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The Effects of Severity of Oligozoospermia on Intracytoplasmic Sperm Injection (ICSI) Cycle Outcome

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The objective of this study was to explore the relationship between the severity of oligozoospermia and the development of embryos and clinical outcome in patients undergoing ICSI. A total of 908 intracytoplasmic sperm injection cycles involving women of ≤ 37 years of age were included in this study. The patients were divided into four treatment groups according to the results of an analysis of their husbands' semen: (A) mild oligozoospermia that ranged from 10×10^6/ml to $< 20 \times 10^6$/ml ($n = 283$), (B) mild to severe oligozoospermia that ranged from 5×10^6/ml to $< 10 \times 10^6$/ml ($n = 192$), (C) severe oligospermia that ranged from 1×10^6/ml to $< 5 \times 10^6$/ml ($n = 259$), and (D) very severe oligozoospermia that ranged from 0 to $< 1 \times 10^6$/ml ($n = 174$).Two pronuclei (PN) oocytes at MII were injected and the development of high quality embryos on day 2, as well blastocyst formation rate on day 5, the implantation rate, clinical pregnancies, and fetal loss, were examined. A lower percentage of two pronuclei (2PN) oocytes in the very severe oligozoospermia group was observed, however, there was no difference in clinical outcome when the oligozoospermic patients were divided by sperm concentration. In addition, no significant difference was detected in zygote production or clinical outcome between spermatozoa with a motility of $< 40\%$ and spermatozoa with a motility of $\geq 40\%$. The results of this study emphasize the importance of selecting good quality sperm for oocyte injection, especially in cases involving very severe oligozoospermia.

KEYWORDS ICSI, semen analysis, sperm motility

Abbreviations: PN: pronuclei; ICSI: intracytoplasmic sperm injection; ART: assisted reproductive treatment; OAT: oligo-asthenoteratozoospermia; DFI: DNA fragmentation index; hCG: human chorionic gonadotropin.

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INTRODUCTION

The development of intracytoplasmic sperm injection (ICSI) created a new era in the field of assisted reproduction and revolutionized the assisted reproductive treatment (ART) protocols for couples with male factor infertility [Palermo et al. 1992]. In general, in the case of male subfertility, ICSI treatment results in higher fertilization rates per oocyte compared with

conventional IVF treatment. Even the most severe cases of oligo-astheno-teratozoospermia (OAT) can now be successfully treated with ICSI. A dominant effect of a single suboptimal semen parameter on the fertilization results after IVF and ICSI was reported for sperm morphology and motility [van der Westerlaken et al. 2006]. However, only a few studies have mentioned semen quality (concentration and motility) in connection with the efficacy of ICSI performed with ejaculated spermatozoa. We determined whether the sperm concentration and motility in cases of oligozoospermia are associated with laboratory or clinical outcomes. The aim of this study was to explore the relationship between sperm quality and embryo development, pregnancy, and implantation rates as well as fetal loss in patients undergoing ICSI.

RESULTS

The background of the male population subjected to the routine semen analysis, as well as the age of the women (mean $\pm$ SD) and the results of the evaluation of zygote production (2PN oocytes - percentage of MII oocytes), proportion of normal cleaved embryos (percentage of 2PN oocytes), and the proportion of high quality embryos on day 2 (percentage of cleaved embryos) after ICSI are given in Table 1. The very severe oligozoospermia group demonstrated a significantly lower percentage of 2PN oocytes compared with the other groups (Groups A, B, and C vs. Group D, $p < 0.05$). There was no significant difference in clinical pregnancy rates, implantation rates, or fetal loss (Table 1).

Even when the treatment groups were selected based on sperm motility of $< 40\%$, there were no significant differences in zygote production or clinical pregnancy rates among any group (Table 2). In addition, no significant differences in zygote production or clinical pregnancy rates were detected between motility of $< 40\%$ and $\geq 40\%$ in any group (Table 2).

DISCUSSION

Conventional semen analysis is the first step when counselling the male partner of an infertile couple towards the clinical diagnosis and management of male infertility. However, it is not always sufficient

TABLE 1 Background and Evaluation of Embryo Development and Clinical Outcome After ICSI.

	Group A	Group B	Group C	Group D
	10 M/ml $\leq$ Co. $<$ 20 M/ml	5 M/ml $\leq$ Co. $<$ 10 M/ml	1 M/ml $\leq$ Co. $<$ 5 M/ml	0 $<$ Co. $<$ 1 M/ml
Number of patients	283	192	259	174
Female's age (years)	33.3 $\pm$ 3.0	32.8 $\pm$ 3.1	32.9 $\pm$ 3.3	32.7 $\pm$ 3.3*
2PN oocytes (% of MII oocytes)	257/349 (73.6%)	120/153 (78.4%)	244/341 (71.6%)	161/252 (63.9%)
Normal cleaved embryos (% of 2PN oocytes)	247/257 (96.1%)	117/120 (97.5%)	240/244 (98.4%)	155/161 (96.3%)
High quality embryos on day 2 (% of cleaved embryos)	159/247 (64.4%)	70/117 (59.8%)	150/240 (62.5%)	102/155 (65.8%)
Blastocyst formation rate on day 5 (%)	36.2	42.5	43.2	44.0
Clinical pregnancy (% per ET cycle)	108/283 (38.2%)	73/192 (38.0%)	95/259 (36.7%)	57/174 (32.8%)
implantation rate (% per embryo)	125/511 (24.5%)	83/387 (21.4%)	101/462 (21.9%)	60/280 (21.4%)
Fetal Loss (%)	17.6	20.5	18.9	19.3

Co.: sperm concentration, *$p < 0.05$, group A, B, and C, vs. D.

TABLE 2 Evaluation of Embryo Development and Clinical Outcome After ICSI

	Group A 10 M/ml ≤ Co. < 20 M/ml	Group B 5 M/ml ≤ Co. < 10 M/ml	Group C 1 M/ml ≤ Co. < 5 M/ml	Group D 0 < Co. < 1 M/ml
The patients whose sperm motility was < 40%				
2PN oocytes (% of MII oocytes)	172/229 (75.1%)	67/94 (71.3%)	133/188 (70.7%)	157/246 (63.8%)*
Clinical pregnancy (% per ET cycle)	63/172 (36.6%)	29/92 (31.5%)	60/160 (37.5%)	48/144 (33.3%)
The patients whose sperm motility was ≥ 40%				
2PN oocytes (% of MII oocytes)	85/120 (70.8%)	53/59 (89.8%)	111/153 (72.5%)	4/6 (66.7%)
Clinical pregnancy (% per ET cycle)	45/111 (40.5%)	44/100 (44%)	35/99 (35.4%)	9/30 (30.0%)

Co.: sperm concentration, *$p < 0.05$, group A, B, and C, vs. D.

for the assessment of sperm function and male infertility. A dominant effect of a single suboptimal semen parameter of sperm morphology or motility on the fertilization results after IVF and ICSI was reported [Bartoov et al. 2001]. Only a few studies have mentioned semen concentration and motility in connection with the efficacy of ICSI especially in cases involving oligoasthenozoospermia.

In general, the selection of the spermatozoa to be injected into the oocyte is based on their gross morphology and motility. In cases of poor quality semen, the risk of inadvertently using spermatozoa with damaged DNA is probably higher. It has been reported that semen of poor quality contains an increased proportion of spermatozoa with DNA fragmentation [Lopes 1998]. The DNA damage in spermatozoa as well as sperm concentration and motility have been found to be negatively correlated with fertilization and pregnancy in conventional IVF cycles [Sun et al. 1997; Tomsu et al. 2002; Henkel et al. 2003]. Nevertheless, in ICSI cycles, there is no consensus of the relationship of the DNA fragmentation index (DFI) with outcome. Unfortunately, we could not examine DFI in this high volume study. However, as for sperm concentration, we only observed a significant relationship for the percentage of 2PN oocyte embryos, but no significant difference was observed for clinical outcome between cases of mild oligozoospermia and very severe oligozoospermia in ICSI. Interestingly, no significant difference in embryo development or clinical outcome was detected between spermatozoa with a motility of $< 40\%$ and spermatozoa with a motility of $\geq 40\%$ for oligozoospermia.

Increasing evidence has suggested that sperm morphology also plays a significant role in ICSI outcome [Bartoov et al. 2001; Chemes et al. 2003; De Vos et al. 2003]. However, these studies were not able to identify cutoff values for one or more sperm parameters that could successfully predict the occurrence of fertilization and thus be used to determine the optimal treatment for individual patients. The optimal period of sexual abstinence, the differences between fresh and frozen sperm, and how to treat cryptozoospermia also require consideration. As we have observed in performing ICSI using spermatozoa from patients with very severe oligozoospermia, morphological selection of spermatozoa could increase the rate of clinical

pregnancy. Patients with very severe oligozoospermia show a significantly lower percentage of 2PN oocytes. This emphasizes the importance of selecting good quality sperm for oocyte injection especially in patients with very severe oligozoospermia.

MATERIALS AND METHODS

Patient Population

In this case study, we retrospectively analyzed data obtained from a total of 908 ICSI-ET cycles performed between March 2000 and December 2008 with fresh ejaculated spermatozoa. Informed consent was obtained from all patients. The project was reviewed and approved by our board of ethics. This study analyzed 908 couples who underwent ICSI treatment. To exclude poor-responding patients, who would influence the results, couples in which the female was older than 38 years were excluded. Then, the patients were divided into four treatment groups according to the results of an analysis of the husband's semen: (A) mild oligozoospermia that ranged from 10×10^6 to $< 20 \times 10^6$/ml ($n = 283$), (B) mild to severe oligozoospermia that ranged from 5×10^6 to $< 10 \times 10^6$/ml ($n = 192$), (C) severe oligospermia that ranged from 1×10^6 to $< 5 \times 10^6$/ml ($n = 259$), and (D) very severe oligozoospermia that ranged from 0 to $< 1 \times 10^6$/ml ($n = 174$). Male-factor subfertility was defined as the presence of an abnormal semen parameter, i.e. a concentration $< 20 \times 10^6$/ml and/or $< 40\%$ motility according to the World Health Organization [WHO 1999] criteria.

Sperm Preparation and ICSI Technique

After 2–3 days of sexual abstinence, semen samples were produced by masturbation, collected in cups, and allowed to liquefy at room temperature. Then, the ejaculated sperm were centrifuged for 10 min at $600 \times$ g. Spermatozoa were prepared by the swim up technique. All ejaculate-related ICSI procedures used fresh (not frozen) ejaculated spermatozoa. During oocyte retrieval, the patients were stimulated using standard GnRH agonist/FSH protocols. Ovulation was triggered when the second leading follicle was > 18 mm in diameter, and oocytes were retrieved transvaginally under ultrasonographic guidance 36 h after human chorionic gonadotropin (hCG) administration. Handling of the injected oocytes was similar in all ICSI procedures. Approximately 16–18 h after microinjection, the oocytes were observed under an inverted microscope for any sign of damage that may have been caused by the microinjection as well as for the presence of pronuclei (PN) and polar bodies. Fertilization was considered normal when two clearly distinct PN containing nucleoli were present. The embryo cleavage of the 2PN oocytes was evaluated after a further 24 h of *in-vitro* culture on day 2. The embryos were scored according to the quality, number, size of the blastomeres, and the percentage of anucleate fragments. The high-quality embryo rate was defined as the number of high-quality embryos divided by the total number of normally fertilized oocytes. In the two-step ET group, one embryo was transferred on day 2 (the first step of ET), but another embryo with a better morphologic score was not used for transfer on day 2, but rather, was cultured until day 5. On day 5, one blastocyst or morula was transferred if available (the second step of ET). Cleavage-stage embryos were graded according to the criteria of Veeck [1991]. Clinical pregnancy was confirmed when the development of a gestational sac was observed by means of echographic screening at 7 weeks of pregnancy and the presence of a fetal heartbeat, and the implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred.

Statistical Analysis

Statistical evaluation was performed using the Chi-square test and t test where appropriate. A p-value of < 0.05 was considered statistically significant.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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