of the 99 transfers, 31 clinical pregnancies were obtained, giving an average pregnancy rate of 31.3% per transfer. There were six cases of twin pregnancy and three cases of triplet pregnancy. Excluding four cases of clinical abortion, the ongoing pregnancy rate was 27.3% per transfer.

Normal Fertilization and Pregnancy Outcome in ICSI Patients Related to the Sperm Motility and Sperm Concentration

The mean sperm motility and sperm concentration were 20.3 ± 16.1% (range, 0 to 50%) and 18.2 ± 25.1 × 10^6/ml (range, <1 to 150 × 10^6/ml), respectively. The relationship between sperm motility and outcome in ICSI is shown in Table I. In four cases in which immotile sperm was used, the fertilization rate was lower than in all of the other sperm motility groups. There were significant differences in the fertilization rate between the 0% motility group and the 20 to 39% motility group ($P < 0.025$) as well as between the 0% motility group and the ≧40% motility group ($P<0.025$). None of the four cases with totally immotile sperm resulted in a pregnancy. There were no significant differences in the pregnancy rates between any of the sperm motility groups.

The relationship between the sperm concentration and the outcome of ICSI are shown in Table II. There were no significant differences between the groups in either the fertilization rate or the pregnancy rate, indicating that the sperm concentration did not seem to have a significant impact on the outcome by ICSI.

Table I. Normal Fertilization and Pregnancy Outcome in ICSI Patients Related to the Sperm Motility*

Sperm motility (%)	No. of cycles	No. of fertilized oocytes per No. of injected oocytes	No. of pregnancies per No. of transfers
0	4	10/23 (43.5%)[a,b]	0/4 (0.0%)
1 to 9	20	76/128 (53.1%)	6/18 (33.3%)
10 to 19	25	95/149 (63.8%)	11/25 (44.0%)
20 to 39	37	135/193 (69.9%)[a]	10/36 (27.8%)
≧40	16	58/83 (69.9%)[b]	4/16 (25.0%)
Total	102	374/576 (64.9%)	31/99 (31.3%)

* Superscripts a and b, $P < 0.025$.

Table II. Normal Fertilization and Pregnancy Outcome in ICSI Patients Related to the Sperm Concentration[a]

Sperm concentration (×10^6/ml)	No. of cycles	No. of fertilized oocytes per No. injected oocytes	No. of pregnancies per No. of transfers
<1	13	36/64 (56.3%)	2/13 (15.4%)
1 to 9.9	27	95/144 (66.0%)	6/27 (22.2%)
10 to 19.9	20	70/112 (62.5%)	6/20 (30.0%)
≧20	42	87/117 (74.4%)	17/39 (43.6%)
Total	102	374/576 (64.9%)	31/99 (31.3%)

[a] There were no significant differences between the groups.

Normal Fertilization and Pregnancy Outcome in ICSI Patients Related to the Sperm Motility Index

Ninety of 102 (88.2%) semen samples were judged as "poor" sperm motility indices according to the three categories classification. In the other 12 cases, 5 were judged as "medium" and 7 were judged as "good." The mean sperm motility index was 31.2 ± 45.0 (range, 0 to 220). As shown in Table III, 14 cases with a sperm

Table III. Normal Fertilization and Pregnancy Outcome in ICSI Patients Related to the Sperm Motility Index (SMI)*

SMI	No. of cycles	No. of fertilized oocytes per No. of injected oocytes	No. of pregnancies per No. of transfers
0	14	34/68 (50.0%)[a,b,c]	3/14 (21.4%)
1 to 20	32	123/191 (64.4%)[a]	8/31 (25.8%)
21 to 40	25	85/138 (61.6%)	7/23 (30.4%)
41 to 80	19	70/101 (69.3%)[b]	7/19 (36.8%)
≧81	12	62/78 (79.5%)[c]	6/12 (50.0%)
Total	102	374/576 (64.9%)	31/99 (31.3%)

* Superscripts a and b, $P < 0.05$; c, $P < 0.001$.

Table IV. Comparison of the Normal Fertilization and Pregnancy Outcome in ICSI Patients with Sperm Having a Motility Index (SMI) of 0[a]

SMI	Sperm motility (%)	No. of cases	No. of fertilized oocytes per No. of injected oocytes	No. of pregnancies per No. of transfers
0	0	4	10/23 (43.5%)	0/4 (0.0%)
0	≧1	10	24/45 (53.3%)	3/10 (30.0%)
Total		14	34/68 (50.0%)	3/14 (21.4%)

[a] There was no significant difference between the groups.

motility index of 0 had a 50.0% fertilization rate, which was significantly lower than three of the other four sperm motility index groups, however, 3 of the 14 cases (21.4%) with a sperm motility index of 0 maintained ongoing pregnancies. There were no significant differences in the pregnancy rates among all these groups.

The fertilization rate and pregnancy outcome in ICSI patients with a sperm motility index of 0 were compared to the sperm motility (Table IV). There was no significant difference in the outcome of ICSI between 4 patients with a sperm motility of 0% and 10 patients with a sperm motility of ≧1. However, the pregnancy rate in patients with a sperm motility of ≧1 seemed to be higher than that with a sperm motility of 0%, although there was no significant difference.

Comparison of the ICSI Fertilization Outcome with the Semen Characteristics and Sperm Motility Index

As shown in Table V, none of the sperm parameters including sperm concentration, sperm motility, and sperm motility index were significantly correlated with the ICSI fertilization outcome.

DISCUSSION

Although IVF is now widely used for the treatment of infertility due to sperm dysfunction (24,25), most male-factor patients with less than adequate sperm numbers have a total or partial failed fertilization. ICSI has recently been reported to facilitate fertilization in couples who could not be helped by conventional IVF treatment or by other microassisted fertilization methods such as partial zona dissection (PZD) and subzonal insemination (SUZI) (1–3). ICSI has now gained worldwide acceptance as the ultimate microassisted approach to male-factor infertility (4). However, low fertilization rates still occur when ICSI is carried out for patients with totally immotile sperm in the ejaculate (26–29). Evaluation of sperm vitality using the eosin Y exclusion test (19) to differentiate between live and dead spermatozoa may predict the outcome of ICSI. Patients with a sperm vitality score below the normal values were recommended to undergo the hypoosmotic swelling test because live sperm can be selected before injection (29) or treated with testicular sperm injection (28). Recently, Barros *et al.* (30) reported a case of successful pregnancy and birth after ICSI with totally immotile ejaculated sperm which presented normal vitality scores using the usual random sperm selection. On the contrary, there is a report that achievement of high pregnancy rates by ICSI in couples with severe male-factor infertility is dependent primarily upon female and not male factors (13). Taking these investigations into consideration, the aim of this study was to determine if the SQA could predict the fertilizing potential of sperm obtained from patients with severe male-factor infertility in the ICSI treatment.

With respect to the sperm parameters, previous studies have demonstrated that there was no sperm criterion required for successful ICSI. Oehninger *et al.* (13) have reported that none of the original semen analyses including sperm concentration, progressive motility, and morphology correlated with ICSI outcome. Svalander *et al.* (14) have used the "strict criteria" of sperm morphology and concluded that the outcome of ICSI was unrelated to the criteria. Sukcharoen *et al.* (31) used a computer (IVOS system) to predict the fertilization rate after ICSI, especially with regard to sperm morphology and also found no correlation between sperm morphology and the outcome of ICSI. However, totally immotile sperm have never been included in these reports. In contrast, Nagy *et al.* (11) and Liu *et al.* (12) have determined that poor viability of the sperm or immotile sperm were the main causes of fertilization failure after ICSI. The authors' group recently reported that the causes of total asthenozoospermia were variable and the problem was a sporadic rather than a permanent condition (32). They suggested that if the injection of ejaculated immotile

Table V. Predictability of the ICSI Fertilization Outcome by the Semen Characteristics and Sperm Motility Index (SMI)

Parameter	Correlation[a]
Sperm concentration (10^6/ml)	0.115
Sperm motility (%)	0.149
SMI	0.175

[a] There was no significant correlation for any parameter (Pearson's correlation coefficient).

sperm in an initial cycle led to poor results, performance of subsequent ICSI cycles with ejaculated sperm was justified (32).

The SQA is a simple and inexpensive device which provides a quantitative estimation of sperm motility. Furthermore, the SQA has a good reproducibility, particularly in comparison to conventional semen analysis, which is highly subjective, and to computer-assisted sperm motility analysis techniques, which have problems associated with the distinction between debris and sperm. As the SQA recognizes only motile particles, problem with debris have been largely overcome (33). Previous investigations have demonstrated significant correlations between the sperm motility index and traditional semen parameters (33,34) as well as the sperm motility index and the fertilization rate in conventional IVF (18). However, it has not been reported if the SQA can be used to predict fertilization failure after ICSI.

The overall normal fertilization rate of 64.9% after ICSI in our present study is as high as those in recent reports (12–14). The average clinical pregnancy rate of 31.3% per transfer is also encouraging compared with the world report of an average clinical pregnancy rate of 21.7% (range, 6.3 to 31.3%) (4). In the present study, correlation between the ICSI outcome and sperm parameters and sperm motility index assessed by the SQA was analyzed retrospectively. Sperm concentration had no impact on the ICSI results (Table II) as shown by other investigators (11–14,31,32). As for sperm motility, the average fertilization rate was 43.5% and no pregnancy could be achieved in four cases with totally immotile sperm (Table I), indicating that the injection of a totally immotile spermatozoon may have a negative effect on the ICSI results. This conclusion supported other investigations (11,12).

In 14 cases with a sperm motility index of 0, the fertilization rate (50.0%) was significantly lower than in two of the other four sperm motility index groups (sperm motility index, 41–80 and ≧81) (Table III), although there was no significant difference in the pregnancy rate. In ICSI patients who had a sperm motility index of 0, the normal fertilization rate in 10 cases with a sperm motility of ≧1% was as low as that in 4 cases with a sperm motility of 0% (Table IV). These findings suggest that in severe male-factor cases with totally immotile sperm or a sperm motility index of 0, including severe asthenozoospermia from the ejaculate, the selection of good-quality sperm should be verified before injection. However, the reason that sperm from an ejaculate with a sperm motility index of 0 show a low fertilization rate is not known. It may be suggested that the SQA can be used as one of the discriminatory tests that can predict fertilization failure after ICSI.

In conclusion, our data suggest that ICSI is the best microassisted approach to severe male-factor infertility. Information on the sperm motility index as well as the sperm motility could help the laboratory providing the hypoosmotic swelling test or testicular sperm before manipulation.

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